



**OPTIONS X**  
for the Control of  
**INFLUENZA**

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SUNTEC SINGAPORE

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# ORAL PRESENTATION

Topic: Clinical Sciences: Avian & Zoonotic Influenza - Human Infections  
Abstract No: 10853

## Incidence and seroprevalence of avian influenza viruses among Egyptian backyard poultry growers: Results from a prospective cohort study

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**Introduction and Objectives:** The highly pathogenic avian influenza H5N1 and H5N8 viruses and the low pathogenic H9N2 viruses are enzootic in Egyptian poultry. Several cases of human infection with H5N1 and H9N2 were reported in Egypt. We previously determined that the seroprevalence of H5N1 and H9N2 antibodies in Egyptians exposed to poultry was 2.1% and 7%, respectively, suggesting that mild or subclinical infections with those viruses occur.

**Methods:** We designed a 7-year, prospective, household-based cohort to determine incidence and household transmission of avian influenza viruses in humans exposed to backyard poultry. A total of 2405 individuals were enrolled from 5 villages in the Nile delta region.

**Results:** At baseline, seroprevalence of anti-H5N1 antibodies was 1.3% and decreased to 0.05% at the first follow-up year. Similarly, the seroprevalence of anti-H9N2 antibodies was 1.6% decreasing to 0.15%. Antibodies against H5N8 were not detected. In the 2017-2018 influenza season, 15% of the cohort reported ILI and 4.7% were laboratory-confirmed influenza A infections. Of those, 4 cases were confirmed as infected with H5N1 (incidence 17/10,000) reporting mild illness and recovered. In the 2018-2019 season, 34% of the cohort reported ILI and 7.2% were influenza A-confirmed. Subtyping a portion of those revealed 8 suspected and 4 confirmed H9N2 infections.

**Conclusion:** These preliminary results suggest that human infection with avian influenza viruses among poultry-exposed humans continues to occur but the patients report mild illness and are hence not detected by the surveillance system that relies on hospital-based severe acute respiratory illness and ILI reporting. The burden of human infection with avian influenza in Egypt is under-reported while severity of infection is over-estimated.

**Keywords:** Seroprevalence; human; poultry; avian influenza; Egypt; incidence

Topic: Clinical Sciences: Clinical Trials – Study Designs, Clinical Endpoints  
Abstract No: 11082

## CORRELATES OF PROTECTION FOR BETTER, FASTER INFLUENZA VACCINE DEVELOPMENT

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**Introduction:** BARDA is pursuing improved influenza pandemic preparedness through the advanced development of more effective vaccines. Such vaccines may use additional and/or alternative mechanisms of protection beyond induction of a humoral response as measured by serum hemagglutination-inhibition (HAI) titer. Licensure of these vaccines for pandemic indications will require development of validated immune correlates of protective efficacy.

**Method:** Recently, a non-replicating, orally administered adeno-based vaccine expressing the HA antigen (VXA-A1.1), and a commercial inactivated vaccine (IIV) were compared using several immune response measures in a large (~150 subject), BARDA funded, human challenge trial. In order to help define the most accurate correlates, we used a random forest model to create an unconditional analysis of the importance of each marker in predicting protection against infection.

**Result:** Post-vaccination, pre challenge serum HAI and neutralizing antibody; post vaccination HA specific IgA and IgG antibody secreting cells; and HA responsive IFN-g and Granzyme B secreting T cells, were all higher in those who were subsequently protected from infection, and correlated with each other. Random forest analysis suggested a substantial difference in the relative importance of immune factors by vaccine. For oral vaccine recipients, the most important predictive factor was the level of HA-specific IgA antibody-secreting cells (ASC) on day 8 post vaccination, followed by IgG ASC, IFN-g producing T cells, serum HAI and Granzyme B producing T cells. For recipients of IIV, the most important predictive marker was the HAI titer prior to challenge, followed by IgG ASC, IgA ASC, neutralizing antibody, and dual expressing T cells. B-cell phenotyping indicated up-regulation of integrin  $\beta 7$  within the plasmablast population of oral but not intramuscular vaccines, suggesting a unique role for B-cell homing in protection achieved with VXA-A1.1.

**Conclusion:** Establishing potential immune correlates of vaccine protection for novel influenza vaccines is in progress.

*Keywords: oral, mucosal, vaccine, correlate, human challenge*

Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention  
Abstract No: 10324

## ***Vaccine Induced Mucosal IgA Contributes to Protection Against Influenza Infection in Humans***

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**Introduction:** Current influenza vaccines are licensed and optimized to elicit serum antibody responses based on HAI titer; however, new vaccines that protect by alternative mechanisms are needed to improve efficacy and cross-protection.

**Methods:** A human challenge study was initiated to directly compare influenza vaccine approaches. 179 subjects were immunized with either an oral vaccine (recombinant adenovirus expressing H1 hemagglutinin), a quadrivalent inactivated vaccine (QIV), or placebo. Three months later, subjects were challenged with wildtype influenza H1 virus. Vaccine efficacy was measured using symptoms of illness and viral shedding. Immunogenicity was assessed by measuring humoral (HAI and MN titers) and cell-mediated responses (antigen-specific circulating IgA/IgG ASC and secretion of IFN- $\gamma$ /granzyme B in T cells) by ELISpot. Flow cytometry was used to investigate mucosal homing markers on plasmablasts and IgA cross-reactivity was measured by mesoscale and Luminex m-Plex assays.

**Results:** 37% of oral immunized subjects developed influenza infection compared to 44% of QIV subjects (and 71% of placebo subjects), yet HAI seroconversion was only found to be the most important immune parameter to predict protection in the QIV immunized subjects. Several cellular responses, in contrast, showed significant differences in ill versus not-ill subjects for the oral immunized subjects. In particular, the numbers of antigen specific IgA ASC most strongly correlated with protection after oral immunization. Mucosal homing markers on plasmablasts were upregulated in the oral immunized subjects, not QIV. Oral influenza protected subjects showed elevated nasal IgA responses against mismatched influenza (H5, avian influenza) compared to unprotected subjects. Serum IgA was cross-reactive against stalk and divergent group 1 HAs as well.

**Conclusions:** An oral influenza tablet vaccine protected against influenza infection as well or better than injectable QIV. Protection was most strongly associated with mucosal homing IgA B cells, rather than HAI. The cross-protective potential of vaccine elicited IgA is currently being explored.

**Keywords:** Human challenge, Mucosal, IgA, plasmablasts



Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention  
Abstract No: 10563

## THE IMPACT OF SYNDROMIC MOLECULAR POINT-OF-CARE TESTING FOR RESPIRATORY VIRUSES IN ADULTS PRESENTING TO HOSPITAL WITH EXACERBATION OF AIRWAYS DISEASE: FURTHER ANALYSIS FROM A RANDOMISED CONTROLLED TRIAL

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### Introduction:

The ResPOC study demonstrated that syndromic molecular point-of-care testing (POCT) for respiratory viruses was associated with earlier discontinuation of unnecessary antibiotics. Subgroup analysis suggests this occurs predominantly in patients with exacerbation of airways disease. Molecular POCT is becoming more widespread but there is a lack of evidence to inform the choice between multiplex syndromic panels versus uniplex tests for influenza.

### Methods:

We evaluated patients with exacerbation of asthma or COPD who were treated with antibiotics. The duration of antibiotics and proportion with early discontinuation were compared between patients testing positive for viruses by POCT, those testing negative by POCT and controls. Patients testing positive for viruses by POCT were compared according to virus types detected. Survival curves were generated for duration of antibiotics and compared using the log-rank test.

### Results:

There were 118 patients with exacerbation of airways disease in the POCT group who received antibiotics and 111 in the controls. In the POCT group 49/118(42%) patients tested positive for viruses. Of those testing positive for viruses by POCT 17/49(35%) had early discontinuation of antibiotics versus 9/81(13%) in those testing negative and 7/111(6%) in controls,  $p < 0.0001$ . Survival analysis showed reduced time to antibiotic discontinuation in patients positive for viruses,  $p = 0.034$ .

Of those positive for viruses by POCT, 20% were positive for influenza, 43% for rhinovirus and 37% for other viruses combined. The proportion with early discontinuation of antibiotics was not different between the virus types and survival analysis did not show any differences,  $p = 0.53$ .

### Conclusion:

Syndromic molecular POCT for viruses in adults with exacerbation of airways disease leads to early discontinuation in those testing positive. Most viruses detected were non-influenza viruses and there was no difference in antibiotic use between virus types. This suggests that syndromic molecular POCT for respiratory viruses should be favoured over uniplex POCT for influenza.

*Keywords: point-of-care testing; diagnostics; influenza; antibiotics; non-influenza respiratory viruses*

Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention  
Abstract No: 10753

## Emergence of Viruses with Reduced Susceptibility to Baloxavir Marboxil: Impact on Clinical and Virologic Outcomes in Patients with Influenza at High Risk of Complications (CAPSTONE-2)

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**Introduction and Objectives:** Single-dose oral baloxavir marboxil (BXM) rapidly reduces virus titers and symptoms in adult/adolescent outpatients with acute influenza and at high risk of complications. Variant viruses with reduced susceptibility due to substitutions at position 38 of polymerase acidic protein (PA/I38) were detected in in 5.2% (15/290) of BXM-treated patients but not before treatment or in placebo (PCB)-treated. Here we report post-hoc analyses on the effects of PA/I38X-substituted virus emergence on clinical and virologic outcomes.

**Method:** CAPSTONE-2 was a multicenter, randomized, double-blind, placebo- and oseltamivir-controlled trial in adult and adolescent outpatients with high risk of influenza complications. The primary endpoint was time to improvement of influenza symptoms (TTIIS). BXM-recipients were divided into two subgroups for outcomes assessment: those with (BXM/I38+) and those without detectable PA/I38X-substituted viruses (BXM/I38-).

**Results:** Median TTIIS in BXM/I38+ (65.2 hours, n=15) was 11.6 hours shorter than in BXM/I38- (76.8 hours, n=236) and 37.1 hours shorter than in PCB (102.3 hours). Median time to resolution of fever in BXM/I38+ was 3.9 hours shorter than in BXM/I38- and 24.2 hours shorter than in PCB. Median time to sustained cessation of viral shedding was 168 and 48 hours in BXM/I38+ and BXM/I38-, respectively, and 96 hours with placebo. In BXM/I38+ mean virus titer increased transiently between Days 3-5. The proportions of patients with recurrence of influenza symptoms and fever after improvement/resolutions showed no differences between BXM/I38+ and BXM/I38-. The emergence of PA/I38X-substituted viruses was associated with lower baseline neutralizing antibody titers, but baseline antibody titer did not cause prolongation of symptoms relief.

**Conclusion:** In acute influenza outpatients at high risk of developing complications, the emergence of PA/I38X-substituted viruses was not associated with symptom rebound and clinical benefit was preserved in those with PA/I38X-substituted virus emergence. However, careful study of variant virus emergence in other high-risk populations is required.

*Keywords: Baloxavir, susceptibility, high-risk, substitutions, outcomes*

Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention  
Abstract No: 10937

## OVX836, A novel universal influenza A vaccine candidate: First results of a phase I clinical trial in humans

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### Introduction

Cellular immunity to the well-conserved influenza nucleoprotein (NP) is associated with protection against influenza disease, providing a strong rationale to develop a NP-based universal vaccine. OVX836 is an unadjuvanted recombinant vaccine candidate obtained by fusing the NP sequence of Influenza A/WSN/1933 (H1N1) virus to Oligodom®, Osivax's proprietary multimerisation platform. We have previously shown that OVX836 induces NP-specific CD8+ T cell response in mice, and protects mice and ferrets from multiple influenza A challenges.

### Method

OVX836-001 is a Phase I, single center, randomized, observer-blind, placebo-controlled (3:1 ratio) study to evaluate safety and immunogenicity of OVX836 after intramuscular or intranasal administration in 72 healthy volunteers aged 18-49 years. Here, we report first results for three dose levels (30µg, 90µg, 180µg) and placebo administered intramuscularly in 2 doses one month apart.

### Result

OVX836 was well-tolerated at all dose levels, with no increased reactogenicity at Dose 2. No safety signal was observed. Differences towards placebo were only seen for solicited injection site reactions (redness, swelling, induration, pain), of which the majority were mild to moderate.

All dose levels showed significantly higher ( $p < 0.05$ ) mean of IFN $\gamma$  NP-specific spot-forming T-cell counts than placebo at Day 7: ratio 4.5 for 30µg, 4.8 for 90µg, and 5.2 for 180µg, with lowest variability in individual counts in the 90µg group.

All subjects had measurable anti-NP IgG levels at baseline, and all dose levels led to significant increase ( $p < 0.05$ ) until Day 28 post-vaccination. There was no significant further increase after Dose 2 for both types of responses.

### Conclusion

The vaccine was well-tolerated, with mild to moderate reactions at injection site. T-cell responses were observed 7 days after Dose 1. Absence of significant increase after Dose 2 was interpreted as the reflect of an anamnestic nature of the response.

These results encourage further evaluation of this vaccine candidate.

*Keywords: First-in-Human; Phase I; Universal-Flu-Vaccine; CMI; NucleoProtei*

Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention  
Abstract No: 10944

## First-in-man clinical trials of influenza vectored vaccines against tuberculosis with intranasal and sublingual routes of administration

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**Introduction and Objectives:** The application of influenza viral vector for mucosal delivery of foreign antigens is an attractive approach for prevention and treatment of respiratory infectious diseases like tuberculosis. In our vaccine construct we used optimal influenza virus vector backbone providing a replicating-deficient phenotype and containing partial NS1 deletion followed by tuberculosis antigen sequences.

**Methods:** First-in-man randomized placebo-control studies were undertaken to evaluate safety and immunogenicity of two Vero-cell manufactured influenza vectored tuberculosis vaccines in 72 healthy adults aged 18 - 50 years. Two doses of vaccine or placebo were administered intranasally or sublingual in 21 days interval. The subjects were closely monitored for 7 days after each vaccination for follow-up of adverse events and viral shedding. Detection of mucosal cytokines was performed in nasal swabs 24h and 48h post first vaccination. Ag-specific CD4 and CD8 T-cell memory response was measured by intracellular cytokine staining in whole blood collected at Day 1, 7, 21 and 42.

**Results:** Influenza vector vaccines showed good safety profile and were well tolerated in both studies. Local cytokines production was determined as early immune response to vaccination. Vaccination induced statistically significant CD4+ and CD8+ memory T-cell response by increasing the number of IFN- $\gamma$  and TNF- $\alpha$  producing cells. Ag specific IFN- $\gamma$  producing CD8+ T cells response was higher in intranasal group, while TNF- $\alpha$  production by this cell population was higher in sublingual group. In both groups CD8+ memory (CD45RA-) T cell response predominated. Overall, 72.2% of subjects from sublingual group and 77.8% of subjects from intranasal group were considered as "responders".

**Conclusion:** First in man clinical trials of two influenza vectored vaccines demonstrated good safety profile and balanced T-cell mediated immune response. Potential of sublingual vaccine delivery as an alternative to the intranasal route was demonstrated.

*Keywords: Influenza vector, vaccine, clinical trial, intranasal and sublingual administration*

Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention  
 Abstract No: 11083

## Towards an Improved Wild-type Sequence Based Hemagglutination Inhibition Assay for the Evaluation of Influenza Vaccines: Challenges and New Developments.

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### Introduction/Objectives:

The utility of the hemagglutination-inhibition (HI) assay as a tool to assess vaccine immunogenicity has recently been threatened. Due to evolutionary changes in receptor specificity, current A(H3N2) strains are largely incapable of agglutinating avian red blood cells (RBCs). Additionally, the assay may fail as a clinically-relevant immunologic surrogate of protection against **wild-type** circulating influenza viruses, due to historical reliance on *egg-adapted* virus reagents which increasingly demonstrate altered antigenicity relative to their **wild-type** counterparts. **Egg-derived** reagents introduce egg-adaptation bias, artificially showing favorable responses to **egg-derived** vaccines, with unintended consequence of hampering development of improved next-generation recombinant vaccines. Consequently, we developed a **wild-type** sequence-based HI assay (WT-HI) to coincide with the development of a *recombinant wild-type* hemagglutinin vaccine; and evaluated assay performance against reference antisera, and sera from a recent vaccine trial.

### Methods:

The WT-HI assay employs virus-like-particles (VLPs)—expressing **wild-type** sequence HAs—as the agglutinating agent, and human type-O RBCs as the indicator particle. We tested CDC reference ferret antisera using **cell-** and **egg-derived** derived *historical* seasonal influenza viruses (no viruses were known to have undergone *egg-adaptive* mutations which are now common) versus **wild-type** VLP equivalents as agglutinins. We then tested sera from a Phase-I trial comparing a *recombinant* HA-nanoparticle vaccine versus a high-dose *egg-derived* influenza vaccine using the WT-HI assay.

### Results:

When tested with reference ferret antisera, the WT-HI assay using **wild-type** VLP reagents yielded titers which closely matched those seen with classical **egg-** or **cell-grown** virus reagents, with no homologous virus titer differences greater than 2-fold. Against a panel of five contemporary A(H3N2) drift variants, the WT-HI assay detected significant increases in post-vaccination antibody responses against **wild-type** HA sequences that were inapparent with egg-derived reagents.

### Conclusion:

The WT-HI assay rehabilitated the ability of the assay to interrogate clinically-relevant **wild-type** HI responses against contemporary A(H3N2) strains, and revealed potential advantages of the recombinant vaccine.

*Keywords: egg-adaptation; wild-type; hemagglutination-inhibition; recombinant; vaccine*

Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention  
Abstract No: 11127

## Intravenous Peramivir in Emergency Department High-Risk Patients with Influenza: A Multicenter Randomized Controlled Study

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**Introduction:** Prior studies conducted in outpatient and hospitalized patients with influenza have shown one-dose intravenously (IV) peramivir to be non-inferior to 5-day oral oseltamivir. We sought to compare outcomes of emergency department (ED) patients at high risk for influenza complications treated with peramivir versus oseltamivir.

**Methods:** A randomized controlled clinical trial was conducted over 2 influenza seasons 2015-2017 in 2 urban U.S. EDs. Patients were randomized to either oral (oseltamivir) or IV (peramivir) treatment. Patients who were  $\geq 18$  years, had a positive rapid molecular influenza test, met CDC criteria for antiviral treatment with symptom onset  $< 96$  hours and able to comply with daily follow-up in person (inpatient) or phone assessments (outpatient) were eligible. Outcomes of antiviral treatment were measured by the validated FLU-PRO score, a 32-question clinical end-point indicator, for 14 days via patients' daily diary. Non-inferior t-test was performed to determine whether patients treated with peramivir were not appreciably worse than those treated with oseltamivir using FLU-PRO score.

**Results:** Overall, 847 influenza-positive patients were encountered. Among them, 575 (68%) were approached, 284 met enrollment criteria, and 179 were enrolled. 95 (53%) patients were randomized to IV treatment arm and 84 were to oral treatment arm. Average FLU-PRO score at baseline was similar between two groups (IV: 2.67 vs. oral: 2.52) and the score decreased over time for both groups (day 5: IV: 1.71 vs. oral: 1.62; day 10: IV: 1.48 vs. oral: 1.37; day 14: IV: 1.40 vs. oral: 1.33; all  $p < 0.05$  for significantly non-inferior). Influenza-related complications were similar between two groups (pneumonia: IV: 12% vs. oral: 14%) and there were no deaths after 28 days of enrollment in both groups.

**Conclusions:** This randomized study demonstrated that the outcomes of one-dose IV-administered peramivir was comparable to oseltamivir, indicating potential use of peramivir in influenza-infected patients in the ED.

**Keywords:** *Influenza; Antiviral Treatment; Peramivir; Emergency Department; Randomized Controlled Trial*

Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention

Abstract No: 11217

## A Randomized Controlled Trial on the Effect of Fever Suppression by Antipyretics on Influenza

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### Introduction

Accumulating evidence from animal and human studies are suggesting that antipyretic use in URTIs might prolong the duration of illness and increase the level and duration of viral shedding. With a double-blind randomized placebo-controlled trial, we examined the potential impact of fever suppression by antipyretics on viral shedding and clinical illness in naturally-occurring influenza infections.

### Method

A total of 1861 young adults aged between 18-30 presenting with  $\geq$  URI symptoms within 48 hours of illness onset were screened in university health clinics in Hong Kong from March 2016 to August 2018. Among whom 319 having a positive QuickVue Influenza A & B rapid testing result were randomized to receive either paracetamol 500mg or matching four times daily for 5 days. Ibuprofen 200mg TDS PRN was provided to all for intolerable fever. Viral identification and subtyping by quantitative RT-PCR was performed on nasal and throat swabs on days 1 (baseline)/4/7/10, self-recording of temperature and ten common influenza symptoms were performed twice daily for ten days.

### Result

No difference in clinical illness duration and symptoms severity was detected between the two groups. Total symptom scores by AUC analysis were also comparable. Among 206 patients with PCR-confirmed influenza infection, no difference was detected between the two groups on the total amount of virus shedding as reflected by the AUC for quantitative influenza viral load, the mean durations of viral shedding estimated, and the time to resolution of viral shedding.

### Conclusion

There is no consistent evidence from our result to support the claim that paracetamol use might significantly increase the amount of viral shedding to substantiate these concerns of enhanced community transmission. Further study on the relationship between fever, viral shedding, infectivity, and disease transmission would be important for better informing the proper use of antipyretics in influenza virus infection.

Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention  
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## Single-dose Baloxavir for the Prevention of Influenza among Household Contacts: A Randomized, Double-blinded, Placebo Controlled Post-Exposure Prophylaxis Study (BLOCKSTONE)

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**Introduction and Objectives:** Baloxavir marboxil (BXM) is a cap-dependent endonuclease inhibitor that has proven clinical efficacy for the treatment of uncomplicated influenza A and B virus infections. This study investigated BXM's prophylactic efficacy among household contacts (HHC) with influenza.

**Methods:** BLOCKSTONE was a multicenter, double-blind, placebo-controlled study in HHC of index patients with confirmed influenza conducted in Japan during the 2018/19 season. All index patients received either BXM or neuraminidase inhibitor (NAI) treatment. Enrolled HHC were randomized 1:1 to either a single, weight-based oral dose of BXM, or placebo. The primary endpoint was proportion of HHC who developed clinical influenza (RT-PCR positivity, axillary temperature  $\geq 37.5^{\circ}\text{C}$ , and at least one moderate to severe respiratory symptom) over 10-day observation period. All randomized HHC who had post-baseline efficacy data were defined as the modified-ITT population (mITT) irrespective of RT-PCR positivity at baseline.

**Results:** Among 545 index patients, 95.6% had influenza A infection, and 53.0%, 31.0%, or 16.0% received BXM, oseltamivir, or other NAI, respectively. Of 752 randomized HHC, 749 (99.6%) constituted the mITT population (BXM n=374 vs. placebo n=375; 19.0% aged <12 years; and 13.1% had  $\geq 1$  risk factor for complicated illness). The proportion of HHC who developed clinical influenza in the BXM group was significantly smaller (1.9%) than that in the placebo group (13.6%), with a risk ratio of 0.14 (95%CI: 0.06-0.30;  $p < 0.0001$ ). Similar findings were observed in subgroups of HHC <12 years and among those with risk factors, with risk ratios of 0.27 (95%CI: 0.08-0.90) and 0.13 (95%CI: 0.02-0.94), respectively. Similar results in subgroups of HHC with RT-PCR negative at baseline were also observed. The incidence of adverse events was comparable in BXM and placebo groups (22.2% vs 20.5%, respectively).

**Conclusion:** A single, oral dose of BXM was effective and well tolerated for post-exposure prevention of influenza illness in HHC.

**Keywords:** *Anti-influenza drug, cap-dependent endonuclease inhibitor, baloxavir marboxil, prophylaxis, household contacts*



Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention  
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**SINGLE-DOSE BALOXAVIR IS WELL TOLERATED AND EFFECTIVE FOR TREATMENT OF INFLUENZA IN OTHERWISE HEALTHY CHILDREN AGED 1 TO < 12 YEARS: A RANDOMIZED, DOUBLE-BLINDED, ACTIVE-CONTROLLED STUDY (miniSTONE-2)**

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**Introduction:** Baloxavir marboxil (BXM) is a cap-dependent endonuclease inhibitor with clinical efficacy for the treatment of uncomplicated influenza A and B. This study investigated the safety (primary objective), pharmacokinetics and efficacy of a single dose of BXM in otherwise healthy children aged 1 to < 12 years with influenza

**Methods:** miniSTONE-2 was a global multicenter, double-blind, active-controlled study in otherwise healthy paediatric patients with influenza, conducted during the 2018/19 season. Patients were randomized 2:1 to receive either a weight-based single oral dose of BXM or standard oral dose of oseltamivir (twice-daily dosing for five days). The primary endpoint was the incidence of adverse events over a 29-day follow-up period. Key secondary endpoints included time to alleviation of influenza signs and symptoms for efficacy, and time to cessation of viral shedding by virus titer for virology. Patients who tested positive for influenza via central RT-PCR testing formed the intent to treat infected (ITTi) population.

**Results:** Of the 176 paediatric patients recruited, 124 formed the ITTi population (BXM, n=81 vs oseltamivir, n=43), 89.7% of which had influenza A infection (65.5% H3N2, 24.1% H1N1). No SAEs, deaths or adverse events of special interest were observed and the safety profile of BXM was consistent with that observed in clinical studies to date. The median time to alleviation of influenza signs and symptoms observed in the BXM group (138 hours [95% CI; 116.6,163.2]) was comparable to the oseltamivir group (150 hours [95% CI; 115.0,165.7]). Consistent with previous phase III studies, there was a clear difference in the median time to cessation of viral shedding between BXM (24.2 hours [95% CI; 23.5,24.6]) and oseltamivir (75.8 hours [95% CI; 68.9,97.8]).

**Conclusion:** A single, oral dose of BXM was well tolerated and effective for the treatment of influenza in otherwise healthy paediatric patients aged between 1 and < 12 years.

*Keywords: baloxavir; influenza; paediatric; oseltamivir; virology*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 10100

## LIVE ATTENUATED INFLUENZA VACCINE INDUCES EARLY TONSILLAR FOLLICULAR T HELPER CELL RESPONSES CORRELATING WITH DURABLE SYSTEMIC ANTIBODY RESPONSES

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### Background

Influenza remains a major global health concern. The live attenuated influenza vaccine (LAIV) is licensed in Europe for children (2-17 years old) in 2012. LAIV is more effective as a priming antigen and has higher efficacy in the children. Follicular T helper (T<sub>FH</sub>) cells are the key cell type in germinal centre (GC) formation, provide B-cell help and play important roles in early and long-term humoral responses. It remains unknown whether LAIV elicits T<sub>FH</sub> cell responses in human. We aim to evaluate the cellular and humoral immune response in children and adults vaccinated with LAIV during the 2013-2014 influenza season.

### Methods

We conducted a clinical study in forty children and thirty-seven adults that were scheduled for elective tonsillectomy. Subjects were vaccinated intranasal with LAIV. Palatine tonsils were collected from all subjects at 3, 7 or 14 days post vaccination and utilized to assess the cellular T<sub>FH</sub> cell response to LAIV. Plasma and saliva samples were collected before vaccination (day 0), 3-14, 28, 56, 150 and 360 days post-vaccination. We dissected the overall antibody responses induced by LAIV against A/H1N1, A/H3N3 and B viruses using hemagglutination inhibition assay (HI), microneutralization (MN) and enzyme-linked immunosorbent assay (ELISA).

### Results

We demonstrated that LAIV increases activation of follicles and CXCR5<sup>+</sup>CD57<sup>+</sup>CD4<sup>+</sup>T-cells (T<sub>FH</sub>-cells) in tonsils and induces influenza specific T<sub>FH</sub>-cell responses in children 3-7 days post-vaccination, but to a lesser extent in adults. LAIV elicited significant antibody responses to influenza A and B viruses in children from day 14 to 1 year, but only moderately to influenza B in adults. Lastly LAIV induced T<sub>FH</sub>-cell responses correlate with durable systemic IgG increases after vaccination

### Conclusions

Our study indicates that early local T<sub>FH</sub> cell responses in the upper respiratory tract predict antibody inductions, and explains the age discrepancy of LAIV's efficacy and provide guidance for optimal use of LAIV.

*Keywords: Influenza vaccine, LAIV, Follicular helper T cell, Serology*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 10966

## CLINICAL EVALUATION OF ADJUVANTED RECOMBINANT HEMAGGLUTININ H7 VACCINE TO HIGHLY PATHOGENIC A(H7N9) INFLUENZA VIRUS

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### Introduction

Since first being reported in March 2013, avian influenza A(H7N9) viruses have been circulating in poultry in China and causing human epidemics yearly between Fall and early Spring. Genetic and antigenic analyses indicated that the prevailing A(H7N9) viruses had evolved into an antigenically distinct group. Antibodies elicited by vaccines prepared with A(H7N9) viruses from 2013 demonstrated substantial loss of neutralization potency against the new viruses. The US Department of Health and Human Services maintains the National Pre-Pandemic Influenza Vaccine Stockpile (NPIVS) comprised of adjuvants and bulk antigens representing viruses with pandemic potential and clinical study data regarding the safety, immunogenicity, and dose-sparing of these adjuvanted vaccines to support their deployment for pandemic mitigation.

### Methods

A clinical study to evaluate the safety and immunogenicity of adjuvanted recombinant Panblok H7 vaccine derived from highly pathogenic A/Guangdong/17SF003/2016 (H7N9) at three antigen dose levels was conducted in healthy adults. Hemagglutination inhibition (HAI) and microneutralization (MN) assays were performed against homologous A/Guangdong/17SF003/2016xPR8 or heterologous A/Hong Kong/125/2017xPR8 and A/Shanghai/02/2013xPR8 reassortants.

### Results

Adjuvanted Panblok H7 vaccination was well-tolerated. Peak HAI and MN antibody titers and proportion of subjects achieving seroprotection were observed by day 50. HAI and MN antibody geometric mean titers (GMTs) were similar. The seroprotection rates (SPRs) to homologous (range: 100% to 81.0%) and heterologous (range: 98.2% to 61.4%) vaccine strains after two doses were sustained above baseline for at least 6 months.

### Conclusion

The majority of the study groups met and exceeded the FDA CBER-specified success criterion of 70% SPR for HAI antibodies to homologous vaccine strain and showed high cross-reactivity to heterologous A(H7N9) influenza strains.

By pairing the recombinant protein H7 vaccine with adjuvants in the NPIVS, the study provides critical insights for the US government to develop a response strategy for an influenza pandemic emergency. ClinicalTrials.gov identifier: NCT03283319.

*Keywords: H7N9 Vaccine, pandemic influenza,*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 11073

## Induction of broadly cross-reactive immune responses against A(H3N2) viruses: results of a phase 2 trial of a novel recombinant hemagglutinin saponin-adjuvanted nanoparticle influenza vaccine

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### Introduction:

We have developed a novel recombinant saponin-adjuvanted (Matrix-M1) seasonal quadrivalent hemagglutinin nanoparticle influenza vaccine (qNIV) for older adults in response to two features impeding performance of currently licensed, predominantly egg-derived, influenza vaccines: 1) limited protection against antigenic drift variants; and 2) antigenic mismatch between vaccine and circulating strains due to egg-adaptive mutations arising during manufacturing. In phase 1, we demonstrated that qNIV induced robust, broadly cross-reactive antibody responses against multiple generations of antigenically drifted H3N2 viruses, which were 47-64% better than the egg-derived comparator Fluzone-High Dose (FHD).

### Methods:

In this phase 2 dose/formulation finding RCT, we randomized 1375 subjects aged  $\geq 65$  years to be immunized with 1 of 7 test vaccines: 5 different formulations of qNIV (Table 1), FHD, or FluBlok; and assessed strain-specific wild-type hemagglutinin-inhibition (wt-HAI) and microneutralization (wt-MN) antibody responses (Day 0/28/56); and polyfunctional T-cell responses (Day 0/7) against vaccine-homologous and drifted H3N2 hemagglutinins.

### Results:

Matrix-M1-adjuvanted qNIV induced 15-29% higher wt-HAI titers across 5 vaccine homologous or drifted H3N2 strains at day 28 relative to unadjuvanted qNIV; significantly superior for 5 of 6 strains tested. At Day 28, treatment groups (formulations) A, B, and C of qNIV induced significantly superior wt-HAI titers versus FHD (39-45%, 17-22%, and 44-48% greater titers for [homologous] A/Singapore/INFIMH-16-0019/2016(H3N2), [historic-drifted] A/Switzerland/9715293/2013(H3N2), and [forward-drifted] A/Wisconsin/19/2017, respectively); and comparable HAI titers versus FluBlok. Wt-MN and wt-HAI data showed concordant patterns across treatment groups. Only qNIV Group C (containing a higher dose of Matrix-M adjuvant) elicited median counts of triple-staining (IFN- $\gamma^+$ ; TNF- $\alpha^+$ ; IL-2<sup>+</sup>) strain-specific CD4<sup>+</sup> T cells >1000 per million cells (ranging 1200-2800) against 4 homologous/drifted strains.

### Conclusion:

Multiple formulations of qNIV induced superior wt-HAI antibody responses versus FHD against several homologous/drifted H3N2 viruses. A higher dose of adjuvant induced potent strain-specific polyfunctional cell-mediated immune responses. qNIV may address several critical challenges confronting current egg-derived influenza vaccines.

*Keywords: recombinant; adjuvant; nanoparticle; vaccine; H3N2*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 11095

## IMMUNE HISTORY TO INFLUENZA IS A NOVEL CORRELATE OF PROTECTION OF INFLUENZA VACCINATION

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### Introduction

Vaccination, the most cost-effective public health intervention, stimulates the immune system to generate protective memory responses. A variety of factors impact an individual's heterogeneity in vaccine induced immune responses, such as age, ethnicity and 'immunological history' - the individual's memory antibody repertoire to previously encountered pathogens and vaccines.

### Methods

To study the role of immune-history to previous influenza infections, we have developed a novel influenza antigen microarray spotted with whole-inactivated influenza viruses, recombinant hemagglutinin and neuraminidase proteins, and overlapping peptides from these proteins. Antibody profiles to this diverse set of antigens provide a rich and high-dimensional representation of the anti-influenza antibody repertoire.

### Results

To profile the effect of immune history on vaccine-induced immune responses and protection from infection, we used samples from FluVacs – a randomized double blind placebo controlled influenza vaccine efficacy trial comparing the inactivated (Fluzone) and live-attenuated (Flumist) vaccines in adults aged 18-65, conducted in 2007-2008. Samples were collected at baseline (d0), post vaccination (d21) and at the end of the season (d90). We used our novel assay to generate serum IgG and IgA antibody profiles at all timepoints. In line with the findings of the trial, we found a significant increase in IgG and IgA titers post-vaccination in the Fluzone group only. We also found significant heterogeneity of responses at baseline, with some individuals lacking detectable anti-influenza antibody responses. We compared the baseline and post-vaccination antibody profiles of all subjects who subsequently became infected to those who did not. We identified several novel correlates of protection (CoP) that were based both on IgG and IgA responses. Surprisingly, the strongest CoP was based on baseline serum IgA antibody levels.

### Conclusion

Our results highlight the role of immune-history to previous influenza infections as a baseline measurement that may be predictive of vaccine-induced immune responses and vaccine-induced protection.

*Keywords: immune-history, influenza, correlates of protection, antigen-microarrays,*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
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## Development of a universal Influenza A T cell-based vaccine

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**Introduction and objectives:** The efficacy of currently licensed seasonal influenza vaccines targeting polymorphic surface antigens has historically been shown to be less than optimal. Cellular immune responses against highly conserved Influenza A virus antigens, such as nucleoprotein (NP) and matrix protein-1 (M1) have previously been shown to be associated with natural protection, and viral vector vaccines are known to be an effective strategy to boost such T cell responses.

**Methods:** Oxford University has conducted 5 Phase 1 and one Ph2a study of a chicken embryo fibroblast-produced MVA-NP+M1 vaccine. Vaccitech has subsequently sponsored a Phase 1, phase 2, and two ongoing phase 2b studies of the vaccine produced in immortalized duck embryonic cell line AGE1.CR.pIX. The first is an H3N2 challenge study of 134 participants of MVA-NP+M1 versus placebo. The second is a 6,000 person field trial conducted at multiple sites across Australia over two seasons in people who have received seasonal influenza vaccine.

**Results:** 145 participants aged 18 and older, have received CEF-produced MVA-NP+M1. The vaccine has proven safe, highly immunogenic, and induces persistent combined CD4 and CD8+ T cell responses above baseline for over one year. The cell-based vaccine underwent one Phase 1 trial that showed at least equivalent immunogenicity to the CEF-produced product. This was followed by a trial with vaccine or placebo co-administered with standard QIV to 860 adults 65 years of age and older prior to the 2018 UK flu season. The vaccine was again shown to be safe and immunogenic, and the impact on ILI and days of related symptoms will be reported.

**Conclusion:** MVA-NP+M1 induces CD4+ and CD8+ T cells that may provide protection against influenza when either given alone or as an adjunct to seasonal vaccination. Two Phase 2b virologic endpoint studies began in the first quarter of 2019 to test this hypothesis.

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 11748

**Vaccination with 1/6<sup>th</sup> standard dose of a split inactivated influenza vaccine using a high-density micro-projection array patch induces comparable immune responses to conventional full-dose intramuscular injection; results from a phase I randomized controlled clinical trial**

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*Background:* The Nanopatch is a high-density micro-array patch (MAP) for vaccine delivery into the skin. We have conducted a phase I trial using the Nanopatch to deliver a monovalent influenza vaccine. This is the first clinical evaluation of the vaccine dose-sparing potential of a MAP.

*Methods:* Nanopatches were coated with a split inactivated influenza virus vaccine (A/Singapore/GP1908/2015 [H1N1]) (A/Sing). Healthy volunteers were vaccinated with doses of 15, 10, 5, or 2.5 µg of A/Sing haemagglutinin (HA) via Nanopatches applied to the forearm (FA), or 15 µg HA via Nanopatches applied to the upper arm (UA). Control groups received uncoated Nanopatches applied to the FA ('placebo control') or commercially available Afluria® quadrivalent influenza vaccine (QIV).

*Results:* The A/Sing vaccine coated onto Nanopatches was stable when stored at 40°C for at least 12 months. Nanopatch vaccination was safe and well-tolerated; any AEs were mild or moderate. 2.5 µg HA administered by Nanopatch induced haemagglutination inhibition (HAI) and microneutralization (MN) titres that were not significantly different to those induced by 15 µg HA injected IM. Nanopatch delivery of 15 µg (FA and UA) and 10 µg (FA) HA resulted in a faster increase in HAI responses than IM injection, with 83%, 95% and 90% subjects respectively seroconverting at day 8, compared with 68% for the IM QIV group. The results indicated that overall, Nanopatch delivery induced a range of responses that were similar or potentially superior to those seen with IM injection of QIV.

*Summary:* Vaccination using the Nanopatch that can be stored outside the cold-chain, was safe and well-tolerated and resulted in immune responses that were equivalent to or enhanced compared with IM injection. Using the Nanopatch, a 2.5 µg dose (1/6 of the standard dose), induced HAI and MN titres equivalent to those seen with 15 µg HA injected IM.

*Keywords:* microarray patch, influenza vaccine, Phase I, skin vaccination

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 11792

## Prevention of Influenza during Mismatched Seasons in Older Adults: a Randomized Efficacy Study of an MF59-Adjuvanted Quadrivalent Influenza Vaccine

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### Introduction

Older adults are particularly susceptible to influenza-induced morbidity and mortality due to frailty and age-related immunosenescence. The aim of this study was to demonstrate clinical efficacy of an MF59-adjuvanted quadrivalent influenza vaccine (aQIV) in preventing RT-PCR confirmed influenza in adults 65 years of age or older.

### Method

Overall, 6790 subjects were randomized to receive one dose of aQIV or a non-influenza comparator vaccine (Tdap). Vaccine efficacy (VE) against any RT-PCR-confirmed influenza and any influenza due to strains antigenically similar to the vaccine strains were assessed. The study was conducted in 12 countries during Northern Hemisphere 2016/17 and Southern Hemisphere 2017 influenza seasons.

### Results

Among 6761 subjects exposed to study vaccines, a total of 273 cases of protocol-specified influenza-like illness (ILI) cases were confirmed as influenza by RT-PCR. Only 21 of the 273 cases were caused by strains matched to the aQIV vaccine. The dominant circulating strain during both seasons was mismatched A/H3N2 (74%). Efficacy of aQIV against any RT-PCR confirmed influenza was 19.8% [95% confidence interval (CI); -5.3, 38.9] using a protocol-specified ILI definition, 32.1% [95% CI; 10.2, 48.7] using a modified CDC ILI definition and 51.1% [95% CI; 28.2, 66.7] using a WHO ILI definition (analysed post-hoc). Using the same definitions of ILI, the efficacy of aQIV in preventing culture-confirmed influenza due to antigenically matched strains was 50.0% [95% CI; -24.0, 79.8], 61.5% [95% CI; -8.0, 86.3] and 75.0% [95% CI; -17.9, 94.7], respectively. aQIV induced a robust immune response against all vaccine strains. The safety profile for aQIV was similar to the licensed non-influenza vaccine comparator.

### Conclusion

In older adults, aQIV induced a robust immune response and demonstrated moderate efficacy against RT-PCR-confirmed influenza during influenza seasons with dominant circulation of antigenically mismatched A/H3N2 strains. The efficacy of aQIV was higher against cases of influenza with fever.

*Keywords: Influenza, MF59-Adjuvanted, Quadrivalent Vaccine, Elderly*



Topic: Clinical Sciences: Development of Human Disease  
Abstract No: 11345

## LOWER COGNITION AMONG TODDLERS WHO EXPERIENCE ACUTE RESPIRATORY ILLNESSES IN PANAMA AND EI SALVADOR

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**Background:** A previous birth cohort suggested that acute respiratory illnesses (ARIs) during infancy may affect early development. We established a larger cohort to ascertain whether findings are replicable after controlling for factors known to affect development.

**Methods:** During October 2014-April 2017, newborns of women participating in a pregnancy cohort in Panama and El Salvador were followed to identify ARIs defined as sudden onset of subjective or measured fever  $\geq 38^{\circ}\text{C}$  and  $\geq 1$  respiratory symptom, asthenia, anorexia, irritability, or vomiting. Staff obtained nasopharyngeal swabs for RT-PCR detection of respiratory viruses. Toddlers were administered Bayley's development tests at  $\sim 12$  and again at 18–24 months of age. We used a repeated measures mixed effect model to explore the association between Bayley's scores and infancy ARIs controlling for country and year of testing with an interaction between ARI and testing year.

### Results:

We enrolled 1,568 (98%) of 1,596 neonates and tested the development of 1,068 (68%) once and 616 (58%) twice. Six hundred and fifty-six (61%) had ARIs at a median age of 5 (2–9 IQR) months. Toddlers scored a median of 84% (63–91%) in cognitive, 50% (IQR 42–73%) in language, and 68% (42–84%) in motor development in their first Bayley's. Children with ARIs before their first Bayley's had an average cognitive score of 72% compared to 77% among children without ARIs ( $p=0.01$ ) but there was no association with language and motor scores or with maternal factors, age at ARI, number or severity of ARI, or ARI viral etiology. There was no association between ARIs before the second Bayley's and cognitive development.

### Conclusion:

Infancy ARIs were associated with depressed Bailey scores during infancy with catch-up by the second year of life. Larger cohorts might be warranted to identify lasting impacts on subpopulations, explore mechanisms of action, and associations with vaccine preventable pathogens.

*Keywords: infant development cognition respiratory virus*

Topic: Clinical Sciences: Diagnostics and Disease Markers  
Abstract No: 10565

## EVALUATION OF THE FEBRIDX HOST RESPONSE POINT-OF-CARE TEST TO DIFFERENTIATE VIRAL FROM BACTERIAL AETIOLOGY IN ADULTS HOSPITALISED WITH ACUTE RESPIRATORY ILLNESS DURING INFLUENZA SEASON

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### Background:

Antibiotics are over-used in patients hospitalised with acute respiratory illness (ARI). Diagnostic uncertainty regarding microbial aetiology contributes to this practice and so a host response test that can distinguish between viral and bacterial infection has the potential to reduce unnecessary antibiotic use. The FebriDx is a host response POCT that uses fingerpick blood samples to distinguish between viral and bacterial infection but has not been evaluated in hospitalised adults.

### Methods:

We took fingerprick blood samples and tested them on the FebriDx from adult patients with ARI, hospitalised during influenza season and with samples tested for viruses on the FilmArray Respiratory Panel. The FebriDx was evaluated for ease of use, failure rate and accuracy of the results (Viral, Bacterial, Negative).

### Results:

149 patients were approached, and 10 patients declined testing. A valid result was obtained from 124/139(89%) overall. Common user comments were the high failure rate due to difficulty getting blood to fill the capillary tube and difficulty in interpreting the results lines due to the variability of colour change.

111/124(89%) were tested for viruses. 69/111(62%) had viruses detected. Of 69 patients with viruses detected, 41(59%) had influenza, 12(17%) rhino/enterovirus and 16(23%) other viruses combined. 44/69(64%) had a viral FebriDx result. For influenza-positive patients 34/41(83%) had a viral FebriDx result, 1/12(8%) of rhinovirus-positive patients had a viral FebriDx result and 9/16(56%) of patients with other viruses detected had a viral FebriDx result.

These are interim results. Full results for 200 patients will be available at presentation.

### Conclusions:

Use of the FebriDx POC was associated with a high failure rate and problems with the interpretation of result lines. FebriDx was not sufficiently accurate to allow the safe withholding of antibiotics, however it did have a high specificity and PPV for influenza detection in this cohort and may have utility in hospitals.

*Keywords: FebriDx; point-of-care test; influenza; diagnostics; host response*

Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 10877

## RAPID MOLECULAR TESTING FOR INFLUENZA IN CHILDREN IMPROVES PATIENT MANAGEMENT IN ACUTE CARE SETTING

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**Introduction and Objectives:** Rapid and accurate laboratory detection of influenza is helpful to optimize patient care. Our objective was to measure the impact of rapid molecular testing for influenza on overall patient management in the acute care setting.

**Methods:** We implemented a rapid, PCR-based test (45 minutes turn-around-time) for influenza for patients seen in our emergency departments (ED) and urgent care centers (UCC). During the 4 weeks in mid-influenza season, we ran a provider survey at the moment of ordering to capture the clinical suspicion for influenza and intention for further management (radiology, additional labwork, antimicrobials, admission). Compliance with intended patient management was determined through a query of orders that occurred during the encounter.

**Results:** A total of 339 surveys were completed for children (median age = 4 yrs, IQR 1-7) seen in the ED (N=140) and UCC (N=199). A total of 164 (48%) influenza test results were positive (Flu A=162, Flu B =2 ). The correlation of influenza test positivity with low, moderate or high clinical suspicion for influenza was 40%, 47%, and 57%, respectively. Overall, decisions to change intended patient management occurred in 77% of the patients, though this differed by test result (negative: 92% vs. positive: 54%;  $p < .0001$ ). Clinicians changed their original plans for antiviral and antibiotic prescription in 53% and 24% of patients, respectively. Additionally laboratory and imaging plans were changed in 31% and 21% of patients respectively. The intention to admit changed in 6% of patients.

**Conclusion:** Clinical suspicion of influenza correlated poorly to the PCR-based influenza test (range: 40%-57%). Influenza test results altered clinicians decisions in 77% of patient encounters. Most of these decisions resulted in avoiding unnecessary expenses with a potential for overall cost savings.

*Keywords: Rapid Influenza PCR, children, patient management; out patient*

Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 10943

## Pre-existing NP specific T-cell response correlates with reduction of symptoms in a human Influenza challenge model

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### Introduction

In recent epidemiological studies, cellular immunity to the well-conserved influenza nucleoprotein (NP) has correlated with reduction of symptomatic PCR-confirmed cases of influenza. We investigated, in a human challenge study, the T-cell IFN- $\gamma$  response to NP stimulation in subjects with low pre-existing antibody levels to the challenge virus ( $\leq 10$  HAI).

### Method

PBMC samples from 54 subjects inoculated with wild type influenza A/Perth/16/2009 H3N2 virus were assayed for their NP specific IFN- $\gamma$  T-cell response by ELISPOT prior to inoculation (n=54), on Day 7 (n=28) and Day 28 (n=17) post-challenge. Total T-cell responses to NP stimulation were assessed for associations with outcomes of infection including symptom scores, viral loads and infection rates.

### Result

At baseline, subjects with an NP-specific T-cell response above the median (103 spots per million PBMCs) had a reduction in incidence of any symptoms (25/27 vs 18/27 p=0.039) and of grade two and above symptoms on multiple occasions (11/27 vs 4/27 p=0.066) when compared to those below the median.

Total T-cell response to NP stimulation at baseline was not associated with infection rate or viral loads within the infected population. However, of the 16 infected subjects for which the response was tested on day 7, subjects generating NP-specific T-cell responses above 500 spots (n=7) had significantly lower median symptom scores than the subjects who did not (n=9) (76 vs 2; p=0.023).

### Conclusion

These results in human influenza challenge model settings reinforce the findings from epidemiological studies by demonstrating pre-existing or rapidly developing NP specific T-cell immunity is associated with lower levels of symptoms. These results support investigations to refine the respective roles of NP-specific CD4 and CD8 T-cells.

Vaccines triggering NP specific T-cell responses may establish broadly protective immunity across multiple influenza strains and represent an attractive approach to improve influenza prevention.

*Keywords: Human-Challenge-Study; Correlate; NucleoProtein; CMI*

Topic: Clinical Sciences: Special Populations  
Abstract No: 11300

**Long-Term Care/Nursing Home admission following hospitalization with influenza and acute respiratory illness: The role of social vulnerability. A report from the Canadian Serious Outcomes Surveillance Network.**

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**Introduction and Objectives:** Social vulnerability is the extent overall social circumstances leave individuals susceptible to adverse health outcomes. We sought to understand the association between social vulnerability and the odds of Long-Term Care (LTC; nursing home) placement within 30 days of discharge following admission to hospital with influenza or other acute respiratory illness, and whether this association varied based on age, sex, or baseline frailty.

**Methods:** Patients admitted to hospital with acute respiratory illness were enrolled in the Canadian Immunization Research Network's Serious Outcomes Surveillance Network during the 2011/2012 influenza season. Participants (N=475) were 65 years or older (Mean=78.6) and over half were women (58.9%). Social vulnerability was measured using a Social Vulnerability Index (SVI) and frailty was measured with a Frailty Index (FI). Due to the rarity of incident LTC placement (N=15), we used penalized likelihood logistic regression.

**Results:** At age 65, social vulnerability was associated with lower odds of LTC placement at high levels of frailty (FI = 0.4; OR=0.15, 95%CI=0.03-0.61), but not at lower levels of frailty. At age 85 social vulnerability was associated with greater odds of LTC placement in the fittest patients (FI =0.0; OR=13.54, 95%CI=1.42, 131.76 and FI =0.1; OR=6.71, 95%CI=1.01, 40.43), but not at higher levels of frailty. Various sensitivity analyses yielded similar results. Odds of LTC placement following laboratory-confirmed influenza were no different than for other acute respiratory illness.

**Conclusions:** Social vulnerability interacted with frailty and age in predicting LTC admission following hospitalization with influenza and other acute respiratory illness. Social circumstances are important to take into account when considering the health needs and outcomes of older adults. Importantly, the risk of LTC admission was the same following influenza and non-influenza hospitalization, suggesting that some LTC placements may be avoided or delayed through prevention of influenza-related hospitalization.

*Keywords: influenza, older adults, frail elderly, long-term care*

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 10475

## Human-to-human transmission of influenza A(H3N2) viruses exhibiting reduced susceptibility to baloxavir due to a PA I38T substitution in Japan

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### Introduction and objectives

The cap-dependent endonuclease inhibitor baloxavir marboxil became available in Japan in March 2018 for the treatment of influenza virus infection in patients 12 years and older and children younger than 12 years weighing at least 10 kg. Between October 2018 and January 2019, baloxavir was supplied to medical institutions for approximately 5.5 million people. Because of the need to monitor influenza viruses for reduced susceptibility to baloxavir, we have been conducting nationwide monitoring of the baloxavir susceptibility of circulating influenza viruses since the 2017/18 influenza season.

### Methods

The susceptibilities of viruses to baloxavir and four neuraminidase (NA) inhibitors approved in Japan, oseltamivir, peramivir, zanamivir and laninamivir, were determined by using a focus reduction assay and a fluorescent NA inhibition assay, respectively. Results were expressed as the 50% inhibitory concentration (IC<sub>50</sub>). The gene sequences of viruses were determined by using deep sequencing.

### Results

Between November 2018 and February 2019, we detected three influenza A(H3N2) viruses carrying an I38T substitution in the polymerase acidic subunit (PA), which confers reduced susceptibility to baloxavir, from three children aged 8 months to 12 years without baloxavir treatment. The viruses exhibited reduced susceptibility to baloxavir but were susceptible to NA inhibitors. We confirmed that family clusters of influenza virus infection occurred in two of the three cases. Deep sequencing analysis revealed that two viruses from a family cluster, isolated from the child without baloxavir treatment and baloxavir-treated brother of this child, possessed the same sequence as each other. This observation indicates transmission of the PA I38T mutant A(H3N2) virus among humans.

### Conclusion

Our results might indicate that the recently circulating A(H3N2) viruses with the PA I38T substitution have, to some extent, retained replication and transmission capability in humans. Therefore, the baloxavir susceptibility of influenza viruses should be closely monitored for public health measures.

*Keywords: Influenza virus; cap-dependent endonuclease inhibitor; baloxavir marboxil; drug resistance*

Topic: Clinical Sciences: Therapeutics – Antivirals  
 Abstract No: 10712

### Effect of treatment with neuraminidase inhibitors on the risk of in-hospital death among influenza patients reported from EU countries, 2010–2019

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Research indicates that timely treatment with neuraminidase inhibitors (NAI) reduces severe outcomes in influenza-infected patients. Our aim was to analyse whether surveillance data on treatment among hospitalised influenza patients in EU countries from 2010/11 to 2018/19 support this.

The European Surveillance System collects weekly case-based data on hospitalised laboratory-confirmed influenza cases. For this study, we included cases with known treatment data, hospital unit type (general ward/intensive care unit (ICU)), age, sex, and outcome. We excluded cases with hospitalisation date before or >10 days (d) after illness onset and treatment initiation before or >22d after onset. Poisson regression models were used to estimate the incidence rate ratio (IRR) for in-hospital death in patients treated with NAIs versus not treated, adjusted for patient demographics, virus type, timing of hospitalisation and treatment, admission to ICU, influenza vaccination, and spatial clustering.

Eleven countries reported 17,281 cases with 2,710 (16%) in-hospital deaths; 5,772 patients (33%) were in ICUs. NAIs were given to 14,237 patients (82% oseltamivir): 4,281 treated within ≤48hours, 3,959 within 3-4d, 3,796 within 5-7d and 2,201 >7d.

The adjusted relative risk of in-hospital death was lower in all NAI-treated patients compared to no treatment (0.71; 95%CI 0.65-0.78). For all patients irrespective of hospital ward, treatment was protective against death given within 48hours (0.59, 95% CI 0.53–0.67), 3-4d (0.69, 95% CI 0.62–0.78), or 5-7d (0.76; 95% CI 0.67-0.86), while treatment after 7d was not protective (1.07; 95% CI 0.93-1.24). For ICU patients, treatment within 48hours of onset was protective against death compared to no or later treatment (0.81; 95% CI 0.67-0.98 and 0.92; 95% CI 0.79-1.08, respectively).

NAI use was common but infrequently given early in illness. Our results support the role of early treatment, even administered later than 48 hours, in hospitalised influenza-confirmed patients to reduce the risk of in-hospital death.

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 10750

## SEASONAL AND OTHER INFLUENZA VIRUSES WITH REDUCED SUSCEPTIBILITY TO BALOXAVIR AND PIMODIVIR

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**Introduction.** Influenza type A, B, C, and D viruses infect a wide range of species and pose threats to human and animal health. Sporadic human infections with influenza A viruses of avian or swine-origin are of global health concern due to their pandemic potential. Antivirals with different mechanisms of action are needed for treatment of influenza virus infections. We assessed susceptibility of seasonal and animal-origin influenza viruses to vRNA polymerase inhibitors: baloxavir, targeting the PA(P3) cap-dependent endonuclease of all four influenza virus types, and pimodivir, targeting the PB2 cap-binding domain of type A viruses.

**Methods.** Next generation sequencing was used to analyze viral genomes. Virus yield reduction, focus reduction, and high content-imaging neutralization (HINT) assays were used for drug susceptibility testing.

**Results.** *In vitro* replication of influenza viruses of four types was inhibited by baloxavir with the EC<sub>90</sub> range 1.2-69 nM (48hpi) and the susceptibility pattern A>B>C>D. Both inhibitors showed similar antiviral effect on influenza A viruses (n=95). In rare instances, substitutions that decrease baloxavir susceptibility by ≥10-fold (e.g., PA-I38M in swine-origin A(H1N1)v virus) were detected in influenza A viruses. A(H7N9) viruses carrying PA-I38M, PA-E199G and PA-A36V associated with reduced baloxavir susceptibility were also found by sequence analysis. Substitutions known to decrease pimodivir susceptibility were rarely detected in seasonal influenza A viruses (e.g., PB2-S342R/C). Among 52 avian and swine-origin viruses tested, one avian A(H4N2) virus displayed ~150-fold decreased pimodivir susceptibility. The role of two rare substitutions PB2-H357N and PB2-L464M identified in the PB2 cap-binding domain of this virus is under investigation.

**Conclusions.** Baloxavir inhibited replication of all four influenza virus types *in vitro*. Baloxavir may offer a therapeutic option for influenza C virus infections; further studies are needed. Because molecular markers of resistance for baloxavir and pimodivir are not well established, susceptibility monitoring requires phenotypic testing and sequence analysis.

**Keywords:** Baloxavir acid, drug susceptibility, zoonotic influenza A, pandemic potential, avian influenza



Topic: Clinical Sciences: Therapeutics – Antivirals  
 Abstract No: 10812

## Reduced Susceptibility Viruses to Baloxavir Marboxil: Prognosis Factors of the Emergence and Impact on Clinical and Virologic Outcomes in Pediatric Patients in Japan

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**Background:** Single-dose baloxavir marboxil (BXM) rapidly reduces influenza virus titers and symptoms in adult and pediatric patients with uncomplicated acute influenza, but variant viruses with reduced susceptibility harboring substitutions at position 38 of polymerase acidic protein (PA/I38) have emerged in some treated pediatrics with higher incidence than in adults.

**Method:** Open-label, multi-center, non-placebo-controlled study conducted during 2016/17 in Japan was analyzed in post-hoc to identify prognosis factors associated with the emergence of PA/I38X-substituted viruses using a logistic regression model for pediatric patients with A(H3N2). The factors assessed included sex, body weight, meal condition before administration, time-to-treatment from influenza onset, baseline hemagglutination inhibition (HAI) antibody titer to A(H3N2), body temperature, symptom scores and infectious virus titer. We also evaluated the relationship of baseline HAI antibody titer to time to illness alleviation (TTIA).

**Results:** PA/I38X-substituted viruses were seen in 23.4% (18/77) patients, which resulted in longer TTIA (median time, 79.6 vs 42.8 hours in patients without PA/I38X) and infectious virus detectability (median time, 180.0 vs 24.0 hours in patients without PA/I38X). Temporary elevations of the viral titer were observed after Day 2 in patients with PA/I38X, accompanying symptom rebound in some cases. A higher frequency of PA/I38X-substituted viruses occurred in patients with baseline HAI antibody titer <40 (42.3% [11/26]) than in those with titer ≥40 (17.1% [7/41]) (p=0.0467). No correlations were found between PA/I38X emergence and the other assessed factors. Among patients with PA/I38X-substituted viruses, the median TTIA in patients with lower baseline antibody titer (<40) was approximately 30 hours longer than that in those with higher antibody titer.

**Conclusion:** Low baseline HAI antibody titers were significantly associated with higher risk of PA/I38X emergence in pediatric patients. Moreover, a clear prolongation of illness alleviation was seen in patients with PA/I38X and low baseline antibody titer, however, the reasons remain currently unclear.

*Keywords: Baloxavir marboxil; pediatric; treatment; study; influenza*

Topic: Clinical Sciences: Therapeutics – Antivirals  
 Abstract No: 10900

## Neuraminidase-targeted Hapten Immunotherapy to Treat Influenza

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### Introduction

Influenza represents a significant threat to public health, however, its current treatment options are limited. In this study, a bifunctional small molecule was designed and synthesized by conjugating the neuraminidase inhibitor, zanamivir, with a dinitrophenyl (DNP) group. This zanamivir-DNP conjugate forms a bispecific molecular “bridge” between influenza virus/virus-infected cells and endogenous anti-DNP antibodies, which are abundantly present in all human serum. This “marking” step then initiates immune responses leading to the clearance of the antibody-coated virus/virus-infected cells via mechanisms such as antibody-dependent cellular phagocytosis (ADCP), antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).

### Methods

Influenza virus-infected MDCK cells were used to measure the binding affinity of zanamivir-DNP conjugate binding to neuraminidases. A neuraminidase transfected HEK293 cell line was generated for the antibody recruiting, ADCP, ADCC and CDC assays. In the mouse protection studies, BALB/c mice were first immunized with DNP-KLH and infected with a lethal dose of influenza virus (100 LD<sub>50</sub>, A/Puerto Rico/8/1934) before the treatment of zanamivir-DNP conjugate.

### Results

We demonstrate that our zanamivir-DNP conjugate not only preserves zanamivir’s high binding affinity binding to both group 1 and group 2 neuraminidases, but also recruits anti-DNP antibodies to the surface of virus-infected MDCK cells. Moreover, zanamivir-DNP conjugate is shown to induce killing of neuraminidase-expressing cells through CDC and ADCP effects. Finally, we demonstrate that zanamivir-DNP conjugate is superior to zanamivir alone in protecting virus-infected mice in three aspects: (1) only one dose of zanamivir-DNP conjugate can cure all infected mice when administrated intranasally 24h post-infection; (2) zanamivir-DNP conjugate preserves its efficacy in protecting infected mice when treatment is delayed until 72h post-infection. (3) low dose of zanamivir-DNP conjugate can be systemically administrated (intraperitoneal injection) to protect mice.

### Conclusion

By combining small molecule drug and antibody therapies, our neuraminidase-targeted hapten immunotherapy represents a novel and potent therapy to treat influenza.

*Keywords: small molecule-hapten conjugate; immunotherapy; neuraminidase inhibitor; zanamivir;*

Topic: Clinical Sciences: Therapeutics – Antivirals

Abstract No: 11025

## Development of a new class of broad spectrum influenza PB2 inhibitors

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### Introduction and objectives

Influenza trimeric polymerase complex (PB1:PB2:PA) is required for viral replication and transcription in the nuclei of infected cells. The PB2 subunit of the viral heterotrimeric RNA polymerase binds the cap structure of cellular pre-mRNA and subsequently a host pre-mRNA bound by PB2 is cleaved by the PA (endonuclease) subunit. We discovered CC-42344 and another distinct class of PB2 inhibitors using our structure-based technology. We present here *in vitro* characterization and mechanism of action of these novel PB2 inhibitors.

### Methods

Seven different influenza A PB2 domains (H1N1, H2N2, H3N2, H5N1, and H7N9) were purified for protein crystallization and biochemical assays. PB2 crystals and cocrystals were diffracted to 1.0 – 2.5 Å. Cytopathic effect (CPE) assays measured antiviral activity.

### Results

CC-42344 is a novel, potent, broad spectrum anti-influenza preclinical lead molecule which targets the cap-binding PB2 domain, and is active against a panel of seasonal, pandemic, and Tamiflu-resistant influenza A strains. High resolution X-ray cocrystal structures of CC-42344 and other PB2 inhibitors further revealed a channel connected to the high conserved m7GTP binding site. We designed and developed a distinct class of PB2 linker inhibitors interacting with both the m7GTP binding site and the channel. These novel PB2 inhibitors showed broad spectrum activity, excellent anti-influenza activity (EC<sub>50</sub> <1 nM), and a strong synergistic effect with approved influenza antivirals including, Tamiflu (oseltamivir) and Xoflaxa (baloxavir). In addition to the *in vitro* studies, we obtained favorable pharmacokinetic and ADMET profiles of these PB2 inhibitors.

### Conclusion

Novel anti-influenza PB2 inhibitors have been developed for the treatment of seasonal and pandemic influenza infections.

*Keywords: influenza antivirals; novel PB2 inhibitors; structure-based drug design; X-ray crystal structures; Cocystal Pharma*

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 11161

## TREATING INFLUENZA WITH ANTIVIRALS IS ASSOCIATED WITH A DECREASED BURDEN OF COMPLICATIONS AND HEALTH RESOURCE UTILIZATION IN HIGH RISK PATIENTS

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### Introduction and Objectives:

Understanding how antiviral use affect rates and severity of influenza-related complications is important to inform treatment decisions, especially in patients at high-risk for developing complications. This study used real world US claims data for 2 flu seasons to understand the frequency of complications in high-risk patients and how intervention with antivirals may affect their occurrence.

### Methods:

This was a retrospective cohort study using Truven US commercial claims data from the 2016-17 and 2017-18 flu seasons. Patients under 65y with a medical claim suggesting flu who met at least one CDC category for being at high risk for complications were identified and required to have continuous insurance coverage for at least 180 days before and 120 days after flu diagnosis. Patients prescribed antivirals were identified and propensity score matched to a comparative cohort without antiviral use but similar baseline health resource utilization and comorbidities. Health resource use (HRU) and outcomes in month 1 and months 2-4 after flu were analyzed and compared.

### Results:

451,262 cases of influenza were identified in high-risk patients, with 65.2% receiving antiviral therapy compared with 63.6% of non-high-risk patients. Greater HRU following flu was identified in the high-risk patients that did not receive antiviral therapy, with 16.2% vs 12.2% ( $p < 0.001$ ) visiting the ER in the first month after diagnosis, and 2.3% vs 1.5% ( $p < 0.001$ ) being admitted for inpatient care within the first month. Similarly, greater utilization was also seen in various categories of respiratory related utilization and costs.

### Conclusions:

Our study suggests that high risk flu patients treated with antivirals have less complications, HRU and incur less overall costs than those who do not receive antiviral treatment. Causal inference cannot necessarily be derived from this retrospective observational study however, and further studies are needed to confirm the findings.

*Keywords: High Risk Burden Cost Complications*

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 11163

## IMPACT OF ANTIVIRAL THERAPY ON SHORT- AND LONG-TERM OUTCOMES OF PATIENTS WITH COPD FOLLOWING INFLUENZA INFECTION

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### Introduction/Objectives:

Especially in high-risk patients, influenza is associated with complications, aggravated by comorbidities, including exacerbation of their underlying disease. The pro-inflammatory cascade triggered in the lung by viral infection is linked to significant morbidity/mortality. We have previously demonstrated the impact of influenza infection on health outcomes of patients with COPD for the following 12 months. However, we didn't assess the influence of antiviral therapy (AT). Using real world U.S. claims data from four seasons, we examined the effects of AT on the clinical course of COPD patients over 13 months post-infection.

### Methods:

Utilizing Truven U.S. commercial claims data from the 2012-2015 flu seasons, this retrospective cohort study compared health-resource-utilization (HRU) among COPD patients diagnosed with influenza +/- AT. Patients receiving AT were propensity score matched to patients without AT (controls). HRU, outcomes, and costs over the 13 months following index influenza diagnosis were analyzed and compared.

### Results:

COPD patients who had influenza infection and were treated with AT (n=4134) were identified and matched 1:1 to respective controls. In the first month following the index influenza episode, fewer patients treated with AT had COPD-related outpatient-(48.0% vs 60.0%, p<.0001), inpatient-visits (2.5% vs. 7.9%, p<.0001) and COPD exacerbations (10.4% vs 18.2%, p<.0001) compared to control patients. HRU rates remained lower up to 1-year post-infection, exemplified in months 10-13 post-flu: compare outpatient-, (49.1% v 54.5%, p<.0001), ER-(5.6% vs 7.1%, p<.007), inpatient-visits (1.1% vs. 2.5%, p<.0001), and COPD exacerbations (7.5% vs. 10.8%, p<.0001). Similarly, there was also significantly less pneumonia-related HRU and associated costs (COPD or pneumonia related) in the following year among patients treated with AT (Table).

### Conclusions:

Employing AT to treat influenza infection in patients with COPD significantly ameliorates short- and long-term complications in this high-risk population, as exemplified by lower exacerbation rates and decreased HRU and costs maintained up to 1-year post-infection.

*Keywords: COPD antivirals cost complications claims*

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 11211

## RESISTANCE DEVELOPMENT IN INFLUENZA A VIRUSES INFECTING MALLARDS EXPOSED TO LOW LEVELS OF PERAMIVIR

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**Introduction and Objectives:** Resistance to neuraminidase inhibitors in influenza A viruses (IAVs) with pandemic potential is a serious threat, as these drugs constitute a pandemic preparedness cornerstone, especially before vaccines are mass-produced. Human pandemics contain genetic material initially originating from wild waterfowl IAVs; thus, resistance development in waterfowl IAVs are of public health concern. Resistance could develop in aquatic environments where IAVs infecting waterfowl can be exposed to neuraminidase inhibitor drug residues discharged from sewage treatment plants. Previous experiments have demonstrated resistance development in IAVs infecting Mallards exposed to low levels of oseltamivir and zanamivir, but no data exist regarding peramivir.

**Methods:** Mallards were infected with H1N1 or H4N2 IAVs while exposed to low concentrations of peramivir in their sole water source. Matrix gene qPCR and neuraminidase gene sequencing were performed on daily fecal samples. Neuraminidase activity inhibition assay was performed on selected isolates after culturing in embryonated chicken eggs.

**Results:** 10 ng/L and 100 ng/L (H1N1 and H4N2), and 330 and 670 ng/L (H4N2) of peramivir exposure did not result in resistance-related substitutions. Exposure of H1N1 IAV to 1 µg/L of peramivir resulted in acquisition of resistance substitution H275Y, rapidly outcompeting wild-type. 1 µg/L (H4N2) and 10 µg/L (H1N1 and H4N2) exposure effectively inhibited IAV replication. Phenotypic evaluation confirmed drug resistance of H275Y-carrying IAV isolates.

**Conclusion:** Compared to previous exposure experiments in the same model, the H1N1 IAV acquired resistance more rapidly (compared to oseltamivir) or at a lower concentration (compared to zanamivir), and comparatively lower levels of peramivir inhibited IAV replication. We could not demonstrate resistance development in the H4N2 virus, indicating a small selective window. Environmental pollution of neuraminidase inhibitors is a potential public health concern; peramivir and oseltamivir seem to be relatively more prone to resistance induction in Mallard IAVs than zanamivir.

**Keywords:** One Health; Drug residues, Environmental pollution, LPAIV, pandemic preparedness

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 11286

## Pharmacokinetics of favipiravir (T-705) in combination with oseltamivir for treatment of critically ill patients with severe influenza

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**Background:** There is an unmet need to determine the pharmacokinetics of favipiravir in critically ill patients.

**Methods:** In this the open-label, dose-escalating study of favipiravir, 4 academic medical centres in China assessed the pharmacokinetics and tolerance in critically ill influenza patients. Hospitalised adults with severe influenza A or B (defined as PaO<sub>2</sub>/FiO<sub>2</sub>≤300 mmHg or on mechanical ventilation) were enrolled to received a licensed dose regimen (1600 mg BID on day 1 and 600 mg BID on the following days) in Japan for uncomplicated influenza and a high explored dose regimen (1800 mg/800 mg BID). The primary endpoint is the proportion of patients with an observed trough concentration above the minimum effective concentration (MEC) at all time points after the second dose.

**Findings:** Between Feb 6, 2018, and Feb 20, 2019, 87 participants were screened for eligibility. Totally, 16 patients and 19 cases were enrolled into low dose and high dose regimen of the study, respectively. No steady state was reached during the dosing period, and concentrations were observed to decrease over time. In the patients receiving 1600/600mg favipiravir, the median values of observed concentrations were 35.9, 23.43, 8.6, 8.6 µg/mL on day-2, 3, 7, 10, respectively. Increasing the loading dose from 1600 to 1800 mg BID on day 1 and the maintenance dose from 600 to 800 mg BID on day 2-10, the median values of observed concentrations were 34.1, 31.15, 8.29, 11.15 µg/mL on day-2, 3, 7, 10, respectively. Proportion of with the target exposure was higher in patients receiving 1800/800mg favipiravir than those in 1600/600 mg regimen.

**Interpretation:** The pharmacokinetics is different in severely ill influenza patients. The favipiravir regimen (1800/800 mg) achieve the target exposure. An escalating dose of favipiravir was associated with an increased proportion of patients with the target exposure.

**Keywords:** favipiravir, population pharmacokinetics, influenza, human, modelling, critical ill; mortality; intensive care units

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 11309

## Baloxavir for the treatment of Influenza virus infection in hematopoietic stem cell transplant recipients who failed oseltamivir

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**Introduction:** Seasonal influenza causes significant morbidity and mortality in hematopoietic stem cell transplant (HSCT) recipients. In this population, influenza virus can replicate for prolonged periods despite repeated courses of neuraminidase inhibitor (NAI) treatment, leading to NAI resistance mutations and treatment failure. Baloxavir targets the influenza polymerase and may be effective for treating these cases.

**Methods:** Influenza was detected in nasopharyngeal (NP) swabs using the FilmArray Respiratory Panel 2 (BioFire Diagnostics). Nucleic acid (NA) from NP specimens was tested by real-time RT-PCR to confirm the presence of influenza. In samples with sufficient viral load, resistance mutations were detected with pyrosequencing and NA was further assessed with whole-genome sequencing (WGS). WGS were analyzed with the IRMA bioinformatics pipeline and examined for minor variant percentages using Geneious Pro (9.1.5). Influenza-positive specimens were cultured in MDCK cells and isolates tested for phenotypic NAI susceptibility (NA-Fluor).

**Results:** Patients symptomatic and positive for influenza after oseltamivir therapy received one or two doses of Baloxavir. We treated five HSCT recipients, aged 61-72y, with complicated influenza infection with Baloxavir: four with A/H1 and one with A/H3. Four of the patients were co-infected with other respiratory pathogens. Two patients carried the H275Y neuraminidase mutation conferring NAI resistance. Four patients cleared their infection after one or two doses of Baloxavir. The fifth patient remained persistently influenza positive.

**Conclusion:** Baloxavir may be a useful treatment option for infections with influenza virus with NAI resistance mutations, and in HSCT patients who fail NAI-treatment for other reasons. Randomized clinical trials are needed to determine the role of Baloxavir for influenza treatment in this immunocompromised population.

*Keywords: Influenza treatment, Baloxavir, Oseltamivir, immunocompromised*



Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 11754

**A GLOBAL, RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY EVALUATING SAFETY AND EFFICACY OF VIS410 IN COMBINATION WITH OSELTAMIVIR VERSUS OSELTAMIVIR ALONE IN HOSPITALIZED ADULTS WITH INFLUENZA A REQUIRING OXYGEN**

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**Introduction:** VIS410, a human monoclonal antibody targeting influenza A hemagglutinin stem, was evaluated in an exploratory phase 2b study for hospitalized patients with influenza A requiring O<sub>2</sub> therapy.

**Methods:** Patients with symptom onset within 120 hours were randomized 1:1:1 to receive oseltamivir and a single IV infusion of VIS410 4000mg, 2000mg, or placebo(PBO). Safety was monitored through 8 weeks. Efficacy endpoints included: seven-level ordinal scale (SLOS), oxygen normalization, clinical response, mortality, hospital/ICU stay, viral load, and patient-reported outcomes.

**Results:** 89 subjects were randomized, 88 received study drug, 85 subjects had confirmed influenza A. At baseline, there were more VIS410-treated subjects in the ICU (37.9%(4000 mg),35.7%(2000 mg) vs 14.3%(PBO), and on mechanical ventilation (13.8%,10.7% vs 3.6%); odds ratio p=0.037. VIS410-treated subjects were older and had a higher rate of bacterial pneumonia at baseline (48.3%,32.1% vs 17.9%). Despite this profound baseline imbalance favoring placebo, median time to normal oxygenation and time to complete clinical response (CCR) were not significantly different across treatment arms. Analysis of patient groups with comparable baseline status demonstrated faster times to oxygenation and vital signs normalization; these differences were significant in subgroups presenting within 72 hours or with positive baseline viral cultures (Table). VIS410 significantly improved time to clearance of infectious virus in culture positive subjects. Serious adverse events were similar across treatment arms (20.7%(4000 mg), 13.8%(2000 mg), and 20.0%(PBO), and were not considered study drug-related. Overall mortality was 10% among placebo recipients versus 5% among VIS410 recipients.

**Conclusions:** VIS410 was associated with significant improvements in time to normal oxygen and vital signs in subgroup analyses of patients with comparable baseline disease severity. These data strongly support the paradigm of broadly-neutralizing antibody therapy for influenza and have informed designs for registrational studies of VIS410.

**Keywords:** SAFETY, EFFICACY, VIS410, ANTIBODY, HOSPITALIZED

Topic: Clinical Sciences: Therapeutics – Immunomodulators, Supportive Care, Others  
Abstract No: 11151

## POTENTIAL THERAPEUTIC ROLE OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN ACUTE LUNG INJURY INDUCED BY HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS A/H5N1

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### Introduction and Objectives

Highly pathogenic avian influenza (HPAI) A/H5N1 virus is one of the causative agents with a high incident rate in acute respiratory distress syndrome (ARDS). Studies on therapeutic administration of bone marrow-derived mesenchymal stem cells (BM-MSCs) in ARDS caused by the viral infection have been limited and shown conflicting results. The aim of this study was to investigate using a mouse model for therapeutic potential of BM-MSCs administration in the virus-caused ARDS.

### Methods

BALB/c mice were intranasal inoculated with HPAI A/H5N1 virus. BM-MSCs were prepared from bone marrow of 9 to 12 week-old BALB/c mice. BM-MSCs were intravenously administered into the mice on day 2, 4, and 6 after virus inoculation. To examine effects of the treatment, we measured body weight, virus growth in lung, lung alveolar protein (an indicator for lung injury), PaO<sub>2</sub>/FiO<sub>2</sub> ratio (an indicator for lung functioning), and duration of survival. In addition, expressions of Sftpc (alveolar cell type II marker), Aqp5+ (alveolar cell type I marker), RAGE (transmembrane receptor for damage associated molecular patterns), NFκβ (transcription factor), TNFα (cytokine), and IL-1β (cytokine) were examined by immunohistochemistry. Lung injury was scored by haematoxylin-eosin staining.

### Results

On days 3, 5, and 7 after virus infection, the induction of Sftpc, Aqp5+, RAGE, Nfkb, TNFα, and IL-1β was observed, and that of RAGE, Nfkb, TNFα, and IL-1β significantly inhibited by BM-MSCs administration compared to mock-treated group, while that of Sftpc and Aqp5+ was enhanced. Lung damage was shown to decrease by histopathological score and also by measuring lung alveolar protein and PaO<sub>2</sub>/FiO<sub>2</sub> ratio. Virus growth in lungs at day 3 significantly decreased and survival period was slightly increased.

### Conclusion

The administration of BM-MSCs had a tendency to inhibit acute lung injury caused by HPAI A/H5N1 virus by their ability for immunoregulation and regeneration, indicating its therapeutic potential

**Keywords:** bone marrow-derived mesenchymal stem cells (BM-MSCs); acute respiratory distress syndrome (ARDS); highly pathogenic avian influenza (HPAI) A/H5N1 virus; therapeutic potential.

Topic: Co-Infection: Bacterial Co-infections with Influenza  
Abstract No: 10904

## Evaluating the window of susceptibility to secondary bacterial infections post-influenza infection in ferrets

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### Introduction and objectives

Influenza virus infections cause epithelial cell damage and reduced Toll-like receptor (TLR) responsiveness, which increase susceptibility to secondary bacterial infections (SBI) resulting in increased morbidity and mortality. *Streptococcus pneumoniae* (SPN) is the most common bacterial pathogen associated with SBIs. We examined interactions between influenza and SPN in ferrets, with the aim of establishing an animal model to evaluate how antiviral treatment may impact SBI progression following influenza infection.

### Methods

Ferrets were infected intranasally with  $10^3$  TCID<sub>50</sub> of influenza A(H1N1)pdm09 virus and 5 or 10 days later were infected intranasally with  $10^3$  colony forming units of the 19F strain of SPN. Control ferrets were infected with either influenza or SPN alone. Nasal washes were collected daily to enumerate viral and bacterial loads, and clinical signs were monitored. Ferrets were euthanised at a humane endpoint and bacterial counts in the lungs and blood determined.

### Results

Compared to ferrets infected with bacteria alone, animals that received SPN 5 days post-influenza showed accelerated disease signs including laboured breathing, lethargy and dehydration. In these ferrets, the bacterial burden in the upper respiratory tract (URT) was approximately 100 to 1000-fold higher than those infected with SPN alone. Conversely, a SBI 10 days after influenza infection resulted in few or no disease signs, although bacterial burden in the URT was approximately 10-fold higher than in animals infected with SPN alone. At both time points, bacteraemia occurred in 1 of ferrets that had been infected with influenza + SPN, but was absent in ferrets infected with SPN alone.

### Conclusions

Morbidity and mortality was enhanced when SBIs occurred 5 days post-influenza infection though SBI could be established 10 days post-influenza infection. Experiments to determine whether treatment with influenza antivirals would reduce the susceptibility to SBI and associated disease severity are ongoing.

*Keywords: Influenza virus; Streptococcus pneumoniae; ferrets; secondary bacterial infections; antivirals*

Topic: Co-Infection: Bacterial Co-infections with Influenza  
Abstract No: 10912

## Epidemiology of influenza-associated community-acquired pneumonia admissions: A 7-year retrospective cohort study in Singapore

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### Introduction

Bacterial and viral co-infected community-acquired pneumonia (CAP) in older individuals can aggravate underlying comorbidities and have severe outcomes.

### Methods

We conducted a retrospective, observational cohort study on patients hospitalized in acute care hospital with a diagnosis of CAP from 1 Jan 2010 to 31 Dec 2016.

### Results

We identified 20,155 first-time hospitalizations during the study period with predominantly elderly (72% aged  $\geq 65$  years), Chinese (79%) and a male-to-female ratio of 1.2:1. 10,026 (50%) had Charlson's co-morbidity score  $\geq 3$ . 1,568 patients (8%) with blood or respiratory culture positive results. The most common organisms overall were *Klebsiella pneumoniae* (14%), followed by *Escherichia coli* (12%) and *Streptococcus pneumoniae* (8%). Of all, 10,837 patients (54%) had an influenza PCR test during the hospitalization. Of these, 9% (967 patients) had influenza detected (53% A/H3, 20% B, 19% A/H1N1-2009 and 7% A/subtype undetermined). Of the 1,568 patients with positive blood or respiratory cultures, 72 (5%) patients were co-infected with influenza virus. Among all, the proportion of severe pneumonia (admitted to the intensive care unit or had died) was significantly higher in patients with co-infections (0.7% Vs. 0.2%,  $p < 0.001$ ). On multivariate analysis, independent risk factors positively associated with severe CAP were age  $\geq 65$  (AOR 1.80, 95%CI 1.66-1.95), male gender (AOR 1.13, 95%CI 1.06-1.21), Chinese ethnicity (AOR 1.31, 95%CI 1.20-1.42), prior hospitalisation in the past twelve months (AOR 1.37, 95%CI 1.28-1.47), Charlson score  $\geq 3$  (AOR 1.96, 95%CI 1.83-2.11), having bacterial and virus co-infection (AOR 4.48, 95%CI 2.74-7.32), and protective factors were history of pneumococcal vaccination before hospital admissions (AOR 0.85, 95%CI 0.75-0.97) and influenza vaccination in the past twelve months (AOR 0.83, 95%CI 0.72-0.96) after adjusting for admission year.

### Conclusion

Influenza and bacterial co-infections have an adverse impact on the severity of pneumonia. Influenza and pneumococcal vaccinations for older adults are highly recommended.

Topic: Public Health: Disease Burden & Severity  
Abstract No: 10362

## Under-Detection of Laboratory-Confirmed Influenza-Associated Hospitalizations among Infants in a Multi-Country Prospective Study

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### INTRODUCTION/OBJECTIVES

Existing surveillance and research platforms potentially under-estimate the true burden of severe influenza disease among infants. The *Influenza and RSV in Infants Study* (IRIS) assessed influenza virus infections by rRT-PCR and serology among acutely ill infants aged <1 year admitted to study hospitals during two influenza seasons (2015-16 and 2016-17) in Albania, Jordan, and Nicaragua and a continuous 34-week period (2015-16) in the Philippines.

### METHODS

Infants admitted with an acute respiratory or non-respiratory illness with onset  $\leq 10$  days were prospectively enrolled. Combined nasal and oropharyngeal swabs and acute sera were collected within 24-hours of admission; convalescent sera was collected  $\sim 28$  days after illness onset. Clinical discharge diagnoses were abstracted from medical records. Seroconversion was defined as  $\geq 4$ -fold rise in acute-to-convalescent antibody titers with convalescent titers  $\geq 40$ . Seroconversions for influenza A viruses were defined by hemagglutination inhibition (HI) assay; seroconversion for influenza B viruses were defined by both HI and microneutralization assay.

### RESULTS

Of 1943 acutely ill hospitalized infants, 152 (7.8%) had rRT-PCR-confirmed influenza and an additional 100 (5.1%) were rRT-PCR-negative but seroconverted to influenza; thus, 40% of all influenza positives were confirmed by serology-only. Of 252 influenza positives, 82 (32.5%) had only non-respiratory clinical discharge diagnoses (e.g., sepsis, febrile seizures, dehydration, other non-respiratory viral illness). The attached Figure illustrates the incremental contribution of serologic and non-respiratory diagnoses to the total number of influenza positive infants. A focus on respiratory rRT-PCR-influenza disease under-detects influenza-associated hospitalizations among infants by a factor of 2.6 (95% CI = 2.0-3.6), after adjustment for study site and season. Findings were unchanged when SARI criteria were applied instead of clinical diagnoses.

### CONCLUSIONS

If the true burden of laboratory-confirmed influenza-associated hospitalizations among infants is at least twice that of previous estimates, this substantially expands the potential preventive value of maternal and infant vaccination.

*Keywords: infant; burden; serology*

Topic: Public Health: Disease Burden & Severity  
 Abstract No: 10448

**PRELIMINARY ESTIMATES OF THE INCIDENCE OF INFLUENZA-ASSOCIATED ACUTE RESPIRATORY INFECTION AMONG ADULTS AGED  $\geq 60$  YEARS IN A MULTI-SITE COMMUNITY COHORT IN INDIA**

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**Introduction and Objectives:** Despite a growing population of persons aged  $\geq 60$  years in middle-income countries, few data are available on the burden of influenza among older adults in India. We conducted a prospective multi-site cohort study among community dwelling adults aged  $\geq 60$  years to estimate the incidence of influenza-associated acute respiratory infection (ARI).

**Methods:** During August 2018-February 2019, which coincides with the influenza season, trained nurses conducted weekly household surveillance for ARI among cohorts at four sites: Delhi, Chennai, Kolkata and Pune. We defined ARI as new onset/ worsening of cough or difficulty in breathing in the last seven days and ALRI as ARI plus dyspnea or chest pain, a respiratory rate of  $>20$  breaths/minute, and either measured fever or a reported symptom complex of fever, sweating, headache and myalgia. Nurses collected nasal and oropharyngeal swabs from all ALRI cases and 20% of randomly selected ARI cases not meeting ALRI criteria for influenza testing by RT-PCR. We extrapolated the monthly influenza positivity rate among tested non-ALRI cases to the monthly non-ALRI incidence in the cohort and then calculated ARI incidence as the sum of non-ALRI and ALRI incidence. We estimated the incidence of influenza associated ARI and ALRI per 1000 person-years(py) with 95% confidence interval using normal approximation method.

**Results:** We followed 5,678 adults for 2,537 person-years. The median age of the cohort was 65 years (IQR 62-70); 57% were female and 71.4% reported pre-existing chronic morbidity. Overall, the incidence of influenza-associated ARI was 122.2/1000py but incidence varied by site (Table-1). The highest influenza-associated ARI incidence rate was in Pune (167.1/1000py); and lowest rate in Chennai (86.4/1000py). The influenza-associated ALRI rates were comparable between sites, with average rate of 23.2/1000py (95%CI 17.3-29.2).

**Conclusion:** The preliminary estimates showed 1-in-50 older adults had influenza associated ALRI each year in this multi-site community based cohort.

*Keywords: influenza, incidence, india, older adults*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 10547

## ESTIMATING THE INFLUENZA DISEASE PYRAMID IN SINGAPORE

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**Introduction and Objectives:** Influenza surveillance in Singapore has been revised over the years to incorporate the use of new influenza-like illness case definitions and more sensitive diagnostic methods. This study aims to provide updated estimates of influenza infection in the community and its severity.

**Methods:** The posterior distribution of the instantaneous reproduction number of the local influenza activity ( $R_t$ ) was estimated using the daily number of influenza-like illness (ILI) samples positive for influenza collected from 2008 to 2017. Median estimates of  $R_t$  were then used to parameterise a deterministic SEIRS (susceptible-exposed-infectious-recovered-susceptible) model to estimate the number of infected cases during seasonal outbreaks and the rates of primary care consultation among influenza cases. Laboratory-confirmed influenza cases admitted to the intensive care unit or died in eight government hospitals from 2011 to 2017 were also analysed to obtain the age-adjusted incidence and mortality rates for severe influenza.

**Results:** The mean number of people infected in a seasonal outbreak was approximately 235 000 and about 20% of the infected persons would seek medical treatment at primary care clinics. Based on similar methods in a previous study by Ang et al (2014), from 2010 to 2017, the overall influenza-associated pneumonia and influenza hospitalisation rate was estimated to be 50.1/100,000 population. Age-adjusted incidence rates exhibited a J-shaped curve and was the highest in persons aged  $\geq 65$  (18.5/100,000 population), second highest among children aged  $\leq 4$  (3.1/100,000 population) and lowest among persons aged 25-34 (0.2 per 100 000 population). Mortality rate was the highest in persons aged  $\geq 65$  (10.7/100,000 population).

**Conclusion:** Constructing the burden of influenza pyramid requires comprehensive surveillance at different level of the healthcare system. While mild infections go largely unnoticed, sensible extrapolations can provide valuable estimates to devise targeted policies and communications related to influenza to protect vulnerable young and elderly persons.

*Keywords: disease burden, influenza, modelling*

Topic: Public Health: Disease Burden & Severity  
 Abstract No: 10667

## COMMUNITY BURDEN OF INFLUENZA IN A RURAL AND AN URBAN SETTING, SOUTH AFRICA, 2016-2017 COMMUNITY BURDEN OF INFLUENZA IN A RURAL AND AN URBAN SETTING, SOUTH AFRICA, 2016-2017

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### Introduction

Data on influenza burden in Africa are limited. We aimed to estimate the attack rate (AR) of influenza infection in South Africa.

### Methods

We conducted a prospective cohort study in a rural and urban site including 100 randomly selected households in 2016 (April–October) and 108 different households in 2017 (January–October). Nasopharyngeal swabs were collected twice-weekly from consenting household members irrespective of symptoms and tested for influenza using a real-time reverse transcription polymerase chain reaction (rtPCR). AR was estimated by dividing the number of rtPCR-positive individuals by the population at risk.

### Results

We collected 65,347 samples from 1,118 participants in 208 households (follow-up rate 86%), of which, 791 (1%) tested influenza positive (292 A(H3N2), 62 A(H1N1)pdm09, 365 B, 55 A-unsubtyped, 17 mixed); 78% of households (163/208) had at least one influenza-positive individual annually. Annual influenza AR was 33% (369/1118) and 16% of influenza-infected individuals had >1 episode during a season (mean 1.2; range 1–3). By age group, the influenza AR was 39% (75/192) <5 years, 36% (169/471) 5–18 years, 28% (112/403) 19–64 years and 25% (13/52) ≥65 years. On multivariable analysis, urban site (odds ratio (OR) 1.4, 95% confidence interval (CI) 1.1–1.8), year 2017 (OR 1.4 95%CI 1.1–1.8) and age groups <5 years (OR 1.9, 95%CI 1.3–2.9) and 5–18 years (OR 1.6, 95%CI 1.1–2.1) (vs. 19–64 years) were significantly associated with influenza AR. HIV was not associated with AR (OR 0.9 95%CI 0.6–1.3). In 2017, 53% (129/242) of influenza-infected individuals reported ≥1 symptom, 31% (40/129) of these included fever and cough, 22% (29/129) of symptomatic individuals sought medical care, and 57% (51/89) reported absenteeism.

### Conclusion

Influenza burden is high in two areas of South Africa with ≥30% of individuals infected annually; approximately half of episodes are symptomatic. Interventions to reduce influenza burden are warranted.

*Keywords: influenza; burden; community; South Africa*



Topic: Public Health: Disease Burden & Severity  
Abstract No: 10858

## Estimating the Number of Deaths due to Influenza — An alternative to regression-based estimates of excess influenza mortality

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**Introduction and Objectives:** Annual estimates of influenza mortality demonstrate the varying severity of influenza across seasons. Regression-based estimates have been widely used, but are sensitive to the model structure and time-interval used, posing a challenge for public health communication. We present an alternative method, leveraging population-based surveillance for laboratory-confirmed influenza-associated hospitalizations.

**Methods:** We estimated age group-specific influenza mortality in the United States using three approaches: 1) using data from 2010/11–2016/17, we constructed a negative binomial regression of weekly all-cause deaths with an indicator multiplying weekly virologic surveillance with influenza-like-illness surveillance; 2) we refit the regression model with data from 2010/11–2017/18; and 3) we applied a season-specific deaths:hospitalizations ratio (D:H) to age-stratified rates of influenza-associated hospitalization and extrapolated to the national population. We calculated the D:H ratio as the probability of death given hospitalization (from hospital-based influenza surveillance; 2010/11–2017/18) divided by the proportion of deaths occurring out-of-hospital. We calculated the latter proportion from cause-specific death certificates (available through 2016/17); first, creating mutually exclusive categories of pneumonia/influenza, respiratory/circulatory, or all others, and then weighting by the occurrence of each category among patients hospitalized with laboratory-confirmed influenza who died. Due to lags in data availability, we assumed the 2016/17 D:H ratio also applied during 2017/18.

**Results:** Excess influenza mortality estimates varied by season (Table). After incorporating data through 2017/18, regression-based estimates for prior seasons declined by 5,000–35,000 deaths (43%–68% decline, respectively). Estimates using the D:H ratio were qualitatively similar to the regression-based estimates using data through 2016/17.

**Conclusions:** Regression-based estimates of influenza mortality use commonly available data; however, refitting the model to an updated time-series can substantially change prior-season estimates. Our multiplier approach requires additional data and is not without limitations; however, the approach uses directly observed data and allows for direct between-season comparisons, facilitating public health communication and decision-making.

*Keywords: Influenza burden; Influenza-associated mortality; Influenza;*

Topic: Public Health: Disease Burden & Severity  
 Abstract No: 11044

## The Burden of In-Hospital and Out-of-Hospital Deaths among Patients Hospitalized with Influenza, FluSurv-NET, 2010–2016

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**Introduction:** The burden of deaths occurring after influenza-associated hospitalization is poorly understood.

**Methods:** We included patients hospitalized with laboratory-confirmed influenza in the United States during 2010-11 through 2015-16 influenza seasons, and identified through clinical testing from 12 of 13 states participating in CDC's Influenza Hospitalization Surveillance Network (FluSurv-NET), to describe deaths that occurred during hospitalization (in-hospital) and within 30 days post-discharge. We used the National Center for Health Statistics Electronic Death Registration system to link FluSurv-NET cases with death certificates. We used  $\chi^2$  tests to compare characteristics of patients who died in-hospital with those that died post-discharge and characterized causes of death (COD) listed in any position on death certificates.

**Results:** Of 57,288 patients hospitalized with influenza, 2661 (4.6%) died; 1444 (2.5%) in-hospital and 1217 (2.1%) post-discharge. The proportion of patients who died varied by season (3.8% during 2011-12 to 5.5% during 2014-15) and age group (0.5% among 0-4 years and 7.3% among  $\geq 65$  years). The highest proportion of post-discharge deaths occurred during H3N2-predominant seasons (2012-13, 2014-15) (Figure A) and among those  $\geq 65$  years (Figure B). Among 1217 patients who died post-discharge, 51% of deaths occurred within 9 days of discharge and 44% at hospice or long-term care facilities. Compared to patients who died in-hospital, those who died post-discharge were older and had more underlying conditions ( $p < 0.01$ ). Influenza was listed as a COD for 52% versus 18% of patients who died in-hospital and post-discharge, respectively. Influenza was the most frequent COD listed for in-hospital deaths and cardiovascular disease (38%) for post-discharge deaths.

**Conclusions:** Among patients hospitalized with influenza who died, 46% of deaths occurred post-discharge. Our data highlights an under-recognized burden of influenza-associated mortality, particularly among vulnerable patients and those  $\geq 65$  years. Capture of post-discharge outcomes by influenza hospitalization surveillance systems would help to better characterize influenza-associated morbidity and mortality.

**Keywords:** influenza, hospitalization, deaths

Topic: Public Health: Disease Burden & Severity  
Abstract No: 11254

## BURDEN AND SEVERITY OF INFLUENZA-LIKE ILLNESS IN AUSTRALIA: 10 YEARS OF FLUTRACKING ONLINE SURVEILLANCE

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### Introduction and Objectives

Flutracking is the largest participatory online surveillance system in the world with over 40,000 participants reporting influenza-like illness most weeks in winter. We analysed and present Flutracking data that provide an alternative perspective on the burden and severity of influenza compared to traditional systems such as laboratories.

### Methods

Incidence of cough, fever, self-reported laboratory testing, healthcare-seeking behaviour were analysed across years and between Flutracking and other systems from 2009 to 2018 and examined laboratory testing rates from 2013 to 2018.

### Results

Flutracking has a high correlation with laboratory notified influenza, however it is less susceptible to aberrations in testing practices. The proportion of participants seeking health care for ILI symptoms ranged from a low of 31% in 2013 to a high of 42% in 2017. Laboratory testing of Flutrackers increased from 1.5% to 5% from 2013 to 2017. There was a 1.7 fold increase in laboratory testing of Flutrackers in 2017 compared to 2016, suggesting the increase in laboratory notifications was partly explained by increased testing. Based on increased testing among Flutrackers, we estimated a 75% increase in laboratory notifications from 2016 to 2017 due to influenza activity (compared to an unadjusted 203% increase based on laboratory data alone). Comparing attack rates and illness duration, the highest attack rates (8% in peak four weeks) were among the < 18 year olds in 2009 and the longest duration of absence from normal duties was among 65+ years (6.7 days in peak four weeks) in 2014.

### Conclusions

Flutracking provides a unique perspective on ILI that allows traditional systems to be adjusted for changes in healthcare-seeking behaviour in the community or modified clinical and laboratory practice and capacity. It also allows assessment of important indicators of severity such as health seeking behaviour and absence from normal duties stratified by age.

*Keywords: syndromic;ILI;burden of disease*

Topic: Public Health: Epidemiology & Transmission

Abstract No: 10352

## INFLUENZA VIRUS TRANSMISSION FROM SYMPTOMATIC AND ASYMPTOMATIC INDIVIDUALS IN A RURAL AND AN URBAN SETTING, SOUTH AFRICA, 2016-2017

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### Introduction

Data on influenza virus transmission in Africa are limited. We estimated factors associated with household cumulative infection risk (HCIR) in two sites in South Africa.

### Methods

During April–October 2016 and January–October 2017, we conducted a prospective cohort study in 208 households (100 in 2016, 108 in 2017). We collected data on the presence or absence of fever, cough and 8 other symptoms, and nasopharyngeal swabs twice weekly from consenting household members (HM) to test for influenza viruses using real-time reverse transcription polymerase chain reaction (rtPCR). We estimated HCIR by dividing the number of rtPCR-confirmed subsequent household infections within 2 mean serial intervals by the total number of exposed HM per introduction. Clusters started with the first rtPCR-positive HM and ended when the last HM tested positive. We performed multivariable logistic regression to determine factors associated with index cases and infected HM.

### Results

Among 1118 participants from 208 households there were 430 influenza virus infections and 276 clusters. The mean number of infections per cluster was 1.6 (430/276). The mean duration of a cluster was 8.5 days (range 3-35). 70% (304/430) of influenza virus infections were community-acquired. The HCIR was 10% (126/1202)—19% (42/225), 8% (6/81) and 5% (16/293) for index cases with  $\geq 2$ , 1 or no symptoms, respectively. Factors associated with HCIR index cases include age <5 years (aOR 3.3, 95% CI 1.4-7.5) or 5-18 years (aOR 2.7, 95% CI 1.8-3.3) vs 19-64 years and presence of 2 or more symptoms (aOR 4.8, 95% CI 2.5-9.2) vs. none. HIV infection (aOR 2.3, 95% CI 1.1-4.9) was a risk factor for influenza infection in exposed HM.

### Conclusion

The HCIR was lower than traditionally measured in household transmission studies of symptomatic illness. Children were more likely to transmit influenza infections. While less infectious, asymptomatic individuals were able to transmit influenza viruses.

**Keywords:** influenza viruses, transmission, symptomatic, asymptomatic

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10492

## DETERMINANTS OF INFLUENZA TRANSMISSION IN HOUSEHOLDS IN RURAL NORTH INDIA

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**Introduction:** Household transmission studies help in estimating secondary infection risk (SIR) and understanding determinants of influenza transmission. Previous studies showed SIR for influenza ranging from 1—38%.

**Methods:** In an influenza vaccine-study platform, we visited weekly 2,954 households in three villages of Faridabad district, to identify febrile acute respiratory illness (FARI) cases defined as reported fever with  $\geq 1$  respiratory symptom (cough, sore-throat, congestion/runny nose, breathlessness, earache). Trained nurses collected nasal and oropharyngeal swabs for RT-PCR testing to detect influenza-associated FARI (I-FARI). A primary case was I-FARI with the earliest onset date in a household cluster. Secondary cases had onset between 2-10 days after a primary case. We used an I-FARI case-free interval of fourteen days to distinguish clusters. SIR was the proportion of secondary cases among household contacts. The serial interval (SI) was the interval between the onset dates of a primary case and secondary cases. We used multilevel logistic regression to estimate the risk of secondary I-FARI cases among household contacts.

**Results:** During November 2009-April 2012, there were 1,744 I-FARI clusters in 1,347 households. The SIR was 2.0%(95%CI:1.6 – 2.4) in November 2009-October 2010; 2.3%(95%CI:1.9 – 2.9) in November 2010-October 2011; and 2.4%(95%CI:1.6 – 3.4) in November 2011-April 2012. The median SI was 4 days (IQR: 2-7 days). Compared to those aged  $\geq 60$  years, adjusted odds ratio (aOR) of I-FARI in children aged  $\leq 5$  years was 15.2(95%CI:4.6-50.1); and 6-15 years was 6.6(95%CI:2.0-21.4) (Table 1). The aOR was 1.6(95%CI:1.2-2.2) among females; 1.5(95%CI:1.0-2.6) among those living in households with no vaccinated members; and 1.6(95%CI:1.0-2.8) among smaller(2-5 members) vs. bigger households( $>10$  members).

**Conclusion:** SIR was comparable across seasons despite variability in predominating viruses, including emergence of A(H1N1)pdm09, but lower than most of the previous studies. Young age of contacts, female gender and smaller household size were associated with secondary infections in these households.

*Keywords: influenza; household; transmission; public-health*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10702

## Impact of influenza antigenic evolution on disease dynamics in the United States

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**Introduction and Objectives:** Influenza viruses continually evolve new antigenic variants, primarily through mutations to the HA1 region of the hemagglutinin (HA) gene. Here we study the poorly understood impact of evolutionary changes on annual influenza dynamics.

**Methods:** We constructed an A/H3N2 phylogenetic tree using a dataset enriched for U.S. sequences and inferred antigenic and genetic distances between strains circulating in successive seasons, using hemagglutination inhibition (HI) data, substitutions at epitope sites in HA1 (N = 129), or substitutions at sites adjacent to the receptor-binding site (N = 7). We obtained weekly epidemiological and virological data from the CDC and explored correlations between A/H3N2 epidemic dynamics (timing, size, peak incidence, transmission intensity, age patterns) and evolutionary distance indicators during 1997 – 2018. We used lasso regression to select predictors of epidemic dynamics, including evolutionary distance, epidemic size in prior seasons, and influenza A/H1N1 and B incidence.

**Results:** Epitope change was the strongest and most consistent predictor of A/H3N2 epidemiology. Increased epitope distance between seasons correlated with earlier ( $r = -0.26$ ,  $P = 0.004$ ) and larger peaks ( $r = 0.73$ ,  $P = 0.004$ ), larger epidemic sizes ( $r = 0.7$ ,  $P = 0.008$ ), and higher transmission rates ( $r = 0.7$ ,  $P = 0.008$ ). Influenza cases shifted from children aged  $\leq 5$  years ( $r = -0.2$ ,  $P = 0.03$ ) to adult age groups ( $r = 0.2$ ,  $P = 0.02$ ) in seasons with high epitope change. Epitope distance was the only predictor consistently retained in multivariate models.

**Conclusion:** The relationship between HA antigenic drift, epidemic impact, and age dynamics is moderate, with genetic distance based on a broad set of epitopes having greater predictive power than HI-based distance. Influenza epidemiological patterns are consistent with increased population susceptibility in high HA epitope change seasons. The impact of neuraminidase evolution and vaccine-related parameters will be studied next.

**Keywords:** *antigenic evolution; epidemic impact; A/H3N2; hemagglutinin; age dynamics*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11001

## The Epidemiological Signature of the Influenza B/Victoria and B/Yamagata Lineages in the 21<sup>st</sup> Century

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**Introduction and Objectives:** We describe the epidemiological characteristics, patterns of circulation, and geographical distribution of influenza B viruses and its lineages using data from the Global Influenza B Study (<https://www.nivel.eu/gibs>).

**Methods:** We included over 1.8 million influenza cases from national surveillance in thirty-one countries during 2000-2018. We calculated the proportion of cases caused by influenza B and its lineages; determined the timing of influenza A and B epidemics; compared the age distribution of B/Victoria and B/Yamagata cases; and evaluated the frequency of lineage-level mismatch for the trivalent vaccine.

**Results:** Influenza B virus caused a median 23.4% of cases in a season (higher in tropical vs. temperate countries,  $p=0.06$ ), and was the dominant virus type in about one every seven seasons. In temperate countries, influenza B epidemics occurred on average three weeks later than influenza A epidemics; no consistent pattern emerged in the tropics. The two B lineages caused a comparable proportion of influenza B cases globally, however B/Yamagata was more frequent in temperate countries, and B/Victoria in the tropics ( $p=0.03$ ). In most countries, B/Victoria patients were significantly younger than B/Yamagata patients. Moreover, influenza B/Victoria cases were distributed according to their age along a unimodal curve peaking at <10 years, while the age distribution of B/Yamagata cases mostly followed a bimodal curve, with a larger peak below 10 years, and a smaller peak between 25 and 50 years of age. A lineage-level vaccine mismatch was observed in >40% of seasons in temperate countries and in 30% of seasons in the tropics.

**Conclusion:** The type B virus caused a substantial proportion of influenza infections globally in the 21<sup>st</sup> century, and its two virus lineages differed in terms of geographical distribution and patients' age. These findings will help inform health policy decisions aiming to reduce seasonal influenza disease burden.

*Keywords: Influenza B, B/Victoria, B/Yamagata, epidemiological characteristics*

**Topic: Public Health: Epidemiology & Transmission**  
**Abstract No: 11088**

### **EFFECT OF MATERNAL PANDEMIC VACCINATION ON SEROPREVALENCE AGAINST INFLUENZA IN CHILDREN AT BIRTH AND AT 4 YEARS**

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**Introduction:** Influenza exposure history through infection and vaccination may influence the immune response to subsequent exposures (“immunological imprinting”). The impact of maternal pandemic influenza immunisation on imprinting in offspring is unclear. The immune response to a novel pandemic virus, or more potent adjuvanted vaccine, may be different to conventional seasonal influenza vaccines. In this study, we aimed to explore transplacentally transferred influenza antibodies at birth and new influenza exposures up to age 4, according to maternal pandemic vaccination status.

**Method:** The Norwegian Influenza Pregnancy Cohort (NorFlu) was established during the H1N1 pandemic in 2009/2010. Blood and questionnaire data were collected from mother-child pairs at delivery and after 4 years (N=255), and exposure data obtained from national health registers. To test for influenza specific antibodies, hemagglutination inhibition (HI) assays were performed against strains that circulated between the pandemic and the second sampling (during 2009-2015).

**Result:** 56% of mothers received a monovalent, adjuvanted pandemic vaccine. At delivery, maternal and cord blood had a high seroprevalence of antibodies against H1N1pdm09, and of antibodies cross-reactive with post-pandemic seasonal H3N2 and influenza B strains. As expected, the H1N1pdm09-specific HI titers were higher after maternal pandemic vaccination compared to no vaccination. At age 4, the seroprevalence was 62%, 67% and 43% against H1N1pdm09, H3N2 and influenza B, respectively. 97% of children had a HI titer  $\geq 10$  against any tested influenza viruses by age 4. Preliminary analysis showed minor differences in seroprevalence at age 4 according to maternal vaccination.

**Conclusion:** Our findings suggest that transplacentally transferred antibodies reflect maternal immune history. Further, these antibodies are cross-reactive to future seasonal strains. Most children had HI titers to any influenza by age 4, reflecting new exposures. Further analysis is on-going to understand the role of maternal pandemic vaccination.

*Keywords: Pregnancy Imprinting Children Seroprevalence Pandemic*



Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11200

## Effect of host genetic polymorphism on transmission of influenza virus infection in a household setting

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### Introduction

Individuals show great variability in susceptibility and disease severity upon influenza infection. The role of host genetic polymorphisms on the transmission of influenza virus infection remained largely unknown.

### Methods

We conducted a genetic association study with a candidate gene approach, based on a household transmission study conducted from 2007-2017. 23 single nucleotide polymorphisms (SNPs) in 15 genes was examined with Sequenom MassARRAY and evaluated with mixed effect logistic and linear regression models.

### Results

2342 subjects from 679 households were studied. Polymorphism OSA1 rs1077467 GT (OR=1.42) and GT/TT (OR=1.37); CCR2 rs1799864 combined AA/AG (OR=1.47) were associated with higher odds of clinical ARI infection. NLRP3 rs1075455 GC (OR=1.60) and GC/GG (OR=1.57) were associated with higher odds; while CCR2 rs1799864 AG (OR=0.36), GG (OR=0.38), combined AG/GG (OR=0.35); IL-8 rs4073 AA (OR=0.52), combined AA/TA (OR=0.68); and CD55 rs2564978 TT (OR=0.53) with lower odds of RT-PCR confirmed influenza infection.

MxA rs2071430 GT was associated with a shorter illness duration of 0.36 days. MxA rs2071430 GG, GG/GT, and IL-1B rs16944 combined AA/GA were associated with fewer symptoms. IL-8 rs4073 combined AA/AT (OR=1.45) was associated with higher odds; while IL-1B rs16944 combined AA/AG (OR=0.59); TLR8 rs3764879 CG (OR=0.39), GG (OR=0.51) and combined CG/GG (OR=0.50); TLR8 rs3764880 AG (OR=0.38), GG (OR=0.50) and combined AG/GG (OR=0.50,) were associated with lower odds of a more serious clinical picture.

For viral shedding, RIG1 rs9695310 CC and CC/CG were associated with a longer, while TLR8 rs3764879 CC/CG and TLR8 rs3764880 AA/AG with a shorter shedding duration. MBL-2 rs7096206 CC, combined CC/CG, and IL-8 rs4073 TA were associated with a higher; and IL-1B rs16944 GG/GA with a lower amount of viral shedding.

### Conclusion

This study identified SNPs affecting disease susceptibility, clinical illness profile and viral shedding pattern, highlighting the role of genetics in affecting influenza transmission in a household setting.

Topic: Public Health: Human Sero-Epidemiology Studies  
Abstract No: 11051

### **Hemagglutinin and neuraminidase antibodies are induced in an age- and subtype- dependent manner after influenza virus infection.**

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**Introduction and Objectives.** Rises in levels of antibodies to influenza virus hemagglutinin (HA) have been the classical serological measure used to detect influenza virus infection. Less is known about the dynamics of the antibody response to the other virus surface glycoprotein, neuraminidase (NA), following infection and whether it is influenced by similar variables as the response to HA. Here, we compared the antibody response to viral HA and NA in two natural-infection human cohorts.

**Methods.** In a serosurvey cohort, pre- and post-influenza season sera from PCR-confirmed influenza cases (N=50) were tested while in an immunology cohort (N=94), paired sera collected at time of PCR confirmation and approximately 14 days later were tested.

**Results.** Seroconversion by HA-inhibition (HAI) assay was detected in 55% (95% Confidence Interval [CI]: 41% - 69%) and 36% (95% CI: 26% - 46%) of the serosurvey and immunology cohort, respectively. Seroconversion by NA-inhibition was 92% (95% CI: 85% - 99%) and 29% (95% CI: 20% - 38%) in the same participants. HAI-assay was more sensitive in detecting infection in those < 20 years old (serosurvey, p=0.01, immunology, p=0.02) for influenza A but not influenza B. The sensitivity of the NAI assay in detecting infection was not influenced by age or virus. Seroconversion to either HA or NA was 100% in the serosurvey and 46% (95% CI: 36% - 56%) in the immunology cohort.

**Conclusion.** Serologic response to only HA or NA appeared to be more common in adults infected with influenza A and in children infected with influenza B viruses.

*Keywords: Influenza; antibody; hemagglutination-inhibition; neuraminidase-inhibition; serology*

Topic: Public Health: Human Sero-Epidemiology Studies  
 Abstract No: 11162

## Antibody response and influenza-like illness among healthcare workers after influenza vaccination

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Annual seasonal influenza vaccination is recommended for health care workers (HCW). However, recent studies of vaccine effectiveness have reported a reduction in serological response among repeat vaccinees. We compared antibody response and the frequency of influenza-like illness (ILI) among HCWs by vaccination history.

A prospective serosurvey of HCWs at the Royal Melbourne Hospital, Australia was performed. HCW received 2016 quadrivalent influenza vaccine. Haemagglutination inhibition (HI) titres were measured pre-vaccination, 21-28d post-vaccination and post-season. HCWs were monitored weekly for ILI during the influenza season and tested for influenza if symptomatic. Geometric mean titres (GMTs) and geometric mean fold ratios (GMRs) were compared between HCWs classified by the number of vaccinations received in the past 5 years. For a subset of 26 vaccine-naïve and highly vaccinated HCWs, HI was extended to include 9 recently circulating cell and egg-grown A(H3N2) antigen pairs, and neuraminidase inhibiting (NI) titres were determined via an enzyme-linked lectin assay.

157/190 HCW completed the study. Titre rises against all vaccine strains were significantly greater among vaccine naïve compared to previously vaccinated HCWs ( $p < 0.001$ ). NI titres against A(H3N2) but not A(H1N1) were also attenuated among previously vaccinated HCWs. GMTs were higher for 6/9 egg-grown H3N2 antigens compared with cell-grown antigens, particularly the vaccine strain, A/Hong Kong/4801/2014 ( $p < 0.0001$ ). Of six HCWs who developed A(H3N2)-positive ILI, all were vaccinated 3+ times, and showed poor response to cell-grown antigen whereas post-infection titres boosted antibodies to all 9 cell and egg-grown antigens, suggesting that infection induces greater breadth and intensity of response than vaccination.

On average, HCWs in our study demonstrated protective antibody titres post-vaccination. However, our findings suggest greater post-vaccination responses among vaccine-naïve HCWs. Repeated vaccination appears to attenuate NI antibody response to A(H3N2), which could contribute to poor vaccine effectiveness against A(H3N2).

*Keywords: healthcare worker; serosurvey; vaccination; antibody response; sero-epidemiology*

Topic: Public Health: Human Sero-Epidemiology Studies  
Abstract No: 11206

## SEROSOLVER: AN OPEN SOURCE TOOL TO INFER EPIDEMIOLOGICAL AND IMMUNOLOGICAL DYNAMICS FROM SEROLOGICAL DATA

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### Introduction and Objectives

Serological data have been invaluable in improving our understanding influenza epidemiology and population immunology. However, interpretation of these data is challenging, as antibody titres depend on the cross-reaction of antibody responses from multiple unobserved prior infections. There is currently a lack of widely available tools to interpret routinely generated influenza serology that account for these phenomena.

### Methods

We developed a Bayesian analysis framework linking latent infection dynamics and antibody kinetics to generate expected antibody titres against multiple strains over time. This was implemented as a flexible, computationally efficient R-package to infer individual infection histories, historical attack rates and key immunological parameters using commonly available serological data. We present two case studies using data from contrasting study designs to answer different questions of epidemiological interest.

### Results

In case study 1, we used *serosolver* to infer influenza A/H1N1pdm09 attack rates in Hong Kong during the 2009/10 outbreak using previously published longitudinal serological samples tested against this virus. We estimated quarterly infection incidence, finding that serology-based incidence agreed with viral-isolate data, but detected a larger second peak. In case study 2, we inferred historical attack rates using previously cross-sectional serological data from China against a panel of historical A/H3N2 strains. We detected age-specific infection patterns in both data sets: individuals were infected less frequently by A/H3N2 as they got older, and the incidence of A/H1N1pdm09 was highest in the 19-64 age group and lowest in the >64 age group.

### Conclusion

*Serosolver* is a flexible, open source tool that may be used to generate biological and epidemiological insights from typical serological survey designs. The package can be easily applied to existing datasets, used to motivate serological study designs, or may be extended to include immunological mechanisms that are important but not yet fully characterised in analyses of human antibody landscapes.

Topic: Public Health: Human Sero-Epidemiology Studies  
Abstract No: 11349

### Risk factors and attack rates of seasonal influenza infection: results of the SHIVERS seroepidemiologic cohort study

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**Background:** Understanding the attack rate of influenza infection and the proportion who become ill by risk group is key to implementing prevention measures. While population-based studies of anti-haemagglutinin antibody responses have been described previously, studies examining both anti-haemagglutinin and anti-neuraminidase antibodies are lacking.

**Methods:** In 2015, we conducted a sero-epidemiologic cohort study of individuals randomly selected from a population in New Zealand. We tested paired sera for haemagglutinin-inhibition (HAI) or neuraminidase-inhibition (NAI) titres for seroconversion. We followed participants weekly and performed influenza PCR for those reporting influenza-like illness (ILI).

**Results:** Influenza infection (either HAI or NAI seroconversion) was found in 321 (35%; 95%CI:32-38%) of 911 unvaccinated participants, of which 100 (31%) seroconverted to NAI alone. Young children and Pacific peoples experienced the highest influenza infection attack rates, but overall only a quarter of all infected reported influenza-PCR-confirmed ILI and one-quarter of these sought medical attention. Seroconversion to NAI alone was higher among children aged <5 years vs. those aged ≥5 years (14% vs 4%; p<0.001) and among those with influenza B vs A(H3N2) virus infections (7% vs 0.3%; p<0.001).

**Conclusions:** Measurement of anti-neuraminidase antibodies in addition to anti-haemagglutinin antibodies may be important in capturing the true influenza infection rates.

**Keywords:** seroepidemiologic cohort, attack rate, hemagglutination inhibition antibody, neuraminidase inhibition antibody, influenza-like illness

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 10466

## ACCESS TO TELEWORK, PAID LEAVE BENEFITS, AND WORK ATTENDANCE IN ADULTS WITH MEDICALLY-ATTENDED ACUTE RESPIRATORY ILLNESS (ARI)

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**Introduction and Objectives.** Workplace contacts play a substantial role in the transmission of influenza. We assessed the association between access to telework, paid leave that could be used for an illness (e.g., paid sick leave, vacation leave, personal time off), and work attendance during the first 3 days following ARI onset.

**Methods.** Participants included workers aged 19-64 years seeking care for ARI at outpatient facilities affiliated with the US Flu Vaccine Effectiveness Network sites during the 2017-18 influenza season. The overall number of days worked was computed by summing days worked at the usual workplace and days teleworked. Negative binomial regression was used.

**Results.** Among 1,362 workers with ARI, 15% reported telework access and 79% reported having paid leave. Compared to workers without access to telework, those reporting telework access worked more days overall while ill (adjusted ratio of overall days worked = 1.28; 95% confidence interval, 1.10-1.48), mainly because of teleworking, but there was no difference in days worked at the usual workplace (Table). Access to paid leave was not associated with days worked overall or at the usual workplace. Participants whose employers discouraged employees with flu-like symptoms from coming to work were less likely to work at their usual workplace while ill (adjusted ratio = 0.83; 95% CI, 0.74-0.94). Results were similar among the subset of 488 participants with laboratory-confirmed influenza.

**Conclusions.** While there are some overall productivity benefits for those who can telework, just having telework and paid leave policies in place may not be sufficient to keep employees from going to their workplace while sick. Liberal business culture with practices that actively discourage employees from coming to work when ill may be necessary to reduce influenza transmission. Our findings pertain to workers with medically-attended ARI and may not be generalizable to persons with non-medically attended ARI.

*Keywords: absenteeism; influenza; paid leave; telecommute; workplace*

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 10910

## Quantifying the effects of school closures on mitigation of influenza epidemics in Hong Kong

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**Background:** School closures are an effective non-pharmaceutical interventions to increase social-distancing and reduce influenza transmission in a population, although they can cause serious social disruption. In Hong Kong, school closures have been used to mitigate seasonal influenza in 2008, pandemic influenza A(H1N1)pdm09 in 2009, seasonal influenza B in 2018, and most recently seasonal influenza A(H1N1) in 2019.

**Methods:** We analyzed surveillance data on influenza activity in Hong Kong during 2008-2019. We estimated transmissibility through the effective reproduction number ( $R_{eff}$ ), assuming the serial interval distribution was a Weibull distribution with mean 3.2 days and standard deviation 1.3 days. We examined changes in transmissibility during the school closure periods. Using multivariate regression model and state-space models, we simulated epidemics with and without school closures to quantify the impact of these closures on peak incidence and overall incidence of infections and hospitalizations. Further we simulated possible counterfactual/experimental scenarios to provide better timing and duration of the school closures.

**Results:** We estimated a 16% (95% CI: 10%, 26%) to 27% (95% CI: 19%, 36%) reduction in transmissibility for four school closure interventions in Hong Kong. The simulated incidence under the counterfactual scenario of no school closures during the implemented school closure, estimating that closures led to a reduction by 4.2% (95% CI 1.5%, 6.7%) to 13.7% (95% CI 8.6%, 17.9%) in the cumulative incidence of infections. There was considerable variation in the impact of closures depending on the timing of implementation (before/around/after peaks).

**Conclusions:** School closures implemented around the peak had higher impact in reduction overall infections compare to after the epidemic peak. Reductions in incidence of infections should have translated to reduced hospitalisations and deaths by a similar fraction, with the caveat that most infections occur in children while most deaths occur in older adults.

**Keywords:** Influenza virus; transmissibility; school closure; statistical models; simulation

Topic: Public Health: Pandemic Preparedness

Abstract No: 10375

## HETEROLOGOUS PRIME-BOOST USING AS03 ADJUVANTED A(H5N1) PANDEMIC STOCKPILED INFLUENZA VACCINES INDUCES BROADER CROSS- CLADE ANTIBODY RESPONSES THAN HOMOLOGOUS PRIME-BOOST

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### Introduction:

Highly pathogenic avian influenza (HPAI) A(H5Nx) viruses continue to pose a pandemic threat. US national vaccine stockpiles are a cornerstone of the influenza pandemic preparedness plans. However, continual genetic and antigenic divergence of A(H5Nx) viruses requires the development of effective vaccination strategies using stockpiled vaccines and adjuvants for pandemic preparedness.

### Methods:

Sera were collected from healthy adults who received either homologous (2 doses of AS03<sub>A</sub>-adjuvanted A/turkey/Turkey/1/2005, A/Turkey at 3.75 ug hemagglutinin per dose), or heterologous (primed with AS03<sub>A</sub>-adjuvanted A/Indonesia/5/2005, A/Indo, followed by A/Turkey boost, at either 7.5 ug or 3.75 ug hemagglutinin per dose) prime-boost vaccination regimens. Pre and post vaccine sera from 105 subjects (N=35 from each of the 3 study groups) were analyzed by hemagglutination inhibition and microneutralization assays against 8 wild-type HPAI A(H5Nx) viruses from 6 genetic clades: A/Turkey (2.2.1), A/Indo (2.1.3.2), A/Vietnam/1194/2004 (1), A/Indonesia/NIHRD-12379/2012 (2.1.3.2), A/Egypt/N04915/2014 (2.2.1), A/duck/Bangladesh/19097/2013 (2.3.2.1a), A/duck/Vietnam/NCVD-1584/2012 (2.3.2.1c), and A/gyrfalcon/Washington/410886/2014 (2.3.4.4). Molecular, structural and antigenic features of the A(H5Nx) viruses that could influence the cross-clade antibody responses were also explored.

### Results:

Compared with homologous prime-boost vaccinations, priming with a clade 2.1.3.2 antigen (A/Indo) followed by one booster dose of a clade 2.2.1 antigen (A/Turkey) administered 18 months apart did not compromise the antibody responses to the booster vaccine (A/Turkey). It also broadened the cross-clade antibody responses to several antigenically drifted variants from 6 heterologous clades, including an antigenically distant A(H5N8) virus (A/gyrfalcon/Washington/410886/2014, clade 2.3.4.4) that caused recent outbreaks in US poultry. The magnitude and breadth of the cross-clade antibody responses against emerging HPAI A(H5Nx) viruses were associated with genetic, structural and antigenic differences from the vaccine viruses, and enhanced by the inclusion of AS03<sub>A</sub> adjuvant.

### Conclusion:

Heterologous prime-boost with AS03<sub>A</sub> adjuvanted vaccines offers a vaccination strategy to utilize existing stockpiled vaccines for pandemic preparedness against new emerging HPAI A(H5Nx) viruses.

*Keywords: Pandemic Preparedness, Heterologous prime boost, A(H5Nx), Highly Pathogenic Influenza viruses*



Topic: Public Health: Pharmaco-economics, Cost-Effectiveness Studies

Abstract No: 10332

## **COSTS AND EFFECTS OF CHILDHOOD INFLUENZA VACCINATION IN THE NETHERLANDS: IMPORTANT RISK OF UNDESIRABLE EFFECTS**

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### **Introduction and Objectives**

Childhood influenza vaccination is expected to reduce the disease burden of influenza at the population level. We aim to assess the cost-effectiveness of childhood influenza vaccination in the Netherlands. To evaluate the long-term costs and effects of such a program, we account for seasonal variation in vaccine effectiveness and a shorter duration of immunity following vaccination as compared to natural infection.

### **Methods**

We use a stochastic dynamic transmission model that has been calibrated to the number of GP-visits with influenza-like illness in the Netherlands over 11 seasons (2003/04 to 2014/15). We analyse the societal costs and effects (measured in quality-adjusted life years [QALYs]) of extending the current influenza vaccination program with vaccinating children aged 2-16 years over 20 consecutive seasons.

### **Results**

Considering outcomes in the entire population, the childhood vaccination program has an average incremental cost-effectiveness ratio (ICER) of €3,944 per QALY gained and will be cost-effective (across 1,000 simulations; conventional Dutch threshold of €20,000 per QALY gained). The childhood vaccination program will not be cost-effective for the target-group itself with an average ICER of €57,054 per QALY gained. Uncertainty analyses reveal that these average ICERs hide a wide range of outcomes. In 23% of the simulations, the childhood vaccination program leads to more (not less) epidemics larger than any observed in the 11 seasons used to fit the model. In 6% of the simulations, the childhood vaccination program causes an overall QALY loss (not gain) in the population.

### **Conclusions**

Childhood influenza vaccination will only be cost-effective in the Netherlands when indirect effects to other age groups are included. In contrast to previous work, and important for policy makers, we show that introducing childhood influenza vaccination leads to an increased frequency of seasons with large epidemics and has a non-negligible risk of causing an overall health loss.

*Keywords: cost-effectiveness, transmission model, childhood influenza vaccination, cost, quality-adjusted life year*

Topic: Public Health: Pharmaco-economics, Cost-Effectiveness Studies  
Abstract No: 10376

## Cost-effective analysis for influenza vaccination coverage and timing in tropical and subtropical climate settings: a modelling study

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**Introduction and Objectives:** The lack of seasonality in influenza epidemics within the tropics complicates vaccination program planning and makes the application of well-established temperate zone national plans challenging. We develop an individual based simulation model to study optimal intra-annual vaccination scheduling for tropical Singapore, assessing the effects of vaccine administration timing and uptake rate. We then assess the differences in cost-effectiveness under no influenza seasonality, and seasonality regimes of increasing strength modelled upon Singapore (i.e. tropics), Taipei (i.e. subtropical) and Tokyo (i.e. temperate).

**Methods:** The simulation models heterogeneities in human contact networks, levels of protective antibodies following infection, the effectiveness of the influenza vaccine and, optionally, seasonality. Using a no intervention baseline, we consider three alternative vaccination strategies: (1) annual and (2) biannual vaccination for a percentage of the elderly (65+yrs) and (3) annual vaccination for all elderly and a fraction of the remaining population. We considered five vaccination uptake rates for each strategy and modelled the estimated costs, quality adjusted life years, and incremental cost-effectiveness ratios (ICERs) indicating the cost-effectiveness of each scenario against a Singapore willingness-to-pay of USD52,961/QALY.

**Results:** In tropical Singapore, annual vaccination for a proportion of elderly are large cost-effective. However, with fixed uptake rates, partial biannual vaccination for the elderly yields a higher ICER than partial annual vaccination for the elderly, resulting in a cost-ineffective ICER. The most optimal strategy is the total vaccination of all the elderly and a proportion of individuals from other age groups, which results in a cost saving ICER. This finding is consistent across different seasonality regimes.

**Conclusions:** Tropical countries such as Singapore can have comparable cost-effective vaccination schemes as those with winter epidemics. The vaccination of all elderly and a proportion of other age groups is the most effective, supporting the need for an extensive national influenza vaccination program.

*Keywords: Influenza; Vaccine; Elderly; Seasonal; Tropics*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 10347

## FluSight: Six Seasons of Forecasting Influenza in the United States, 2013–14 to 2018–19

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**Introduction and Objectives:** The magnitude and timing of influenza activity varies from season to season in the United States and around the world. Accurate and timely infectious disease forecasts could inform the basis of public health decisions and policy development during both influenza seasons and pandemics.

**Methods:** For every influenza season since 2013–2014, CDC's Epidemic Prediction Initiative has facilitated efforts to engage decision-makers and researchers through FluSight, an annual real-time influenza forecasting challenge. These challenges include quantitatively defined forecasting targets, a web application for the submission of weekly forecasts, forecast communication products for public health officials and the public, benchmark forecasts based on the submitted forecasts or historic surveillance data, and post-season evaluation of forecast accuracy.

**Results:** Over six seasons, CDC has received and evaluated forecasts from more than 125 different models produced by 30+ teams for the timing, intensity, and short-term trajectory of influenza-like illness (ILI) activity in the United States. Generally, the top performing models have become more accurate since FluSight's inception and outperform forecasts based on historic data. However, decreases in forecast accuracy were observed during 2017–18, which was a geographically widespread, record-setting high severity season in the United States. FluSight has provided a platform to evaluate novel approaches, including forecasting at the U.S. state level and influenza-associated hospitalizations, the creation of forecast ensembles, and piloting new methods of forecast visualizations. Forecasts are available in real-time at <https://predict.cdc.gov/> and forecast summaries at <https://www.cdc.gov/flu/weekly/flusight/index.html>.

**Conclusions:** FluSight represents an operational disease forecasting system, and FluSight activities have provided the scientific community experience in real-time forecasting; public health officials experience in interpreting and communicating the results; and both groups the ability to evaluate forecast performance across different targets, seasons, and methods. CDC and researchers continue to work together to make further improvements in forecast accuracy and communication.

*Keywords: forecast; predict; model;*

Topic: Public Health: Surveillance & Forecasting

Abstract No: 11184

## Globalization complicated emergence and spread of H3N2 seasonal influenza A viruses

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**Introduction and Objectives:** During the past nearly 50 years, antigenic variants of subtype H3N2 influenza A viruses have frequently emerged, causing significant public health challenges. The manner in which these variants emerge and their patterns of spread are important for vaccine strain selection but not well understood.

**Methods:** By characterizing antigenic evolution of >44,000 H3N2 viruses, we identified 16 antigenic drift events, including 15 antigenic variants with antigenic changes primarily in HA in the H3N2 viruses causing outbreaks during 1968–2016 and one with antigenic changes primarily in NA in the H3N2 viruses contributing to the outbreaks in the 2017–2018 influenza season. A novel Bayesian method was then developed to evaluate the patterns for emergence and spread of these H3N2 viruses.

**Results and Conclusion:** Results suggested that new antigenic variants emerged from certain locations in Asia, Africa, Australia, Europe, and North America rather than being restricted to Asia, and variants emerged year-round and took <2 months to spread across multiple continents. With the growth of global transportation in the past 20 years, emergence and spread of H3N2 viruses have become more dynamic. The uncertainty of the location of antigenic variant emergence and the rapidity of viral spread pose great challenges for influenza surveillance. Our findings suggest that a more robust influenza surveillance strategy, including sampling from underrepresented areas (e.g., tropical locations) and outside established influenza seasons, would help identify the emergence of antigenic variants.

*Keywords: H3N2, antigenic drift, globalization, bayesian, vaccine strain selection*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10311

## VARIATIONS IN SEASONAL INFLUENZA VACCINE EFFECTIVENESS DUE TO BIOLOGICAL CHARACTERISTICS: A SYSTEMATIC REVIEW AND META-ANALYSIS OF TEST-NEGATIVE DESIGN STUDIES

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**Introduction/Objectives:** Test-negative design (TND) study using influenza surveillance data can minimize biases due to health-care-seeking behaviour and case misclassification. We sought to summarize seasonal influenza vaccine effectiveness (SIVE) from TND studies according to study hemisphere, influenza season characteristic, respiratory specimen swab time, and age group.

**Methods:** We systematically searched appropriate bibliographic databases and websites from January 2011–July 2018 for full-text articles from TND studies of SIVE against laboratory-confirmed (PCR/Culture) influenza in primary-care settings during the 2010/11–2017/18 influenza seasons. Two reviewers independently screened retrieved citations against the eligibility criteria using a two-stage sifting approach (screening of titles/abstracts and full-text articles) and extracted data from included studies. Disagreements were resolved by consensus or by a third reviewer. We included only final SIVE estimates. Pooled SIVE was calculated using inverse variance, random-effects model for all–Influenza, H1N1, H3N2, influenza A, and influenza B.

**Results:** Seventy articles met our eligibility criteria. Pooled SIVE was higher in the Southern hemisphere compared with the Northern hemisphere: 55% (CI: 49–60%;  $I^2=0\%$ ) and 40% (CI: 34–46%;  $I^2=78.8\%$ ) for all–Influenza, 66% (CI: 53–75%;  $I^2=0\%$ ) and 51% (CI: 45–56%;  $I^2=55.1\%$ ) for H1N1, 40% (CI: 26–51%;  $I^2=0\%$ ) and 25% (CI: 18–31%;  $I^2=61.7\%$ ) for H3N2, 53% (CI: 29–69%) and 40% (CI: 28–50%;  $I^2=71\%$ ) for influenza A, and 58% (CI: 48–66%;  $I^2=0\%$ ) and 43% (CI: 36–50%;  $I^2=68.1\%$ ) for influenza B. Pooled SIVE across all–Influenza, influenza-types/subtypes differed by vaccine antigenic similarity with circulating viruses (SIVE highest when similar), specimen swab time (higher SIVE with swab time <8days compared with swab time  $\leq 4$ days), and age group (reduction in SIVE with increasing age).

**Conclusion:** The available evidence suggests SIVE estimates from TND studies are influenced by study hemisphere, seasonal influenza vaccine antigenic similarity with circulating viruses, specimen swab time, and participants' age.

**Keywords:** *Test-negative design; Seasonal influenza vaccine; Effectiveness; Systematic review; Meta-analysis*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10385

## COMPARISON OF INFLUENZA ANTIBODY TITERS AMONG WOMEN WHO WERE VACCINATED IN THE 2<sup>ND</sup> AND 3<sup>RD</sup> TRIMESTERS OF PREGNANCIES

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**Introduction and Objective:** Influenza vaccination during pregnancy protects infants during their first few months, but few data are available on optimal timing of maternal vaccination. In Thailand, influenza vaccine is recommended for pregnant women in the second trimester onwards. We compared cord blood titers at delivery in unvaccinated pregnant women and those vaccinated during the 2<sup>nd</sup> and 3<sup>rd</sup> trimesters.

**Methods:** Throughout 2016, pregnant women aged  $\geq 18$  years had cord blood collected at delivery for hemagglutination inhibition assay (HI) using guinea pig erythrocytes against vaccine reference viruses for A/H1N1pdm09, A/H3N2, and B/Phuket. Log-transformed antibody geometric mean titers (GMT) were compared between unvaccinated women and those vaccinated using Student t-test. Proportions of preterm births and those with titers  $\geq 1:40$ , 1:80, and 1:160 were compared using the Chi-squared test.

**Results:** Of 337 women, 215 were unvaccinated and 122 (36%) were vaccinated (2<sup>nd</sup> trimester, n=54 vaccinated at 18-27 weeks gestation; 3<sup>rd</sup> trimester, n=68 vaccinated at 28-37 weeks gestation). Similar proportions of women delivered preterm among unvaccinated women compared to 2<sup>nd</sup> or 3<sup>rd</sup> trimester vaccinated women (8% vs. 7% vs. 3%, p-value=0.31). Cord blood GMTs did not differ between 2<sup>nd</sup> or 3<sup>rd</sup> trimester vaccinated women (A/H1N1pdm09 46 vs. 56, p-value=0.48; A/H3N2 121 vs. 147, p-value=0.38; B/Phuket 70 vs. 83, p-value=0.41), but GMTs were higher for vaccinated women compared to unvaccinated women (A/H1N1pdm09 51 vs. 10, p-value<0.01; A/H3N2 135 vs. 19, p-value<0.01; B/Phuket 77 vs. 15, p-value<0.01). The proportion of women with titers  $\geq 1:40$ ,  $\geq 1:80$ , and  $\geq 1:160$  for the three vaccine reference viruses did not differ between 2<sup>nd</sup> or 3<sup>rd</sup> trimester vaccinated women.

**Conclusions:** Pregnant women vaccinated against influenza had more placental transfer of influenza antibodies to their infants than unvaccinated women but transfer did not differ by vaccination timing. These findings support early influenza vaccination during pregnancy to provide protection to both mother and infant.

*Keywords: Influenza; Vaccination; Pregnancy; Antibody, Thailand*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10390

## Serologic response to sequential influenza vaccination in older adults from a randomized trial

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**Introduction.** Prior vaccination may influence vaccine protection, but studies have not directly compared immunogenicity of sequential vaccination with enhanced vaccines in older adults. We conducted a two-year open label randomized trial to assess immune response to sequential vaccination with high dose (HD-IIV3) and adjuvanted (aIIV3) vaccine.

**Methods.** Adults aged 65-74 years were randomized to receive 2016-17 trivalent standard dose inactivated influenza vaccine (IIV3), HD-IIV3, or aIIV3. HD-IIV3 and aIIV3 recipients received the same vaccine in 2017-18; IIV3 recipients were re-randomized to receive either HD-IIV3 or aIIV3 in 2017-18. Pre- and post-vaccination sera were collected for each dose. Microneutralization (MN) assays were performed using cell-propagated A/Hong Kong/4801/2014 vaccine strain. Hemagglutination inhibition (HI) assays were performed using B/Brisbane/60/2008; H1N1 response was not assessed due to vaccine strain change. Geometric mean titer (GMT) 28 days following vaccination in the second season was compared across study arms using linear regression.

**Results.** 160 were randomized and 153 were included in the two season analysis; 56 received HD-IIV3 both seasons (HD-IIV3-->HD-IIV3) and 58 received aIIV3 both seasons (aIIV-->aIIV). Of 39 who received IIV3 in 2016-17, 19 and 20, respectively, received HD-IIV3 and aIIV3 in 2017-18. Prevacination GMTs were similar across study arms in 2016-17. Postvaccination GMT to A/Hong Kong/4801/2014 in 2017-18 was higher in participants who were sequentially vaccinated with HD-IIV3 or aIIV3 compared to those who received IIV3 in the first year [GMT (95% CI), aIIV3-->aIIV3 95 (70-127) vs IIV3-->aIIV3 34 (20-56),  $p=0.0006$ , and HD-IIV3-->HD-IIV3 74 (55-100) vs IIV3-->HD-IIV3 43 (26-72),  $p=.07$ ]. Postvaccination GMT to A/Hong Kong/4801/2014 in 2017-18 was similar among HD-IIV3-->HD-IIV3 and aIIV-->aIIV recipients ( $p=.26$ ). There were no differences in GMT to B/Brisbane/60/2008 across study arms.

**Conclusion.** Sequential vaccination with HD-IIV3 or aIIV3 over two seasons generated higher titers than a single dose of either vaccine after prior receipt of standard dose IIV3.

*Keywords: High-Dose vaccine, adjuvanted vaccine, sequential vaccination, older adults*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10395

**Vaccine effectiveness against influenza hospitalization in the 2018-2019 season:  
comparison between cell-based and egg-based influenza vaccines**

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**Introduction and Objectives:** It is speculated that low vaccine effectiveness (VE) of egg-based influenza vaccines may be due to mutations in the hemagglutinin protein in viruses passaged in eggs. Cell-based influenza vaccine can be more similar to circulating viruses and potentially more effective.

**Methods:** We conducted a test-negative case-control study to evaluate the VE against influenza hospitalization of cell-based and egg-based vaccines at Kaiser Permanente Southern California (KPSC) during the first half of the 2018-2019 influenza season. KPSC members ages  $\geq 4$  years hospitalized during 10/01/2018-02/15/2019 with an influenza test 14 days prior to 3 days after admission were included. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for receipt of cell-based or egg-based vaccines  $\geq 21$  days prior to admission in cases (positive test result) compared to controls (without a positive test result) were estimated by multiple logistic regression. VE was calculated as 1-OR.

**Results:** Among 207 cases and 4356 controls, 53.1% and 60.5%, respectively, were ages  $\geq 65$  years. Adjusted VE of cell-based vaccine was 46% (95% CI: -28% to 77%) for patients ages  $< 65$  years and -11% (95% CI: -115% to 43%) for  $\geq 65$  years. Adjusted VE of egg-based vaccines was 28% (95% CI: -20% to 57%) for patients ages  $< 65$  years and -6% (95% CI: -78% to 37%) for  $\geq 65$  years. Adjusted relative VE of cell-based versus egg-based vaccines was 24% (95% CI: -76% to 67%) for patients ages  $< 65$  years and -4% (95% CI: -83% to 40%) for  $\geq 65$  years. VE estimates for influenza A followed similar patterns.

**Conclusions:** VE against influenza hospitalization was moderate in the first half of 2018-2019 season for  $< 65$ -year-olds. Cell-based vaccine seems to offer better protection among members  $< 65$  years, but the difference was not statistically significant. Full season data will be available for this meeting in September.

*Keywords: Influenza, Cell-based vaccine, Egg-based vaccine, Hospitalization, Vaccine Effectiveness*



Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10499

**Relative vaccine effectiveness of high dose versus adjuvanted influenza vaccine: a retrospective cohort study**

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**Introduction and Objectives**

Adults 65 years and older (seniors) experience more complications following influenza infection. Two influenza vaccines are licensed in the U.S. exclusively for seniors: a trivalent inactivated high dose (HD) influenza vaccine (Fluzone® High-Dose, Sanofi Pasteur) and a trivalent inactivated adjuvanted (aTIV) influenza vaccine (Fluad®, Seqirus). A recent study reported a propensity score-adjusted 5-way comparison where HD was more effective in preventing influenza-related hospital encounters than aTIV. Propensity score methods only adjust for measured confounders. We estimated the relative vaccine effectiveness (rVE) of HD versus aTIV in seniors using an approach that adjusts for measured and unmeasured time-fixed confounders.

**Methods**

We compared UnitedHealth Group claims data for respiratory hospitalization rates between of HD and aTIV recipients during two influenza seasons: 2016/17 and 2017/18. Subjects were 65 years and older at time of vaccination. We counted the first and any following admission for respiratory disease between two weeks after vaccination and June 30. Rates were adjusted for demographics, comorbid conditions, previous influenza vaccination, and geography. We used the previous event rate ratio (PERR) approach to address bias by time-fixed unmeasured confounders.

**Results**

We identified 842,282 HD and 34,157 aTIV recipients for the 2016/17 season and 1,058,638 HD and 189,636 aTIV recipients for the 2017/18 season. The pooled rVE of HD versus aTIV for hospitalizations for respiratory disease over two seasons was 12% (95% confidence interval: 3.3% – 20%). We estimated an rVE of 13% (-6.4% – 32%) and 12% (2.1% – 21%) for the 2016/17 and 2017/18 seasons, respectively. We cannot fully adjust by insurance plan type; readmission differences may not be vaccine preventable.

**Conclusion**

Pooled over two respiratory seasons, HD was associated with fewer respiratory hospital admissions than aTIV for seniors in the UnitedHealth population in two predominantly A/H3N2 influenza seasons.

This study was funded by Sanofi Pasteur

*Keywords: high dose influenza vaccine; adjuvanted influenza vaccine; vaccine effectiveness*

**Topic: Public Health: Vaccine Effectiveness/Impact**  
**Abstract No: 10561**

## **Repeat vaccination reduces antibody affinity maturation irrespective of influenza vaccine platform in humans**

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### **Introduction and Objectives**

Several vaccines using various platforms have been approved for seasonal influenza vaccination in all age groups that are recommended to be administered every year. The current study was designed to compare the impact of repeat influenza vaccination on immune responses during two consecutive vaccination seasons, 2015-2016 and 2016-2017, following vaccination with three seasonal influenza platforms: egg-based, inactivated vaccine (Fluzone); mammalian cell-based, inactivated vaccine (FluCelvax); and an insect cell-based recombinant hemagglutinin (FluBlok) vaccine.

### **Methods**

In addition to measuring hemagglutination inhibition (HI) titers, samples from all subjects were evaluated for antibody binding to individual HA domains, HA1 and HA2, using surface plasmon resonance (SPR). We also measured antibody affinity maturation against HA0, HA1 and HA2 following vaccination.

### **Results**

Fold change in HI and total antibody binding to HA1 globular heads trended higher for H1N1pdm09 and H3N2 but not against B strains in the group vaccinated with FluBlok compared with FluCelvax and Fluzone. Measurement of antigen-antibody dissociation rates against HA1 domain of H1N1pdm09, H3N2 and B revealed that antibody-affinity maturation occurred after vaccination with all three vaccine platforms against all vaccine strains that correlated significantly with change in HI titers. In contrast, no affinity maturation was observed against HA2 of H1N1pdm09. Importantly, subjects that reported prior year vaccination as well as subjects that had undergone repeat vaccinations in the two consecutive years of this study, demonstrated reduced antibody-affinity maturation to the HA1 domains of all three influenza virus strains irrespective of the vaccine platform.

### **Conclusion**

Our study for the first time identified an important impact of repeat vaccination on antibody-affinity maturation following vaccination, which may contribute to lower vaccine effectiveness of seasonal influenza vaccines.

*Keywords: Repeat vaccination, Vaccine, Antibody affinity, Platforms, Immune response*

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10609

## DOES STRAIN CHANGE INFLUENCE VACCINE EFFECTIVENESS AGAINST INFLUENZA A(H3N2)?

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**Introduction and Objectives:** Studies of vaccine effectiveness (VE) and repeated vaccination have yielded inconsistent results, but negative effects have been reported most often for H3N2. We assessed H3N2 vaccine failure after sequential vaccination with the same vs. different H3N2 antigens in the US Flu VE Network.

**Methods:** Patients with outpatient acute respiratory illness were enrolled during H3N2 dominant seasons with (2011-12, 2017-18) and without (2012-13, 2016-17) a strain change. Circulating viruses were antigenically similar to cell-propagated vaccine strains. Exclusion criteria included unvaccinated or partially vaccinated in current season, and PCR-confirmed H1N1pdm09 or B. Vaccination status was determined from electronic records. The odds of H3N2 vaccine failure was modelled adjusting for season, age, site and other covariates. The primary analysis compared sequential vaccination with different strains vs. same strain (referent).

**Results:** The analysis included 8,760 eligible enrollments. Among 6,156 sequentially vaccinated participants, strain change was associated with vaccine failure (aOR 1.30, 95% CI 1.04-1.63, p=0.02). Strain change was associated with vaccine failure in persons born before 1967 (aOR 1.42, 95% CI 1.17-1.72, p<.001), but not in those born 1967-77 (when only H3N2 circulated) or after 1977 when both H3N2 and H1N1 circulated. In a secondary analysis using current (not prior) season vaccination as the referent group, strain change was also associated with vaccine failure among persons born before 1967 (aOR 1.43, 95% CI 1.13-1.81, p<.001), but not among those born 1967-77. Any sequential vaccination (with or without strain change) was associated with vaccine failure among persons born after 1977.

**Conclusion:** H3N2 strain change was associated with vaccine failure, but the effect size was modest. Most persons born before 1967 were not imprinted with H3N2 in childhood, and they had the highest risk of failure after strain change. These findings may be attributable to residual confounding, but further research is warranted.

*Keywords: effectiveness; H3N2; strain change; sequential vaccination*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10749

## VIRAL SHEDDING IN RECIPIENTS OF LIVE ATTENUATED INFLUENZA VACCINE IN THE 2016/17 AND 2017/18 INFLUENZA SEASONS IN THE UNITED KINGDOM

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### Introduction and objectives

Live attenuated influenza vaccine (LAIV) use in children was suspended in the USA due to an apparent lack of protection against (H1N1)pdm09 viruses in the 2015/16 influenza season. As a result the (H1N1)pdm09 vaccine strain was changed in 2017/18 to improve viral fitness. We conducted LAIV virus shedding studies to assess the effect of this change.

### Methods

Two to 18 year-old children with varying LAIV histories were recruited to receive quadrivalent LAIV in the 2016/17 and 2017/18 influenza seasons. Viruses from nasal swabs taken 1, 3 and 6 days post-vaccination were quantified by real-time reverse transcription-PCR and area under curve titres determined. Presence and quantity of shedding was compared between strains and seasons with adjustment for age and prior vaccination history.

### Results

Influenza B virus detection (positivity) in 2017/18 and 2016/17 (33.9 % and 28.9% respectively for B/Victoria) was greater than that of influenza A, either H3N2 (18.7 and 19.7% respectively) or (H1N1)pdm09 (3.9 and 11.2% respectively), with (H1N1)pdm09 positivity lower in 2017/18 than 2016/17 ( $p=0.005$ ). Within those that shed, H3N2 and influenza B titres were similar between seasons, while the (H1N1)pdm09 titre increased in 2017/18 ( $p=0.02$ ). Positivity rates declined with age and prior vaccination reduced the likelihood of shedding influenza B but not (H1N1)pdm09.

### Conclusions

Increased viral shedding titres imply the 2017/18 (H1N1)pdm09 strain replicates more efficiently in children than the 2016/17 strain and correlates with the high clinical protection observed in UK children. Low rates of (H1N1)pdm09 shedding despite high vaccine effectiveness suggests this is not a suitable correlate of protection.

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10811

## The United Kingdom's childhood influenza vaccination programme: Review of uptake and vaccine effectiveness over the first six seasons

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**Introduction and objectives:** In 2013, the United Kingdom (UK) initiated a childhood influenza national vaccination programme (NVP), starting with children aged 2–3 years in primary care, along with several school-based pilots for children aged 4–11 years. The programme has since expanded to older age groups, through primary care, national school programmes and additional pilots. Here, we review the UK's experience of the programme over six influenza seasons, in terms of vaccine uptake and vaccine effectiveness (VE) of the live attenuated influenza vaccine (LAIV) used in the programme.

**Methods:** Vaccine uptake in children during the programme's first 6 years (2013/14 to 2018/19) was sourced from Public Health England (PHE) for England, Northern Ireland, Scotland and Wales. End-of-season LAIV VE for the 2014/15 to 2017/18 seasons was obtained from PHE and published reports. Interim LAIV VE for 2018/19 was obtained from the I-MOVE in Europe network.

**Results:** The UK's influenza NVP has successfully expanded over the last six seasons to include healthy children. Vaccine uptake ranged from 30.0% to 80.5% in primary care and schools across the UK (**Figure**). Adjusted LAIV VE (95% CI) was 35% (–30, 68) against A/H3N2 and 100% (17, 100) against B in the 2014/15 season, 58% (25, 76), 66% (30, 83) and 27% (–33, 60) against all strains in 2015/16, 2016/17 and 2017/18, respectively. Interim LAIV VE for the 2018/19 season was 80% (–54, 97) against influenza A.

**Conclusions:** The UK's childhood influenza NVP demonstrates that national influenza vaccination programmes for healthy children are feasible and can achieve sustained good vaccine coverage when delivered through primary care and schools. LAIV VE has varied, nevertheless there have been positive individual and community outcomes. From 2019/20, all primary school-aged children will be offered the influenza vaccine as part of the programme.

*Keywords: influenza vaccination; childhood; national vaccination programme; United Kingdom; live attenuated influenza vaccine*

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10881

## INFLUENZA VACCINE EFFECTIVENESS AGAINST LABORATORY-CONFIRMED INFLUENZA MORTALITY IN OLDER ADULTS

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**Introduction and Objectives:** Older adults are at increased risk of mortality from influenza infections. While there is good evidence of influenza vaccine effectiveness (VE) against laboratory-confirmed influenza *infection* in older adults, high-quality evidence of VE against laboratory-confirmed influenza *mortality* is scant. We sought to estimate VE against laboratory-confirmed influenza mortality among adults aged >65 years.

**Methods:** For the 2010-11 to 2015-16 influenza seasons, we conducted a test-negative study using linked laboratory and health administrative databases from Ontario, Canada and multivariable logistic regression to estimate VE against laboratory-confirmed influenza mortality (defined as death from any cause within 30 days of testing positive for influenza). In sensitivity analyses, we corrected for misclassification of influenza vaccination status and we considered death within 7 days and 90 days of testing positive for influenza.

**Results:** Across the 6 seasons, among 6,795 older adults who died within 30 days of specimen collection, 922 (14%) tested positive for influenza and 3,448 (51%) were vaccinated. For the 6 seasons combined, adjusted VE was 20% (95%CI, 7%-30%) against laboratory-confirmed influenza mortality for any influenza, 48% (95%CI, 16%-68%) for H1N1, 29% (95%CI, 10%-44%) for H3N2 and 25% (95%CI, -3%, 46%) for influenza B. These estimates increased to 35% (95%CI, 21%-46%), 68% (95%CI, 52%-83%), 51% (95%CI, 33%-64%), and 25% (95%CI, -15%, 52%), respectively, after correcting for misclassification of vaccination status. We observed significant VE against influenza deaths even during 2014-15 (VE=26% [95%CI, 5%-42%], and 43% [95%CI, 20%-59%] after correcting for misclassification of vaccination status), an H3N2-predominant season with low VE against influenza infection because of vaccine mismatch. Results were similar when considering death within 7 days or 90 days after specimen collection.

**Conclusions:** Influenza vaccination appears to be associated with reduced risk of influenza-associated deaths, even against H3N2, and during a season when the vaccine was mismatched to the circulating strain.

**Keywords:** influenza; mortality; vaccine effectiveness

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10913

## FIRST TRIMESTER SEASONAL INFLUENZA VACCINATION AND MAJOR CONGENITAL MALFORMATIONS: A 2010-2016 UK RETROSPECTIVE COHORT STUDY

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**Introduction/Objectives:** There is good evidence that seasonal influenza vaccination in pregnancy in any trimester provides protection from serious illness in women and their infants, and is safe, but concerns persist especially with first trimester vaccination. Several safety studies of the 2009 monovalent pandemic vaccine exist but the few on seasonal vaccination have been limited in malformation ascertainment and settings. This study explored the safety of first trimester vaccination with respect to major malformations in a UK cohort.

**Methods:** Anonymised, electronic general practice data (the Clinical Practice Research Datalink, CPRD), including a recently developed Pregnancy Register, were used to identify singleton live-birth pregnancies delivered between September 2010 and March 2016. Influenza vaccine exposure was determined using CPRD data. Major malformation ascertainment was maximized by searching for evidence of these outcomes in infants' records in CPRD and linked hospitalisation and death certificate data. Multivariable Cox regression was used to assess the association between vaccination and malformations identified in the first year of life.

**Results:** A total of 78,150 pregnancies were identified. Of these, 6,872 (8.8%) were vaccinated in the first trimester, 11,678 (14.9%) in the second and 12,931 (16.5%) in the third. Overall, 5,707 major congenital malformations were identified. The adjusted HR for vaccination in the first trimester compared to no vaccination was **1.06 (99%CI: 0.94-1.19; p=0.2)**. Results were similar for the second and third trimesters; the adjusted HR for vaccination anytime in pregnancy was **1.02 (99%CI: 0.94-1.10; p=0.5)**.

**Conclusion:** In this large UK cohort, there was no evidence to suggest that exposure *in utero* to seasonal inactivated influenza vaccine, including first trimester exposure, was associated with major malformations.

**Funding:** This research is funded by the National Institute for Health Research Health Protection Research Unit in Immunisation at the London School of Hygiene and Tropical Medicine in partnership with Public Health England.

*Keywords: Influenza; Vaccines; Safety; Pregnancy; Malformations*

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10983

**VIRAL GENOMIC VARIATION AND VACCINE EFFECTIVENESS ACROSS  
 CONSECUTIVE INFLUENZA A(H3N2) EPIDEMICS IN CANADA, 2016-17 AND 2017-  
 18**

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**INTRODUCTION/OBJECTIVES:** The community-based Canadian Sentinel Practitioner Surveillance Network (SPSN) reports influenza vaccine effectiveness (VE) during back-to-back influenza A(H3N2) epidemics in 2016-17 and 2017-18. In 2016-17, the vaccine was updated to newly include an A/Hong Kong/4801/2014(H3N2) clade 3C.2a strain that was unchanged for 2017-18. The egg-adapted version of this vaccine strain bore three amino acid substitutions believed to negatively affect immunogenicity.

**METHODS:** Influenza A(H3N2) was diagnosed by RT-PCR and characterized by Sanger sequencing of the hemagglutinin gene. Sequences were compared to the egg-adapted vaccine and clade distribution was assessed by phylogenetic analysis. VE was assessed by test-negative design based on self-reported vaccination status and adjustment for relevant confounders. Repeat vaccination effects were explored among patients ≥9-years-old.

**RESULTS:** Analyses included 1,940 participants in 2016-17 and 2,358 participants in 2017-18 of whom most (≥60%) were adults 20-64-years-old. In 2016-17, 423/574 (74%) sequenced A(H3N2) viruses belonged to sub-clade 3C.2a1 distinguished by N171K with other substitutions conferring substantial sub-group heterogeneity; only 81/574 (14%) viruses belonged to sub-clade 3C.2a2 distinguished by T131K+R142K+R261Q substitutions. Conversely, in 2017-18, 540/620 (87%) viruses belonged to sub-clade 3C.2a2 and just 39/620 (6%) to 3C.2a1.

In 2016-17, VE against A(H3N2) was 37% (95%CI=20-51%), comparable among recipients of both current and prior season's non-identical vaccines (45%;95%CI=26-59%) and those receiving current season's vaccine only (36%;95%CI=-16-64%). In 2017-18, VE was 14% (95%CI=-7-31%), lower among recipients of both current and prior season's identical vaccines (10%;95%CI=-17-31%) versus current season's vaccine only (46%;95%CI=-4-72%). In 2016/17, VE against dominant 3C.2a1 and minority 3C.2a2 viruses was 51% (95%CI=13-73%) and 35% (95%CI=14-51%), respectively. Conversely, in 2017/18, VE against minority 3C.2a1 and dominant 3C.2a2 viruses was 42% (95%CI=-35-75%) and 16% (95%CI=-7-33%), respectively.

**CONCLUSIONS:** Low VE during consecutive A(H3N2) epidemics in Canada may be explained by the combined effects of viral genomic variation and annual repeat vaccination with unchanged vaccine antigen bearing egg-adaptation substitutions.

*Keywords: influenza; vaccine effectiveness; genomics; A(H3N2); A(H1N1)pdm09*



Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 11047

**BIRTH COHORT-SPECIFIC VACCINE EFFECTIVENESS AGAINST INFLUENZA A(H1N1)PDM09 IN SEASONS WITH DIFFERENT A(H1N1)PDM09 VIRUS VACCINE COMPONENTS, I-MOVE MULTICENTRE PRIMARY CARE STUDY, EUROPE, 2013/14 TO 2018/19**

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**Introduction**

Since 2013, influenza A(H1N1)pdm09 genetic variants with the K163Q substitution in the hemagglutinin glycoprotein predominantly circulated in Europe. Serological data suggest a less effective immune response to these variants in birth cohorts 1965–1979, possibly related to K163 childhood priming. In North America, 2015/16 vaccine effectiveness against A(H1N1)pdm09 among similar age groups was lower.

In 2017/18 the A(H1N1)pdm09 vaccine virus recommendation changed from the post-pandemic A/California/07/09-like strain to the A/Michigan/45/2015-like strain (including Q163).

We use the I-MOVE multicentre primary care test-negative design study to compare birth cohort-specific vaccine effectiveness against A(H1N1)pdm09 (bVE<sub>a</sub>) in Europe pre- and post-vaccine switch.

**Methods**

We combined data from 2013/14, 2014/2015, 2015/16 seasons (pre-vaccine switch) and 2017/18 and 2018/19 (post-vaccine switch).

We categorised birth cohorts as “before-1965”, “1965–1979”, “after-1979”. We calculated pre-and post-vaccine switch bVE<sub>a</sub> with an interaction between birth cohort and vaccination, adjusting for birth year, sex, onset time, season, study and chronic conditions. We used the likelihood ratio test to assess interaction significance. In a sensitivity analysis we measured bVE<sub>a</sub> by season.

### Results

Pre-vaccine switch bVE<sub>a</sub> was 41% (95%CI: 16–59), 49% (95%CI: 23–66) and 38% (95%CI: 22–50) among “before-1965”, “1965-79” and “after-1979” birth cohorts, respectively (p=0.710). Post-vaccine switch bVE<sub>a</sub> was 61% (95%CI: 39–75), 30% (95%CI: -15–57) and 68% (95%CI: 54–77) among “before-1965”, “1965-79” and “after-1979” birth cohorts, respectively (p=0.049). Season-specific sensitivity analyses showed similar results.

### Conclusions

We observed similar bVE<sub>a</sub> in pre-vaccine switch seasons. The switch improved bVE<sub>a</sub> in “before-1965” and “after-1979” birth cohorts, but not in the “1965–1979” birth cohort, resulting in post-vaccine switch differences in bVE<sub>a</sub>.

More research into European and North American childhood priming is needed to understand bVE<sub>a</sub> differences in middle-aged adults. Factors other than previous exposure may be influential, including chance, A(H1N1)pdm09 vaccine virus egg-adaption, previous vaccination and vaccine types used.

*Keywords: Influenza; Vaccine effectiveness; birth cohort; priming; multicentre study*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 11171

## PRIOR INFECTION ENHANCES THE MAGNITUDE AND BREADTH OF ANTI-H3N2 ANTIBODY RESPONSES TO INFLUENZA VACCINATION AND REDUCES THE RISK OF SUBSEQUENT A/H3N2 VIRUS INFECTION

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**Introduction:** Influenza vaccine viruses are frequently updated due to virus evolution, which is most rapid among A/H3N2 viruses. Vaccine effectiveness (VE) is often poor against A/H3N2 viruses and is sometimes further reduced among previously vaccinated individuals. The antigenic distance hypothesis postulates that when successive vaccines are antigenically similar, vaccine is cleared/masked by existing antibodies, whereas the focusing hypothesis postulates that recall and dominance of memory B cells focuses antibodies on limited shared epitopes, both of which have the potential to reduce protection against drifted viruses. It is unclear if antibodies or memory B cells induced by infection could also limit vaccine antibody responses. Our objective was to examine how prior infection affects the magnitude and breadth of A/H3N2-reactive antibodies induced by vaccination.

**Methods:** 100 influenza vaccine-naïve adults from Vietnam who have participated in active influenza surveillance since 2007 received TIV containing the A/Hong-Kong/4801/2014 (HK14) H3N2 antigen in November 2016. Sera were assessed in HI with 34 A/H3N2 viruses spanning 1968 to 2016.

**Results:** 72 vaccinees had been infected with A/H3N2 virus at least once since 2007 and 28 had not. A/H3N2<sup>+</sup> ILI was detected in 5% of vaccinees compared to 1.4% of 421 unvaccinated adults when drifted A/Singapore/0019/2016-like viruses circulated in 2017, including 4 (14%) vaccinees who lacked recent A/H3N2 infection and only 1 (1.4%) vaccinees who had recent infection. Prior infection was associated with higher pre- and post-vaccination titres against the vaccine strain and against past and future A/H3N2 strains. Moreover, titres rose more rapidly and were better maintained among previously infected vaccinees.

**Conclusion:** Prior infection enhances the magnitude, kinetic and breadth of influenza vaccine responses. We postulate that unlike vaccination, infection induced an unfocused B cell response and that vaccination recalled memory B cells against an array of epitopes that were conserved in vaccine, past and future strains.

*Keywords: A/H3N2; vaccine; antigenic distance; antibody focusing; epitope masking*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 11195

## IMPACT OF THE INTRODUCTION OF THE PAEDIATRIC LIVE ATTENUATED INFLUENZA VACCINE (LAIV) PROGRAMME: AN INTERCOUNTRY COMPARISON ACROSS THE UNITED KINGDOM AND THE REPUBLIC OF IRELAND

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### Introduction and objectives

The universal paediatric influenza LAIV programme commenced in the United Kingdom (UK) in 2013/14 and is being incrementally introduced in England and Wales with additional school age cohorts being vaccinated each season. Since 2014/15, in Scotland and Northern Ireland, all primary school age children and all pre-school children (aged 2-4 years) have been offered the vaccine. The Republic of Ireland (RoI) does not have a universal childhood programme. These differences provide an opportunity to compare the population impact of vaccinating children of primary school age in the UK, up to 2017/18.

### Methods

We compare the potential impact of vaccinating primary school age children across the five countries, by evaluating the incidence of influenza using the following indicators; primary care influenza-like illness (ILI) consultation rates, confirmed influenza ICU admissions rates, and all-cause excess mortality rates in both targeted and non-targeted age-groups using standardised methods such as the Moving Epidemic Method (MEM) and the EuroMOMO mortality algorithm.

### Results

Results show that primary care ILI rates in countries (Scotland & Northern Ireland) who offered the vaccine to all primary school age children were lower in the period 2013/14 to 2016/17 and only breached baseline MEM thresholds in 2014/15, in targeted and non-targeted age-group compared to countries (England & Wales) who were incrementally introducing the vaccine or not (RoI) where the primary care ILI rates breached the low/moderate and very high thresholds. These differences were less noted for severe end points, ICU admissions and excess mortality, where no apparent differences were observed pre and post introduction of the programme between countries and during the 2017/18 season which was an exceptionally intense season.

### Conclusion

The findings of this study suggest that the vaccination of primary school age children can provide population-level benefits, in particular in reducing primary care ILI consultations.

*Keywords: Influenza, Vaccination, Live-attenuated influenza vaccine, Children, United Kingdom, Ireland*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10698

## DIFFERENTIAL REGULATION OF POST-TRANSLATIONAL MODIFICATION (PTM) STATUS OF INFLUENZA A VIRAL RIBONUCLEOPROTEINS (RNPs) DURING DIFFERENT STAGES OF THE VIRAL LIFE CYCLE

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### Background:

The RNAs of influenza A virus are encapsidated by viral nucleoprotein (NP) and associated with RNA polymerase subunits (PB2, PB1 and PA), to form a helical nucleoprotein-RNA structure for transcription and replication in the host. Post-translational modifications are crucial for rapid regulation of protein function in human cells. Emerging evidence shows that PTMs occur on NP and are regulated by host machinery to affect viral replication. We investigate the role of viral RNP PTMs in host adaptation of influenza virus.

### Methods and Results:

Comprehensive analysis of the PTM status of RNP proteins was conducted, identified dozens of different PTM events on RNP proteins (NP, PA, PB2), including acetylation, methylation, phosphorylation and ubiquitination. Parallel reaction monitoring quantitation assay was developed on an ultra-high resolution orbitrap mass spectrometry, to compare the relative abundance of each PTM between human- (PB2-627K) and avian-like (PB2-627E) influenza polymerase complexes. Significant differences in the abundance of over a dozen PTM events were observed between the two viral strains. More importantly, a distinct regulation pattern was observed between human- and avian-like strains at different stages during viral replication. Four key PTM sites on NP and PB2 proteins were then chosen for point-directed mutagenesis, to mimic the loss or gain of PTMs on the amino acid residues of viral proteins. Growth kinetics of viruses carrying point-mutations indicated that these mutated PTM sites significantly affected viral replication.

### Conclusion:

This study identified both known and novel PTM sites on RNP proteins that are crucial for viral replication. The differential regulation patterns of PTMs on avian and human viral RNPs are likely to account for the replication efficiency of these viruses in mammalian cells. Distinct PTM patterns at different viral replication stages suggest that particular PTMs on RNP proteins are required for activation of viral transcription and replication machinery.

*Keywords: host adaptation; viral replication; PTM;*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 11313

## Dissecting the mechanism of signaling-induced nuclear export of influenza virus vRNPs

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**Introduction:** Influenza viruses (IV) are nuclear replicating viruses and have to ensure that newly produced viral genomes are transported to the cytoplasm in a temporally controlled manner. While it is long known that IV exploits the cellular Crm1 export pathway, we have shown previously that nuclear export of newly synthesized viral ribonucleoproteins (vRNP) is not a constitutive process but is controlled by virus-induced activation of the cellular Raf/MEK/ERK signaling cascade. The exact mechanism by which the kinase pathway supports vRNP export remained elusive so far.

**Objectives:** The aim of the study was to unravel the different steps of the induced vRNP export process.

**Methods:** STORM high resolution microscopy, cell fractionation experiments, mass spectrometry, reverse genetics to create recombinant virus mutants. Specific kinase inhibitors and siRNA mediated knock-down of target kinases.

**Results:** Raf/MEK/ERK pathway inhibition via MEK-specific inhibitors readily blocks vRNP export but does not generally interfere with the Crm1 export pathway, indicating that viral proteins involved in vRNP formation might be modified. Cell fractionation assays revealed that kinase inhibition lead to a nuclear retention of vRNPs at the chromatin and a reduced binding ability of vRNPs to the viral matrix protein (M1). Mass spectrometry analysis showed that this was due to a missing phosphorylation of two serine residues (S269, S392) within the viral nucleoprotein (NP), which under normal conditions confer the binding to the viral (M1) protein. This has been confirmed by recombinant virus mutants. Furthermore, we could show that the Raf/MEK/ERK effector kinase RSK1 is mediating the export-supporting function and appears to be the direct NP kinase.

**Conclusion:** Our results indicate an essential role of the Raf/MEK/ERK pathway, that results, via activation of RSK1, in NP phosphorylation at S269 and S392, which is needed for binding to M1 and proper assembly of the vRNP export complex.

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 10649

## COMPREHENSIVE MAPPING OF ADAPTATION OF THE AVIAN INFLUENZA POLYMERASE PROTEIN PB2 TO HUMANS

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**Introduction and Objectives:** Viruses like influenza are infamous for their ability to adapt to new hosts. Retrospective studies of natural zoonoses and passaging in the lab have identified a modest number of host-adaptive mutations. However, it is unclear if these mutations represent all ways that influenza can adapt to a new host. Here we take a prospective approach to this question by completely mapping amino-acid mutations to the avian influenza virus polymerase protein PB2 that enhance growth in human cells.

**Methods:** We leveraged deep mutational scanning to measure the functional effects of all single amino-acid mutations to an avian influenza PB2 protein in both human and avian cells.

**Results:** Comparative deep mutational scanning in human versus avian cells identifies numerous previously uncharacterized human-adaptive mutations. These mutations cluster on the surface of the PB2 protein, highlighting potential interfaces with host factors. Some of these mutations are enriched in avian-to-human transmission of H7N9 influenza, demonstrating the utility of our experiments for anticipating PB2's adaptation in nature. However, other new human-adaptive mutations identified here have not yet been observed in nature because they are currently inaccessible by single-nucleotide mutations.

**Conclusion:** Overall, our complete map of human-adaptive mutations sheds light on how selection at key molecular interfaces and evolutionary accessibility shape influenza virus's evolution to new hosts.

*Keywords: PB2; deep mutational scanning; zoonosis; H7N9; pandemic*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 10960

## Mitigating Pandemic Risk with Influenza A Virus Field Surveillance: *Mia* (Mobile Influenza Analysis)

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### Introduction:

The portability of nanopore sequencing has created new possibilities in the investigation of viral outbreaks by allowing on-site and real-time generation of critical genomic information during an outbreak or specific transmission interface. However, the necessity to run complex analyses with high computational power limits the utility of on-site genomic data. We designed a Mobile influenza analysis (*Mia*) platform to both generate and analyze genomic information during a swine influenza virus outbreak while providing an actionable public health response.

### Methods:

Working at a swine exhibition, we collected nasal wipes from 24 swine, purified influenza viral RNA, and amplified the genomes. Following nanopore-sequencing, codon complete genome sequences were assembled and analyzed for 13 viruses within 18 hours.

### Results:

*Mia* successfully sequenced 13 full genomes and identified three swine influenza virus subtypes: 1 H1N1 (gamma lineage), 1 H3N2 (2010.1 human-like lineage), and 11 H1N2 (delta-2 lineage) viruses. On-site analysis of the H1N2 delta-2 viruses revealed >30 amino acid differences compared to the nearest WHO candidate vaccine virus (CVV). Consensus sequences were sent to CDC for synthetic CVV development. Retrospective analysis of other specimens from the same exhibition confirmed that *Mia* identified much of the overall influenza virus diversity at the study site. Further, similar viruses to the H1N2-delta-2 outbreak detected by *Mia*, spread to other swine exhibitions in several states resulting in 13 human infections.

### Conclusion:

We successfully created *Mia*, an inexpensive, portable integrated molecular and bioinformatic system for the analysis of influenza A virus outbreaks and demonstrated its utility in the field. The broad utility of *Mia* extends beyond field surveillance and has practical applications for outbreak response and pandemic preparedness. *Mia* may also help in areas where natural disasters have crippled public health infrastructure. Moreover, the affordability and scalability are well-suited to initiate genome sequencing capacity in small or developing countries.

*Keywords: Nanopore sequencing; Animal human interface; outbreak response*



Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 11179

## FEW SUBSTITUTIONS OF H5 GENE BELONGING TO CLADE 2.3.4 HAVE ALTERED THE NA GENE PREFERENCES OF THE VIRUS OTHER THAN N1

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**Introduction and overview:** Despite the recent outbreak of novel highly pathogenic avian influenza (HPAI) H5Nx virus worldwide, it is largely unknown how HPAI H5N1, which stably maintained its HA and NA combination for more than 10 years, switched the N1 gene for other NAs. The balance between HA and NA protein in influenza A virus has been shown critical for its viral fitness. Thus, any potential imbalance between H5N1's HA and NA may impair it.

**Methodology:** Therefore, we have investigated the molecular changes that abolished the balance between HA and NA of clade 2.3.4 virus resulting in the large outbreak of novel HPAI H5Nx viruses. A NA Selective assay was devised and performed to determine the NA preference by substitutions in HA proteins. We further demonstrated the functional balance between HA and NA through its HA elution, receptor binding affinity and NA enzymatic activity.

**Results:** A cluster of 3 substitutions in HA gene significantly altered the N1 preference of clade 2.3.4 H5 into NA genes including N2, N5, N6, and N8. Furthermore, the changes significantly reformed the receptor binding affinity as well as the NA enzymatic activity indicating the alteration of HA and NA balance of the 2.3.4 virus.

**Conclusion:** Our results represent that a few substitutions in HA gene can alter the NA preference of the virus resulting in the current outbreak of novel emerging 2.3.4.4 H5Nx virus worldwide.

*Keywords: H5Nx; clade 2.3.4; molecular evolution; functional balance;*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 10428

## SPATIAL AND TEMPORAL QUANTIFICATION OF PUTATIVE LUNG REGENERATING CELLS DURING EARLY RECOVERY FROM INFLUENZA PNEUMONIA

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### Introduction

Influenza virus infection may result in infection of the lower respiratory tract, resulting in pneumonia. P63-KRT5-positive distal airway stem cells (DASCs) and proliferating alveolar type II (ATII) cells have been described as the putative regenerating cell types in the lungs, but their roles in pulmonary repair following influenza pneumonia are still unclear. Using a murine model of influenza pneumonia, we quantified the spatial and temporal changes of these cell types in order to elucidate the global processes that occur during infection of the lungs and their subsequent recovery.

### Methods

Female BALB/c mice were infected intra-tracheally with sub-lethal doses of influenza H1N1 PR8 virus. Mice (n=3) were euthanized every two days, starting from 5 days until 25 days post-infection (dpi). The lungs were harvested and stained with hematoxylin and eosin for histopathologic analysis, and stained for the putative stem cell markers via immunofluorescence. For correlation with the cellular changes, the lungs of additional infected mice (n=2) each from 7 and 15 dpi were subjected to proteomic analysis by mass spectrometry.

### Results

DASCs appeared from 7 dpi onwards, branching out from bronchioles before reaching the peak of 20% of the damaged lung area at 21 dpi, and these cells persisted at 25 dpi. No differentiation of DASCs to ATII cells was observed by 25 dpi. However, ATII cells began proliferating from 7 dpi to replenish the population of lung cells. The maximum average numbers of proliferating ATII cells were: 21±1 cells per field in the undamaged lung area (at 13 dpi), 27±19 cells per field in the damaged lung area (at 19 dpi), and 39±3 cells per field in the boundary zone between the damaged and undamaged lung areas (at 13 dpi). Mass spectrometry and gene ontology analysis revealed prominent innate immune responses at 7 dpi, which shifted towards adaptive immune responses by 15 dpi.

### Conclusion

Proliferating ATII cells but not DASCs contribute to ATII cell regeneration, following alleviation of innate immune responses during the early phase of recovery from influenza pneumonia up to 25 dpi.

*Keywords: Influenza, Pneumonia, Stem Cells, Regeneration, Quantification*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
 Abstract No: 10591

## MHC class II proteins mediate cross-species entry of bat influenza viruses

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**Introduction and objectives:** In 2012 and 2013, two novel influenza A-like viral genome sequences have been identified in Central and South America bat specimens and provisionally designated as H17N10 and H18N11. Unlike conventional influenza A viruses (IAV), the hemagglutinin and neuraminidase surface glycoproteins of bat IAV are highly divergent and lack detectable sialic acid binding and neuraminidase activity, respectively. Several lines of evidence suggest that H17 and H18 plays the crucial role in cell entry. However, the putative receptor(s) of bat IAV has not been identified yet. Hence, the specific tropism, potential host range and zoonotic potential of bat IAV could not be fully characterized.

**Methods:** In this study, we performed a genome-wide CRISPR forward screening assay and identified cellular factors that mediate the entry for bat IAV using a susceptible Cas9-expressing human cell line and H18 reporter virus. We further validated the identified receptor using reverse genetic approaches and gain of function experiments.

**Results:** We identified the MHC-II proteins as receptors for bat IAV. We demonstrated that knockout of HLA-DR in bat-IAV susceptible cells blocked the entry of bat IAV, whereas the entry of other viruses was unaffected. The ectopic expression of HLA-DR or HLA-DR homologs from bat, pigs, and chicken in non-susceptible cells conferred susceptibility to bat IAV infection. Remarkably, in mice, bat IAVs robustly replicated in the upper respiratory tract, whereas mice lacking MHC-II were resistant to the infection.

**Conclusion:** We identified MHC-II from multiple species as receptors for bat influenza virus, suggesting their zoonotic potential across species barrier.

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 10740

**REPEATED SEASONAL INFLUENZA VACCINATION RESULTS IN REDUCED PROTECTION AGAINST INFLUENZA A(H3N2) INFECTION IN FERRETS COMPARED TO SINGLE VACCINATION**

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**Introduction and objectives.** Some epidemiological studies suggest that humans who receive repeated annual immunization with influenza vaccine may be less well protected against influenza than are those who receive vaccine in the current season only. We tested this observation using the ferret model of influenza infection.

**Methods.** We vaccinated influenza-naïve ferrets either twice, 10 months apart (Repeated Vaccination group; RV), or once (Current Season only group; CS), with commercial inactivated vaccine containing A/Hong Kong/4801/2014(H3N2) ("HK/4801"). We then challenged these ferrets or naïve controls with cell-grown HK/4801, which is antigenically moderately different from the egg-adapted vaccine virus.

**Results.** Ferrets in the RV group had similar or higher serological anti-influenza titers to both egg-adapted and cell-grown HK/4801. At the time of viral challenge, HI geometric mean titers (GMT) for RV and CS groups were 55.1 and 36.1 (vs egg-propagated HK/4801) and 34.1 and 30.1 (vs cell culture-propagated), respectively. As measured by ELISA, the RV group had significantly higher GMT than did the CS group. However, while ferrets that received either vaccine regimen were protected against influenza disease and infection relative to unvaccinated ferrets, the RV group were less well protected than the CS group. The RV group shed more virus than did the CS group, particularly early after infection. While all three groups lost weight after viral challenge, the CS group began to regain weight after about 5 days post-infection, while the RV and naïve groups continued to lose weight until 7 or 8 days post-infection before starting to recover.

**Conclusion.** Both repeated vaccination, and vaccination in the current season only, protected against influenza disease compared to no vaccination. However, vaccination in the current season was more protective than repeated vaccination, despite having similar or lower antibody titers. Qualitative differences in the antibody response may affect protection after repeated A(H3N2) influenza vaccination.

*Keywords: vaccination;repeated vaccination;immunity;ferret*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 11112

## **A history of obesity reduces the immune response to influenza virus in an NLRP3 dependent manner**

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**INTRODUCTION:** Obesity significantly increases the risk of death following an influenza virus infection. Consistent with these clinical observations, we and others have shown that mice with diet-induced obesity develop much more severe influenza than their lean-fed counterparts. Traditionally, it has been assumed that this increased susceptibility can be reversed by weight loss. However, this remains to be tested experimentally.

**METHODS:** Here, a novel mouse model was developed to study the long-term effects of obesity on anti-viral immunity. Four week old C57BL/6 mice were fed a high fat or lean diet for 10 weeks. After 10 weeks, mice fed a high fat diet had a significantly higher total body weight and percentage body fat compared to mice fed the lean diet. Obese mice were then swapped to a lean diet for 10 weeks.

**RESULTS:** After 10 weeks on the lean diet, mice that were previously obese (PO) had an equivalent body weight and percentage body fat to mice that received the lean diet for the entirety of the 20 week treatment period. However, upon infection with influenza virus (A/Auckland/09(H1N1)), PO mice displayed increased viral replication, inflammation, body weight loss and pulmonary dysfunction compared to lean-fed mice. Cells in the lung lumen of PO mice also had an altered metabolic state compared to those of lean fed mice. Importantly, in mice deficient in the NLRP3 inflammasome, obesity had no long term effect on susceptibility to influenza virus infection.

**CONCLUSIONS:** We propose that obesity can have long-term, NLRP3 dependent, effects on the metabolism of innate inflammatory cells such that they are impaired in their anti-viral response. Understanding the long-term effects that obesity has on anti-viral immunity will help pave the way for the development of novel therapeutics to improve the health of the billions of people who are, or previously have been, obese.

**Keywords:** Diabetes; influenza; glycaemic variability; obesity; oxidative stress

Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 11138

### Obesity increases the cardiac complications of influenza virus infection

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**INTRODUCTION:** Influenza A virus (IAV) causes a wide range of extra-respiratory complications, ranging from central nervous system disease to cardiac complications. However, the role of host factors in the extra-respiratory complications of IAV remains to be defined. Obesity has previously been identified as an independent susceptibility factor for both severe influenza and cardiac disease. We therefore sought to investigate whether obesity increases the cardiac complications of influenza.

**METHODS:** Four week old C57BL/6 mice were fed a high fat or lean diet for 10 weeks. After 10 weeks, mice fed a high fat diet had a significantly higher total body weight and percentage body fat compared to mice fed the lean diet. Mice were then infected with IAV (A/Auckland/1/2009(H1N1)) and disease progression was assessed. The relevance of key experimental findings was confirmed using clinical data.

**RESULTS:** Compared to their lean-fed counterparts, IAV-infected obese mice had elevated levels of creatine kinase-MB, increased cardiac inflammation, reduced cardiac output and increased left ventricular mass. Consistent with these observations, patients with a chronic metabolic disease (the majority of whom had type II diabetes) were significantly more likely to develop cardiac complications after pandemic H1N1 infection (aOR:1.34), even after the data was adjusted for patient age, gender, the presence of chronic pulmonary disease and pre-existing cardiac disease. Transcriptional profiling of the hearts of IAV-infected lean and obese mice showed that obese mice had impaired inflammatory signalling after viral infection. Cardiac disease in obese mice was not associated with a typical hypertrophic expression profile and may instead be associated with the altered inflammatory and/or pulmonary function observed in these mice.

**CONCLUSIONS:** Together, these data provide the first evidence that obesity should be considered as an important risk factor for the development of cardiac complications following IAV infection.

**Keywords:** Influenza; cardiac disease; obesity; heart; extra respiratory complications

Topic: Virology and Pathogenesis: Immune Response to Infection  
 Abstract No: 10471

## MOLECULAR AND FUNCTIONAL DISSECTION OF THE INFLUENZA VIRUS-SPECIFIC CD8<sup>+</sup> T-CELL RECEPTOR REPERTOIRE DURING AGING

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### Introduction

Influenza viruses remain a constant global threat, causing significant morbidity and mortality. Although age is a major factor in determining disease duration and outcome during seasonal epidemic and pandemic outbreaks, the underlying mechanisms that drive age-related changes and disease severity are not well understood. CD8<sup>+</sup> T-cell receptors (TCRs) can recognize conserved regions derived from internal influenza proteins, resulting in broad cross-reactivity across distinct influenza viruses. A robust CD8<sup>+</sup> T-cell response plays an important role in protection against novel influenza virus strains and subtypes, which makes them an attractive target for universal influenza vaccine strategies. As memory CD8<sup>+</sup> T-cells gradually change throughout the human lifetime, we investigated how TCR composition and diversity relate to memory CD8<sup>+</sup> T-cells across immunologically-distinct phases of human life.

### Methods

We combined *ex vivo* peptide-HLA tetramer-associated magnetic enrichment with single-cell multiplex-nested RT-PCR to analyse paired abTCR repertoires directed against the most prominent human influenza epitope, HLA-A\*02:01-M1<sub>58-66</sub> (A2<sup>+</sup>M1<sub>58</sub>) in cord blood, adults and elderly individuals. We compared and contrasted the TCR repertoire dynamics to the magnitude and phenotype of the A2<sup>+</sup>M1<sub>58</sub>-specific CD8<sup>+</sup> T-cell response.

### Results

Our data shows that A2<sup>+</sup>M1<sub>58</sub><sup>+</sup> TCRs in adults differ to those in cord blood and the elderly, both of which have reduced frequencies of the public TRAV27-TRBV19 TCR clonotype, increased proportion of private TCR signatures, broader usage of TRAV-TRBV gene segments, clonal expansion of private TCR clones with shorter/longer CDR3-loops. Furthermore, our study dissects the first *ex vivo* data on paired antigen-specific TCR-ab clonotypes across a 2-9 year time period within the same individual, showing how influenza-specific A2<sup>+</sup>M1<sub>58</sub><sup>+</sup> TCR repertoires change across human life.

### Conclusion

Overall, our findings suggest that priming T-cell compartments at different stages of life, e.g. with T-cell-targeted vaccines, might influence the clonal composition and diversity of responding TCR repertoires against viral infections.

**Keywords:** Influenza; CD8 T cells; TCR; Aging

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10490

## BROADENED CROSS-REACTIVE IMMUNITY IN FERRETS AFTER REPEATED INFLUENZA A/H3 EXPOSURES

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**INTRODUCTION AND OBJECTIVES** Seasonal influenza remains a significant public health concern. Vaccination is the most effective approach to block influenza virus transmission in humans and prevent severe influenza-like illness. However, influenza viruses frequently undergo mutations (particularly in the viral hemagglutinin (HA) protein) resulting in antigenic changes to escape host immunity. Generally, antibodies raised against the highly variable head domain of HA are strain-specific and are limited in capacity to neutralize antigenically drifted viruses. In contrast, immunity directed toward more conserved viral components, e.g. antibodies against the HA stalk region or T cells targeting viral internal proteins, have much broader cross-reactivity and thus are highly desirable for universal vaccine development. The objective of the current study was to profile the humoral and cellular immune responses in ferrets infected with influenza A/H3 viruses and determine how repeated influenza exposures affect the functionality of induced antibodies and T cells.

**METHODS** Seronegative ferrets were infected sequentially with antigenically distant influenza A/H3 viruses. Virus-specific antibodies were analyzed using HA inhibition assay, ELISA, Bio-layer interferometry and antibody-dependent cell cytotoxicity assay. Virus-specific T cell responses were assessed by cell proliferation and ELISPOT assays.

**RESULTS** Although both affinity and avidity of HA-specific antibodies were enhanced by repeated influenza exposures, fewer *de novo* HA head-specific antibodies were elicited. Instead, HA stalk-specific antibodies were gradually enhanced as the number of infections increased. The resultant HA-specific ferret antibodies exhibited broadened cross-reactivity against a wide range of influenza A/H3 viruses. Repeated influenza infections also broadened T cell cross-reactivity toward antigenically distant viruses than single exposure.

**CONCLUSIONS** These results suggest that cross-reactive antibodies and T cells against a broad range of influenza viruses could be elicited via repeated exposures such as natural infections. Further investigations are needed to identify optimal vaccine design strategies to promote antigen-specific immunity with broad cross-reactivity against seasonal influenza.

*Keywords: Seasonal influenza, repeated exposure, HA stalk-specific antibody, ferret, cross-reactivity*



Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10528

## Annotation and Recovery of Ferret-Specific Immunoglobulin Sequences

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### **Introduction and Objectives**

Ferrets are an important model for studying virology, as well as evaluating vaccines against human respiratory diseases such as influenza. Analysis of ferret immune responses, however, are limited to serological or molecular analyses due to the lack of well-validated monoclonal antibodies for flow cytometry and immunohistochemistry. To address the lack of such tools in the field to study B-cell responses in ferrets, we aimed to (1) Identify and Annotate the germline Variable(V), Diversity(D), Joining(J) and Constant(C) immunoglobulin gene segments in the ferret genome (2) Design Ferret-Specific primers to recover and validate the annotated sequences in naive single ferret B-cells (3) To recover HA specific B-cell sequences from influenza infected ferrets.

### **Methods**

Using available human and canine sequences, we identified V, D,J and C gene segments from the Heavy, Kappa and Lambda loci in the draft copy of the ferret genome by sequence homology. Functional sequences were defined as having an ORF as well as functional RSS and Leader Sequences. We subsequently designed Ferret Specific primers targeting the leader and constant regions to amplify and recover paired heavy and light chain sequences from single ferret naive B-cells. To recover HA-specific antibodies from ferrets, we subsequently used a novel murine antibody targeting ferret IgD and tetrameric HA probes to recover HA-specific sequences from infected ferrets

### **Results and Conclusion**

We successfully annotated and validated the germline sequences from the immunoglobulin loci gene segments from single cells using a novel set of ferret-specific primers we designed. Subsequently, we recovered and observed clonally expanded sequences when we sequenced IgD-HA+ single sorted ferret B-cells. This study provides the foundation for a detailed study of B-cell responses in ferrets and the methodology as described will be improved as more ferret specific reagents are developed in the near future.

*Keywords: ferret; reagents; monoclonal antibodies; flow cytometry; recombinant*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 11035

### Innate-like signatures of influenza-specific CD8+ resident memory T cell responses in the human lung

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Resident memory T (Trm) cells confer protection in murine models of influenza but have been little studied in humans. Here we have shown, for the first time, the CD8+ Trm cell-mediated response *in vivo* following human influenza challenge. H1N1(2009)pdm was intranasally administered to 24 healthy adult volunteers, 12 of whom underwent bronchoscopic lower airway sampling at days 0, 7 and 28. Blood and nasal samples were obtained throughout infection in all 24 individuals. Thirteen volunteers (54%) were symptomatically infected while 11 (46%) remained uninfected. Symptoms peaked at day 4 resolving by day 8 post-inoculation.

CD8+ T cells in blood and bronchoalveolar lavage (BAL) were analysed by flow cytometry with tetramer-labelling of influenza-specific T cells. Antigen-specific CD8+ T cells in BAL were significantly enriched compared with blood and predominantly expressed the canonical Trm markers CD69 and CD103. Activation and proliferation peaked at day 7 before contracting to leave an enlarged memory pool. Matched blood and BAL CD8+ T cells were sorted by FACS and analysed by RNA sequencing, with genes known to differentiate naïve and memory T cells removed. This defined differentially expressed genes (DEGs) between anatomical compartments over the course of infection. At baseline, 3928 DEGs were identified (reflecting the marked phenotypic divergence between circulating and tissue populations), with all major known Trm cell markers represented among the most significantly DEGs. However, pathways analysis showed that the most significant enrichment was not in T cell-related modules. Instead transcriptional signatures previously associated with dendritic cells and monocytes were over-represented. These were subsequently validated using selected markers by fluorescent microscopy of bronchial biopsies, thus defining a set of hitherto unrecognised differentiation pathways that may represent novel targets for promoting local cell-mediated immunity against respiratory infection.

Topic: Virology and Pathogenesis: Immune Response to Infection  
 Abstract No: 11216

## SUBDOMINANCE AND DIMINISHED TFH ELICITATION CONSTRAIN HUMORAL IMMUNITY AGAINST THE INFLUENZA HA-STEM

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**Introduction and Objectives:** Influenza infection and seasonal influenza vaccines primarily induce neutralising antibodies against “head” epitopes of the haemagglutinin (HA). There is interest on redirecting immunity towards the conserved HA-stem for broader protection, but stem antibodies are found at low concentrations, with limited increases after immunisation or infection. We sought to elucidate mechanisms driving the establishment and maintenance of immunodominance hierarchies of HA-head and -stem by interrogating B cell and T-follicular helper (Tfh) responses.

**Methods:** HA-specific B cells and Tfh were examined in influenza infection or immunisation of mice, monkeys and humans. B cells were investigated using HA flow cytometric probes and by serological antibodies. Tfh responses were examined by activation-induced marker assays.

**Results:** During influenza infection, the stem domain was immunologically subdominant to the head with poor elicitation of serum antibodies along with low antigen-specific B and Tfh responses. Following immunisation of naïve animals, HA-stem immunogens were poorly immunogenic, with limiting Tfh as a potential constraint to anti-stem immunity. In animals with pre-existing immunity, the poor immunogenicity of HA-stem observed during primary immunisation translated into a diminished capacity to recall stem-specific memory responses. We find that anti-stem responses in primary immunisation or selective recall can be rescued when HA-stem immunogens were linked to the HA-head domain or the keyhole limpet haemocyanin (KLH) protein, both increasing Tfh responses. Finally, we confirm that licensed seasonal vaccines can boost pre-existing memory responses against HA-stem in humans.

**Conclusion:** Our results suggest that fundamental constraints exist at the level of B and Tfh cells to limit HA-stem immunogenicity during infection or immunisation. Strategies that maintain the coupling of head and stem domains might be favourable in boosting HA-stem immunogenicity. A greater understanding of the molecular basis of HA immunogenicity and immunodominance hierarchies is required to guide the design of improved vaccines against influenza viruses.

*Keywords: B cells; Tfh cells; Stem; Immunodominance; HA*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10822

## THE EVOLUTIONARY DYNAMICS OF INFLUENZA A AND INFLUENZA B VIRUSES IN NATURALLY INFECTED HUMAN HOSTS

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**INTRODUCTION:** The evolutionary dynamics of influenza virus ultimately derive from processes that take place within and between infected individuals. These processes have not been well characterized in naturally infected people.

**METHODS:** We used the Illumina platform to deeply sequence upper respiratory specimens collected over seven seasons from individuals enrolled in HIVE, a prospective, community-based household cohort. Within-host diversity was assessed in 249 IAV samples from 200 individuals and 106 IBV samples from 91 individuals. Viruses from 123 households with concurrent infections were used to assess between-host diversity and to estimate the size of the transmission bottleneck. We used an experimentally-validated analysis pipeline to identify intra-host single nucleotide variants (iSNVs) at >2% frequency.

**RESULTS:** For IAV, 243 samples had fewer than 10 minority iSNV (Median 2, IQR 1–3). IBV exhibited lower within-host diversity (Median 0, IQR 0-2). Most iSNV were present at <10% frequency and were only rarely found in more than one individual. The number of iSNV identified was not affected by day of infection, viral load, subtype, or vaccination status. In 49 serially-sampled individuals with IAV, we observed the dynamic turnover of synonymous and nonsynonymous iSNV with little evidence for positive selection. In infected households, we identified 41 sequence-validated transmission pairs for IAV and 15 for IBV. Maximum likelihood optimization of multiple transmission models estimated an effective bottleneck of 1-2 genomes for IAV, which limits the spread of newly arising variants. The IBV bottleneck is also tight, but low within-host diversity precluded an accurate size estimate.

**CONCLUSIONS:** This systematic, large-scale analysis of naturally infected people shows that most hosts exhibit low viral diversity. Consistent with its lower mutation rate, IBV is less diverse. While common on a global scale, within-host positive selection is rare. Limited within-host diversity and tight transmission bottlenecks impose a constraint on influenza virus evolution.

*Keywords: evolution; sequencing; transmission; bottleneck; diversity*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10995

## Predicting evolutionary pathways to 'fit' oseltamivir resistant influenza viruses

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### Introduction

Oseltamivir-resistant influenza viruses arise due to amino-acid mutations in key residues, but these changes often reduce their replication and transmission fitness. While widespread oseltamivir-resistance has not yet been observed in A(H1N1)pdm09 viruses, it is known that permissive mutations in the N1 of former seasonal A(H1N1) viruses from 2007-2009 buffered the detrimental effect of NA/H275Y mutation, resulting in fit oseltamivir-resistant viruses that circulated widely. This study explored two approaches to predict permissive mutations that may enable a fit A(H1N1)pdm09 NA/H275Y variant to arise.

### Method

A computational approach used phylogenetic and *in silico* protein stability analyses to predict potentially permissive mutations, which were then evaluated by *in vitro* NA enzyme activity and expression analysis. The second approach involved the generation of a 'mutant library' of viruses which encompassed all possible individual  $2.9 \times 10^4$  codon mutations in the NA whilst keeping H275Y fixed. To select for variant viruses with the greatest fitness, the virus mutant library was serially passaged in ferrets (via contact and aerosol transmission) and resultant viruses were deep sequenced.

### Results

The computational approach predicted three NA permissive mutations; however they only offset the *in vitro* impact of H275Y by approximately 10%. In our experimental approach, a diverse virus library (97% of 8911 possible single amino-acid substitutions were sampled) was successfully transmitted through ferrets, and sequence analysis of resulting virus pools in nasal washes indicated a high degree of correlation (>90%) across different replicates. We also identified seventeen mutations that were under consistent selection pressure in the presence of H275Y. Further *in vitro* analysis is needed to understand the possible permissive/compensatory role of these mutations for H275Y.

### Conclusion

This study provides valuable insights into the evolution of the influenza NA protein and identified several mutations that may potentially facilitate the emergence of a fit H275Y A(H1N1)pdm09 variant.

**Keywords:** oseltamivir resistance, permissive mutations, H275Y, viral fitness, viral evolution

Topic: Virology and Pathogenesis: Influenza Glycobiology  
Abstract No: 10964

## SEASONAL H1N1 AND ANTIGENICALLY DRIFTED H3N2 INFLUENZA VIRUSES THAT HAVE LIMITED BINDING TO SIALIC ACID BIND TO PHOSPHORYLATED HIGH MANNOSE GLYCANS FROM THE HUMAN LUNG

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**Background:** Influenza A viruses (IAV) bind to sialylated N-glycans, via hemagglutinin (HA), in a species-specific fashion (avian- 2,3-Sia and human- 2,6-Sia). The receptor binding properties have been characterized using erythrocyte agglutination and binding to synthetic receptor analogs, however, the biological significance of these substrates is unknown. To understand what glycan structures are available at the site of infection, and in light of the changing receptor recognition of antigenically drifted H3N2 viruses, our group has pursued shotgun glycomics techniques to identify the glycans from tissue that are bound by IAV.

**Methods:** We generated the human lung shotgun glycan microarray (HL-SGM) and present the first examination of the N-glycome of the total human lung for identifying natural IAV receptors. N-glycans were isolated from homogenized tissue, purified and printed as microarrays for binding assays, including comparative studies of binding after enzymatic treatment and hapten competition. MALDI-TOF mass spectrometry was used to structurally characterize the glycans printed on the arrays.

**Results:** The lung contains complex and high mannose type N-glycans. Both  $\alpha$ 2,3- and  $\alpha$ 2,6-linked sialylated glycans are present. Core fucosylation and polylectosamine chains of varying lengths are also detectable. A panel of ten representative IAV seasonal isolates and vaccine strains bound to sialylated glycans on the HL-SGM. Remarkably, all viruses also bound to phosphorylated, non-sialylated glycans but not through the canonical sialic acid binding site on HA. H3N2 drift strains from 2001 to 2017 exhibit waning Sia recognition following 2004 yet maintain robust phosphorylated glycan recognition regardless of year of isolation.

**Conclusions:** All IAV tested, regardless of subtype, host species, or sialylated glycan recognition, bind to phosphorylated high mannose glycans from the lung. This interaction, which is not impacted by antigenic drift, indicates a potential function for these glycans in processes of viral receptor binding, entry, or assembly.

**Keywords:** Receptor Binding; Human Lung Glycome

Topic: Virology and Pathogenesis: Influenza Glycobiology  
Abstract No: 11089

## INFLUENZA A VIRUS SURFACE PROTEINS ARE ORGANIZED TO HELP PENETRATE HOST MUCUS

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### Introduction and Objectives

Influenza A virus (IAV) enters cells by binding to sialic acid on the cell surface. To accomplish this while avoiding immobilization by sialic acid in host mucus, viruses rely on a balance between the receptor-binding protein hemagglutinin (HA) and the receptor-cleaving protein neuraminidase (NA). Although genetic aspects of HA-NA balance are well-characterized, little is known about how the spatial organization of these proteins in the viral envelope may contribute.

### Methods

We developed replication-competent variants of IAV harboring small peptide tags on five structural proteins, including HA and NA. These tags can be non-disruptively and quantitatively labeled with small-molecule fluorophores, allowing us to measure the composition and spatial organization of individual virus particles. Combining these measurements with super-resolution and time-lapse microscopy, we characterize how the spatial distribution of HA and NA on the surface of individual virus particles influences their dynamics while binding to sialic acid (see figure).

### Results

We find that HA and NA are asymmetrically clustered on the surface of filamentous viruses, with NA strongly enriched at one virus pole. This polarized organization of binding and cleaving activities causes viruses bound to sialic acid (both on surfaces and in mucus gels) to move steadily away from their NA-rich pole (see figure). This directionally-correlated motion increases rates of virus diffusion approximately fivefold. Deletion of the NA cytoplasmic tail abolishes both asymmetric HA-NA distributions, as well as directional correlations in particle diffusion, supporting a link between these characteristics that we model computationally using Brownian dynamics simulations.

### Conclusions

These results demonstrate that filamentous morphology and a polarized spatial organization of HA and NA allows IAV particles to diffuse via a Brownian ratchet-like mechanism that could help resolve the virus's conflicting needs to both penetrate mucus and stably attach to the underlying cells to begin a new round of replication.

*Keywords: Fluorescence imaging, mucus, sialic acid, hemagglutinin, neuraminidase*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10535

## A HUMAN BROADLY CROSS-REACTIVE ANTI-NEURAMINIDASE ANTIBODY PROTECTS AGAINST DIFFERENT SUBTYPES OF INFLUENZA A AND B VIRUSES IN THE MOUSE MODEL

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### Introduction

Neuraminidase is one of the two surface glycoproteins of influenza A and B viruses and plays an important role in the virus life cycle. Antibodies directed against neuraminidase can inhibit its enzymatic activity, thereby interfering with virus replication and have been shown to provide protection *in vivo*. While broadly cross-group reactive, protective human monoclonal antibodies against influenza virus hemagglutinins have been isolated from human subjects, antibodies against neuraminidase with similar breadth have not yet been described.

### Methods

In this study, we characterize three clonally related anti-neuraminidase monoclonal antibodies that were isolated from an individual acutely infected with influenza A virus. The anti-neuraminidase antibodies were analyzed in serological assays including ELISAs, enzyme linked lectin neuraminidase inhibition assays, microneutralization assays and antibody-dependent cell cytotoxicity reporter assays. Additionally, the prophylactic and therapeutic efficacy of the broadly reactive antibodies was assessed in mouse passive transfer-viral challenge experiments. An atomic structure of one of the antibody Fabs was determined in complex with an N2 NA by x-ray crystallography.

### Results

The anti-neuraminidase antibodies exhibited broad cross-reactivity and potent neuraminidase-inhibiting activity *in vitro* across antigenically diverse influenza A and B viruses. One neuraminidase-reactive antibody conferred protection from lethal challenge against all influenza A NA subtype viruses (N1-N9) tested as well as against an influenza B (B/Victoria/2/87-like) virus in a prophylactic setting. The crystal structure of the antibody Fab in complex with an N2 NA provided structural insights for the broad protection.

### Conclusion

These novel, broadly protective anti-neuraminidase antibodies, elicited by natural influenza virus infection, constitute a potential therapeutic resource and further demonstrate the importance of the neuraminidase as an antigenic target. Current influenza virus vaccines mostly induce antibodies toward the hemagglutinin; however, our findings strongly suggest that influenza virus vaccines should be modified to improve the targeting of neuraminidase, which could aid in broad heterosubtypic immunity.

**Keywords:** Neuraminidase; Anti-neuraminidase monoclonal antibodies; Neuraminidase inhibition; Broad protection; Antigenic target



Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 10880

## PRE-EXISTING IMMUNITY TO THE CONSERVED HEMAGGLUTININ STALK OF INFLUENZA VIRUS MAY DRIVE SELECTION FOR AN ESCAPE MUTANT VIRUS IN HUMANS

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### Introduction and Objectives:

The conserved influenza HA stalk has gained much attention as a potent target for universal influenza vaccines. While this strategy has shown potential in different experimental settings, it remains unclear if immune pressure applied to the HA stalk would result in the emergence of escape mutants in humans.

### Methods:

This analysis was performed using samples from an influenza human challenge study where participants were challenged with an influenza virus stock containing a polymorphism in the HA stalk at position 388; Alanine (wildtype) or Valine (mutant). An association was tested between the level of pre-existing anti-stalk immunity and the selection outcomes (388A or 388V) from the study participants. The effect of the A388V mutation on the structure and on the virus was probed. The effect of immune pressure on the mutant selection was investigated using a broadly neutralizing monoclonal antibody (bNAbs) as well as human serum.

### Results:

We found a significant association between a higher level of pre-existing anti-HA stalk antibodies and selection for the mutant virus in humans. The stalk mutation significantly changed the binding epitope for various bNAbs. The mutant virus showed substantially increased resistance to a bNAbs without showing any evidence of decreased viral fitness. Co-culture of the wild type and the mutant virus with a bNAbs resulted in a rapid selection for the mutant virus. Lastly, pooled human serum from the challenge study participants with the mutant virus also selected for the mutant virus in a co-culture setting.

### Conclusion:

This study suggests that pre-existing immunity to the HA stalk may drive selection for an escape mutant virus in humans. This study sheds light on a potentially serious hurdle for developing universal influenza vaccines targeting the HA stalk and calls for further investigation to overcome this hurdle for the success of truly “universal” influenza vaccines.

*Keywords: Influenza; selection pressure; HA stalk; immune-escape*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10980

## RNACTIVE<sup>®</sup>: A PROMISING mRNA BASED INFLUENZA VACCINE

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### Introduction and Objectives

messenger RNA (mRNA) based technologies are increasingly applied in vaccine development. RNActive<sup>®</sup>, an mRNA based vaccination technology developed by CureVac, which uses sequence-optimized mRNA, provides a powerful new platform for prophylactic vaccines. RNActive<sup>®</sup> vaccines have achieved promising results against a variety of viral pathogens such as influenza, rabies, rotavirus and RSV in several animal models. Specific advantages of this platform for influenza include the possibility to rapidly adapt to newly circulating strains, a fast production process as well as inclusion of further protective antigens providing broader and longer-lasting protection.

### Methods

RNActive<sup>®</sup> vaccines encoding for influenza virus hemagglutinin (HA) were formulated in lipid nanoparticle (LNP) and tested in different species (e.g. mice, ferrets, non-human primates (NHP)) for immunogenicity and/or protection upon intramuscular vaccine administration.

### Results

Vaccination of mice with RNActive<sup>®</sup> vaccine encoding for influenza HA led to the induction of both, humoral and cellular immune responses. In mice doses below 1 µg of the HA RNActive<sup>®</sup> vaccines elicited functional antibody responses associated with protection. Further experiments demonstrated that the vaccine was able to induce potent and long lasting immune responses against influenza HA in NHP following intramuscular administration. A multivalent cocktail RNActive<sup>®</sup> vaccine containing influenza HA encoding mRNAs induced immune responses to all components of the vaccine in mice. A quadrivalent influenza HA-based RNActive<sup>®</sup> vaccine protected ferrets upon challenge infection with either Influenza A or B virus.

### Conclusion

LNP-formulated multivalent influenza RNActive<sup>®</sup> vaccines are promising next generation vaccines that can address the limitations of currently licensed vaccines. In the context of Influenza addition of further protective antigens as well as increasing the antigenic breadth covered by encoding additional HAs from circulating strains might increase protection against vaccine mismatch.

*Keywords: mRNA, antigenic breadth, protection, challenge*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11110

## Intranasal M2SR (M2-deficient Single Replication) Influenza Vaccine Induced Protection Against Challenge with a Substantially Drifted H3N2 Virus in a Phase 2 Study

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**Introduction:** Protection against diverse influenza strains is a necessary characteristic for a universal influenza vaccine. Demonstration of such protection by an M2SR H3N2 vaccine was assessed in a phase 2a clinical trial in which the challenge virus was substantially drifted from the vaccine. M2SR is an investigational, intranasal (IN) live virus vaccine that undergoes only a single round of infection in the respiratory epithelium, evokes an immune response profile similar to wild-type influenza viruses, and protects ferrets against both homologous and heterologous influenza strains.

**Methods:** A phase 2a human influenza challenge study (EudraCT: 2017-004971-30) was conducted at SGS in Belgium. M2SR contained HA and NA from A/Brisbane/10/2007 (H3N2). Adults, 18-55 years old, were randomized 1:1 to receive either a single dose of placebo or vaccine. Four weeks later, participants were challenged with H3N2 A/Belgium/4217/2015, and assessed for safety, infection and symptoms.

**Results:** Adverse events and reactogenicity were similar between placebo and M2SR recipients following immunization. After challenge with A/Belgium/4217/2015, 35% of vaccine recipients experienced influenza infection and illness, compared to 49% of placebo recipients. An 18% reduction in viral load was noted after challenge of vaccine recipients. Vaccine recipients with serum microneutralization response to vaccine (54%) demonstrated 34% reduction in viral shedding and 51% reduction in symptom scores. Among the 29% of persons with post-vaccine antibody response to both vaccine and challenge HA antigens, 62% reduction in viral load (P=0.0211) and 56% reduction in symptom scores was noted after challenge.

**Conclusion:** A single IN dose of M2SR protected healthy adults against infection and illness from a highly drifted H3N2 challenge influenza strain. This is believed to be the first human challenge study to demonstrate protection against challenge with a substantially drifted influenza strain and indicates the potential for M2SR to provide improved breadth of protection compared to currently licensed vaccines.

*Keywords: challenge; drifted; H3N2; intranasal; vaccine*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11170

## Characterising human memory B cells with cross-lineage recognition of the influenza B hemagglutinin following seasonal immunisation

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**Introduction and Objectives:** Influenza B viruses (IBV) account for a significant proportion of influenza-related hospitalizations, particularly in young children, yet are relatively understudied compared to influenza A viruses. Given the restricted host range and limited antigenic diversity, IBV infection could be significantly reduced, or even eliminated, through effective vaccines. However, the ability of human B cells to generate antibodies with inter-lineage specificities, and the capacity of seasonal vaccines to elicit such responses is not well understood.

**Methods:** We developed novel IBV HA probes to interrogate IBV-specific memory B cells in humans. Serological and B cells responses were analysed in two clinical cohorts, one receiving seasonal trivalent (2016) and one receiving seasonal quadrivalent (2017) inactivated vaccines.

**Results:** A significant proportion of IBV HA-specific B cells expanded following vaccination could recognize both B/Victoria/2/87-like and B/Yamagata/16/88-like lineages in a distinct pattern of cross-reactivity. Cross-reactive B cells showed greater expansion in subjects receiving a quadrivalent vaccine. A panel of monoclonal antibodies (mAbs) was reconstituted from sequenced IBV HA-specific B cell receptors, including multiple mAbs capable of providing broad protection in murine models of lethal IBV infection. Protection was mediated by broadly-neutralizing antibodies targeting the receptor binding domain, or alternatively via Fc-mediated functions of cross-reactive antibodies binding alternative non-neutralizing epitopes including sites within the HA stem region. Mutational studies showed lineage-restricted and broadly cross-reactive antibodies with HI-activity *in vitro* bound the IBV HA similarly.

**Conclusions:** The human B cell response to IBV contains extensive antigenic cross-recognition of both IBV lineages. Cross-reactive monoclonal antibodies confirm the protective potential of such responses. This and similar studies provide critical guidance for the rational design of vastly improved IBV vaccines for broad and durable protection.

*Keywords: B cell, immunology, memory, influenza B*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 11735

**Immunogenicity of chimeric hemagglutinin-based universal influenza virus vaccine candidates: interim results of a randomized, placebo-controlled, phase 1 clinical trial**

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**Introduction and Objectives:** Influenza viruses cause significant annual morbidity and mortality globally. Current vaccines protect against influenza only when well matched to the circulating strains. However, antigenic drift can cause considerable mismatches between vaccine and circulating strains, substantially reducing vaccine effectiveness. Moreover, current vaccines are ineffective against pandemic influenza until they are remade against the pandemic virus strain, requiring months. Therefore, there is an unmet medical need for a broadly protective influenza virus vaccine.

**Methods:** We conducted a randomized, observer-blinded multicenter phase I study in healthy adults to test the ability of H1 chimeric hemagglutinin-based universal influenza virus vaccine candidates to induce broadly cross-reactive antibodies targeting the stalk domain of group 1 hemagglutinin-expressing influenza viruses. Vaccine regimens tested included, i) a group receiving a chimeric H8/1 hemagglutinin (cH8/1)-based live attenuated vaccine (LAIV) followed by a boost with a non-adjuvanted chimeric H5/1 hemagglutinin (cH5/1) based inactivated vaccine (IIV), ii) the same regimen but with the IIV being adjuvanted with AS03 and iii) a prime-boost regimen including an adjuvanted cH8/1 IIV prime followed by an adjuvanted cH5/1 IIV boost. A planned interim analysis assessed anti-H1 hemagglutinin stalk, anti-H2, anti-H9 and anti-H18 IgG antibody titers as well as plasmablast and memory B cell responses in peripheral blood.

**Results:** The adjuvanted IIV, but not the LAIV, induced a significant serum IgG antibody response after the prime with a 7-fold increase in anti-H1 stalk titers. Post-boost, all vaccine regimens induced detectable H1 stalk (2.2-5.6 fold induction over baseline), cross-reactive serum IgG antibody and peripheral blood plasmablast responses.

**Conclusion:** The chimeric hemagglutinin-based universal influenza virus regimens tested elicited broadly-reactive serum IgG antibodies that target the conserved hemagglutinin stalk domain.

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 10108

## THE BAT INFLUENZA H17N10 CAN BE NEUTRALIZED BY BNMABS AND ITS NA FACILITATES VIRAL EGRESS

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### Introduction and Objectives

The diversity of subtypes within the Influenza A virus genus has recently expanded with the identification of H17N10 and H18N11 from bats. In order to further study the tropism and zoonotic potential of these viruses, we have successfully produced lentiviral pseudotypes bearing both haemagglutinin H17 and neuraminidase N10.

### Methods

Pseudotype production: Transfection of HEK293T/17 cells was performed using a variety of combinations of plasmids pl.18-H17, pl.18-N10, p8.91 and pCSFLW using polyethylenimine transfection reagent. Pseudotype entry into cell lines and neutralization assays were performed in 96 well plates and evaluated by luciferase readout on a Glomax 96.

### Results

These pseudotypes were shown to be efficiently neutralized by the broadly-neutralizing monoclonal antibodies CR9114 and FI6. Our studies also confirm previous reports that H17 does not use sialic acid as its cellular receptor, as pseudotypes bearing the H17 envelope glycoprotein are released into the cell supernatant in the absence of NA. However, we demonstrate that N10 facilitates heterosubtypic (H5 and H7) influenza HA-bearing pseudotype release in the absence of another source of NA, significantly increasing luciferase pseudotype production titres. Despite this, N10 shows no activity in the enzyme-linked lectin assay used for traditional sialidases. These findings suggest that this protein plays an important role in viral egress, but is perhaps involved in further accessory roles in the bat influenza lifecycle that are yet to be discovered.

### Conclusions

We show the lentiviral pseudotype system is a useful research tool, and amenable for investigation of bat influenza tropism, restriction and sero-epidemiology, without the constraints or safety issues with producing a replication-competent virus, to which the human population is naïve.

*Keywords: Bat influenza, H17N10, pseudotype, neutralization*

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 10355

### **Low literacy program for safe slaughter of poultry in developing countries to reduce human infection with avian influenza virus**

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In developing countries, H5 highly pathogenic avian influenza (HPAI) viruses have caused severe and sometimes deadly zoonotic infections through exposure to infected poultry during slaughter at home or in live-poultry markets. Bioaerosol samplers detected airborne infectious virus during laboratory-simulated processing of asymptomatic infected chickens and ducks. Naive chickens and ferrets exposed to the same air space as the slaughter of infected chickens became infected and died. Exposure of ducks and ferrets to the same air space as processing of infected ducks inconsistently produced infections. A mitigation strategy was developed, specifically designed for a low-literacy audience. A behavior change communication campaign using posters and handouts was nationally applied in Egypt. The mitigation process was illustrated in an ordered sequence of simple colorful pictures. The mitigation targeted capturing the viral plume created by the slaughter and death struggle inside a covered container. The illustrations use a "halla", the domestic cooking pot that is a common kitchen implement in Egypt, but any other suitable container with a cover can be used. The bird is slaughtered, immediately dropped into the container which is then immediately covered and kept closed till the death struggle is completed. The cover then is partially pushed horizontally and the scald water is poured to precipitate and inactivate the virus particles. The goal was to reduce airborne virus, thus reducing or preventing the human exposure and any potential infection. Boiling the inedible viscera in the scald water was requested to ensure safe waste disposal. Audience meetings were applied by the governmental veterinarians in health units, veterinary units, feed stores, agricultural units, agricultural equipment distributors, churches, mosques, schools, and youth units.

*Keywords: avian influenza, mitigation, non-pharmaceutical intervention, transmission, zoonoses*

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 11198

## IMPROVING AVIAN INFLUENZA SURVEILLANCE THROUGH WETLAND SAMPLING

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**Introduction and objectives:** Avian influenza virus (AIV) outbreaks in poultry across the globe have resulted in significant poultry mortality and identification of AIV is critical for rapid detection of viruses that could spillover into humans to cause devastating pandemics. A One Health approach recognizes that human, animal, and environmental health are linked and a comprehensive influenza surveillance program can improve the health of each species. We aim to develop a more efficacious AIV surveillance system from environment samples to replace the current labour-intensive live-bird capture surveillance, which failed to detect the 2014/2015 AIV outbreak in British Columbia, Canada.

**Methods:** Using farm and wetland sediment as a population-level proxy of AIV in waterfowl, we collected and extracted total RNA from 345 samples. Due to AIV RNA being rare in environmental samples, we tested a commercial probe-based targeted resequencing (TR) method using AIV specific probes to enrich for AIV genomic sequences. TR results were compared to the standard method of MP gene RT-qPCR.

**Results:** The TR approach was able to capture numerous influenza viruses within a single sample; in total we detected 13 HA (H1-H7, H9-13, H16) and nine NA (N1-9) subtypes, MP, PB2, and PA. Influenza was detected by RT-qPCR in 42/345 (12.2%) samples and by TR in 65/345 (18.8%) samples. We are exploring sources of disparity including complex environmental factors (soil type, pH, *E.coli* concentration, etc.).

**Conclusion:** The commercial TR method detected a wide diversity of influenza from sediment samples, corroborating the belief that wetlands could be the reservoir to facilitate AIV reassortment. This environmental surveillance strategy can be used to design a more efficient early-warning plan for potential AIV outbreaks. We are developing a customizable and flexible TR probe panel targeting pan-flu to cover influenza pandemic threats.

*Keywords: avian influenza virus; targeted resequencing; influenza surveillance, one health*



Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 11253

## Repeated crow (*corvus splendens*) mortality events linked to H5N1 influenza virus circulation in live bird markets, Bangladesh

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### INTRODUCTION AND OBJECTIVES:

H5N1 avian influenza caused >550 reported outbreaks in poultry and wild birds in Bangladesh since 2007. Multiple crow mortality events occurred during the winter season (Nov-March) between 2016- 2018 within the same areas of Dhaka and Rajshahi in Bangladesh. A One Health approach was used to investigate the crow mortalities, identify the etiologic agent, assess the extent of the outbreak, and identify the possible source of infection.

### METHODS:

Cloacal and oropharyngeal swabs were collected from moribund and dead crows (N=375) and offal and environmental samples from nearby live bird markets (LBMs; N=430). All samples were tested using a pan-influenza A consensus PCR assay as well as specific q-PCR for influenza A (M gene) H5/H7/H9/N1/N6.

### RESULTS:

The team observed crows feeding on poultry offal and waste in neighboring LBMs. Of the total, 61% (n=228) of crows and 22.6% (n=97) of LBM samples tested positive for H5N1. 20.5% (n=77) of crows and 15% (n=64) of LBM samples tested positive for H5Nx. Phylogenetic analysis based on a partial sequence of the HA gene of H5N1 subtypes suggests that the strain found in the crows is similar to the Bangladeshi 2.3.2.1a clade that circulated in 2011.

### CONCLUSIONS:

The findings suggest that multiple subtypes of H5 influenza viruses are circulating in LBMs without mortality in poultry. The virus may have been transmitted to crows while they were feeding on poultry waste in the LBMs. But when the virus spilled over to crows it become pathogenic and causes mortality creating a dead-end host. Crows may play a role in transmission between LBMs or to people. Improved LBM biosecurity measures are needed to reduce the risk of influenza virus spillover to wild birds or people in Bangladesh.

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 11794

## CHARACTERIZING THE FUNCTIONALITY OF THE WUHAN SPINY EEL INFLUENZA VIRUS SURFACE GLYCOPROTEINS

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In a study to assess the diversity of RNA viruses in vertebrates, a panel of novel influenza viruses were documented in jawless fish, ray-finned fish, and amphibians. Of these, the Wuhan Spiny Eel Influenza Virus (WSEIV) was specifically found to phylogenetically cluster with influenza B viruses as a sister clade. Influenza B viruses historically have been documented to circulate only in humans, with certain virus isolates found in harbour seals. This is in stark contrast to influenza A viruses which have a broad avian and mammalian host tropism. Analyses of the surface hemagglutinin (HA) and neuraminidase (NA) glycoproteins of the WSEIV could provide valuable insight into understanding the corresponding proteins of influenza B viruses. To do so, the HA and NA of the WSEIV were recombinantly expressed using a baculovirus expression system, and virus like particles expressing the same were produced. To probe the degree of conservation of target epitopes, binding of known broadly cross-reactive monoclonal antibodies targeting the influenza B HA and NA, respectively, were assessed through enzyme linked immunosorbent assays against recombinant WSEIV glycoproteins. Upon functional characterization of the NA, we identified that the WSEIV NA-like protein indeed has sialidase activity when tested in an NA-STAR assay at levels comparable to B/Malaysia/2506/2004 influenza B virus. For the HA, the extent of cross-reactivity of hemagglutination inhibition (HI) responses induced by WSEIV VLP vaccination in mice were determined to provide insight into the conservation of the receptor binding site of the HA. Human serum samples of patients previously infected with influenza B viruses were also used to determine the cross-reactivity against these novel glycoproteins. The receptor target specificity and affinity of WSEIV HAs will also be determined through glycan microarray base technologies to further study the presence of this virus in Spiny Eels. Overall, we have completed a preliminary functional characterization of the novel WSEIV in order to show that it is indeed a bona fide influenza virus remarkably found in ray-finned fish.

**Introduction and Objectives:** In a study published by Shi and colleagues (Shi *et al.*, Nature, 2018) to assess the diversity of RNA viruses in vertebrates, a panel of novel influenza-like viruses were documented in jawless fish, ray-finned fish, and amphibians. Of these, the Wuhan Spiny Eel Influenza Virus (WSEIV) was specifically found to phylogenetically cluster with influenza B viruses as a sister clade. Influenza B viruses historically have been documented to circulate only in humans, with certain virus isolates found in harbour seals. This is in stark contrast to influenza A viruses which have a broad avian and mammalian host tropism. Analyses of the surface hemagglutinin (HA) and neuraminidase (NA) glycoproteins of the WSEIV could provide valuable insight into understanding the corresponding proteins of influenza B viruses.

**Methods:** To do so, the HA and NA of the WSEIV were recombinantly expressed using a baculovirus expression system, and virus like particles expressing the same were produced in mammalian cells. To probe the degree of conservation of target epitopes, binding of known broadly cross-reactive monoclonal antibodies targeting the influenza B HA and NA, respectively, were assessed through enzyme linked immunosorbent assays against recombinant WSEIV glycoproteins. Human serum samples of patients previously infected with influenza B viruses were also used to determine the cross-reactivity against these novel glycoproteins.

**Results and Conclusions:** Upon functional characterization of the NA, we identified that the WSEIV NA-like protein indeed has sialidase activity when tested in an NA-STAR assay at levels comparable to B/Malaysia/2506/2004 influenza B virus NA, making it a bona fide neuraminidase. Testing of the functionality of HA is currently ongoing. In summary, we are conducting a functional and antigenic characterization of the glycoproteins of the novel WSEIV in order to assess if it is indeed a bona fide influenza virus circulating in ray-finned fish.

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 10606

## NEW GENOMIC APPROACHES TO UNDERSTAND THE HETEROGENEITY OF VIRAL REPLICATION IN SINGLE CELLS

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### Introduction and Objectives

At the single-cell level, viral infection is extremely heterogeneous. Recent advances in sequencing have made it possible to study the transcriptomes of individual, virus-infected cells, and these studies have shown extensive heterogeneity at the transcriptional level. However, it remains unclear how variation in *viral transcription* within cells relates to the production of *infectious progeny virions*--which are ultimately the critical unit for understanding viral evolution.

### Methods

To address this need, we have developed new methods to study progeny virion production in single cells. We insert a 16 bp barcode into the influenza genome, which uniquely identifies each virion in an experimental sample. These barcodes are maintained during replication. The progeny of each virion are quantified in the viral population using deep sequencing. Simultaneously, we sequence the transcriptome of each infected cell and the complete genome of the virion that infected each cell.

### Results and Conclusion

Using this approach, we measure (1) how many replication-competent progeny each infected cell contributes to the viral population, (2) the transcriptome of each infected cell, and (3) the sequence of the virion that infected each cell. These data are analyzed to discover host and viral factors that regulate the production of fully-infectious progeny.

*Keywords: single-cell; sequencing; progeny; lineage tracing; replication*

Topic: Virology and Pathogenesis: Viral Replication

Abstract No: 10843

## **ANP32 PROTEINS FROM DIFFERENT MAMMALIAN SPECIES ACT AS HOST RANGE BARRIERS AND SHAPE INFLUENZA POLYMERASE ADAPTATION.**

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Although the natural reservoir host of influenza A viruses is wild birds, the virus can adapt for stable circulation in mammalian species. One key adaptation occurs in viral polymerase to utilise the truncated forms of ANP32 proteins found in mammals. Here we investigate the role different mammalian ANP32 proteins play in polymerase adaption and as species barriers in non-human mammalian species.

We utilised a human cell line lacking endogenous ANP32A and B proteins to investigate the ability of range of mammalian ANP32 proteins to rescue replication of polymerase constellations from different influenza viruses. We also assessed the compatibility of ANP32 proteins from different species with a range of mammalian-adapting PB2 mutations introduced to an avian influenza polymerase and the mechanism for this interaction.

Mammalian ANP32 A and B proteins differ in their potency to support influenza polymerase activity. The pattern of interactions can partially explain different patterns of adaptation seen in different mammalian species. For example, we find that swine ANP32A can partially support 'non-adapted' avian polymerases, potentially allowing this host to be more readily infected by these viruses, and that viruses that have evolved in swine show a strong ability to co-opt this factor. In addition, several well known mammalian adaptations, including PB2-E627K and -Q591R, specifically shift ANP32 preference towards ANP32B across multiple mammalian species.

We conclude that species specific difference in ANP32 proteins may drive divergent host adaptations in these species. Furthermore, we hypothesise swine may act particularly well as evolutionary intermediates due to possessing an ANP32A that is partially permissive to avian viruses while simultaneously being fully supportive to human adapted influenza polymerases. Overall this work helps assess the relative zoonotic threat posed to humans by different mammalian influenza viruses while also giving insight into the function and specificity of ANP32 proteins in influenza virus replication.

*Keywords: Influenza, ANP32A, ANP32B, polymerase, adaptation*

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission  
Abstract No: 10440

## AUTOPHAGY-MEDIATED RESTRICTION OF AVIAN INFLUENZA VIRUS REPLICATION IN MAMMALIAN CELLS

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### Introduction and Objectives:

Adaptive mutations in avian influenza A viral polymerase subunits are critical for cross species infection and mammalian adaptation, but mechanistic details of this process are not fully understood. We present evidence to demonstrate that mammalian host cells utilize autophagy to restrict avian influenza virus replication.

### Methods:

To explore the molecular basis of adaptive mutations in avian influenza virus polymerase, intracellular trafficking of vRNPs in influenza virus infected cells was examined by combined immunofluorescence and fluorescence in situ hybridization (IFA-FISH) assays.

### Results:

Our study revealed distinct differences between vRNP trafficking patterns in mammalian cells infected with H7N9 and other avian viruses isolated from human and avian hosts. Through analysis of a series of reassortant viruses, we confirmed that the presence of avian-like PB2 causes the formation of vRNP aggregates in mammalian cells, whereas acquisition of adaptive mutations in the PB2 subverts accumulation of vRNP aggregates. Treatment with BafA1 disrupts the formation of vRNP aggresomes in mammalian cells, leading to enhancement of vRNP trafficking efficiency and virus replication. LC3 lipidation, the hallmark of autophagy, was found to closely associate with the formation of vRNP aggresomes. Further characterization showed that influenza virus vRNPs interact with the autophagy cargo receptor p62, Rab11, ubiquitin, and LAMP1 (a lysosomal marker) in influenza virus infected cells. A gene knockout experiment suggests that p62 may be responsible for targeting vRNP to autophagosomes. Influenza virus adapts to alter viral gene expression and the post translational process to elude host-mediated autophagic restriction, facilitating efficient replication in mammalian cells.

### Conclusion:

This study identifies a mechanism by which mammalian cells restrict intracellular trafficking of influenza vRNPs through targeting avian-type PB2 subunits of newly synthesized vRNP to the autophagy pathway, and details viral adaptive strategies that alter PB2 to evade such restriction.

*Keywords: Influenza virus; PB2; Adaptive mutations; Autophagy*

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission  
Abstract No: 10855

### **Haemagglutinin Mutation and Higher Neuraminidase Activity Enhanced the Adaption of H5N6 Avian Influenza Viruses to Mammalian Hosts**

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Different subtypes of Clade 2.3.4.4 H5NX virus, including H5N2, H5N6, and H5N8 show unprecedented intercontinental spread. Since 2014, human case of H5N6 avian influenza virus (AIV) infection have been reported in china . These H5NX viruses, especially H5N6 viruses, pose a severe threat to poultry industry and human health. However, the critical molecular features that determined H5N6 viruses infectivity in mammals remain unclear. Here, we evaluated the pathogenicity and transmissibility of representative H5N2, H5N6, and H5N8 viruses in mammals. All viruses studied replicated well in mice and ferrets, whereas only H5N2 and H5N6 viruses could transmitted efficiently among ferrets. Receptor binding assays demonstrated that these H5NX viruses acquired the affinity for human-like SA $\alpha$ 2,6Gal receptor and displayed a higher binding affinity for avian-like SA $\alpha$ 2,3Gal receptors. Based on reverse genetics and crystallographic data, we confirmed that combined mutations at the receptor binding site (RBS) and deglycosylation site at residue 158 of haemagglutinin (HA) are responsible for the Clade 2.3.4.4 viruses recognition of SA $\alpha$ 2,6Gal receptor. The neuraminidase (NA) of H5N6 viruses possessed significantly higher enzyme activity than that of H5N2 or H5N8 viruses. This higher NA activity could help to facilitate the viral release, thereby conferring their readily transmission in mammals. In summary, our results highlight that HA-NA balance plays a critical role in adaptation of H5N6 AIVs to mammalian hosts.

*Keywords: H5N6 Avian Influenza Viruses, Transmission, Receptor binding, Structure, Neuraminidase activity*

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission

Abstract No: 10945

## ASSESSMENT OF ZONOTIC TRANSMISSION OF SWINE INFLUENZA A VIRUSES FROM PIGS TO NAÏVE OR VACCINATED FERRETS

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**Introduction and Objectives:** An *in vivo* study was conducted to assess zoonotic infection dynamics of two swine-origin H1N1 influenza A viruses – a pandemic 2009 (Sw-pdm09) strain and human isolate (A/Pavia/65/16) which is a phylogroup 1C Eurasian avian-like swine influenza A virus (H1<sub>av</sub>N1). The efficacy of the human 2016-17 seasonal influenza vaccine, which contained the human pdm09 strain A/California/7/2009, was assessed in ferrets representing a human model.

**Methods:** Five donor pigs were housed in different rooms and infected with either Sw-pdm09 or A/Pavia/65/16 strains. Each group of pigs was co-housed with five naïve and five vaccinated ferrets held in separate cages. Virological and immunological parameters were then assessed in longitudinal samples.

**Results:** Both virus strains readily infected pigs producing similar, mild, disease profiles, and reached peak shedding at 2-4dpi, which ceased by 7dpi. Vaccinated, but not naïve, ferrets exposed to Sw-pdm09 virus showed a significant reduction in nasal shedding of virus and didn't develop influenza-specific antibody titres, indicating (partial) vaccine-mediated protection. These naïve ferrets, as well as both ferret groups exposed to the A/Pavia/65/16 strain had a virus shedding profile characteristic of infection and seroconverted. All infected ferrets exhibited mild clinical signs and controlled the infection. The A/Pavia/65/16 strain was found to transmit, in the ferret model, by direct contact but not via the airborne route.

**Conclusions:** This study confirms that vaccine strains must be antigenically matched to the challenge strain in order to afford protection. Pre-existing immunity to Human-origin pdm09 strains may not provide protection to all circulating swine influenza A virus H1N1 strains. There was no evidence of increased risk to human health - using animal models - from the A/Pavia/65/16 strain compared to the swine-origin pdm09 strain assessed in parallel. While zoonotic potential of A/Pavia/65/16 was confirmed in this study, onward transmission only occurred by close contact.

**Keywords:** zoonosis; swine; animal models, vaccines

# POSTER PRESENTATION



Topic: Clinical Sciences: Clinical Trials – Study Designs, Clinical Endpoints  
Abstract No: 10354

## EFFICACY AND SAFETY OF ANTI-INFLUENZA A MONOCLONAL ANTIBODY, CT-P27, IN SUBJECTS WITH ACUTE UNCOMPLICATED INFLUENZA A INFECTION: UPDATED PHASE IIB STUDY RESULTS

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**Introduction/Objectives:** CT-P27, a mixture of human Immunoglobulin G1 monoclonal antibodies, targets stem region of influenza A viral hemagglutinin protein. This trial evaluated efficacy and safety including antibody-dependent enhancement (ADE) of CT-P27 in subjects with acute, uncomplicated influenza A infection.

**Methods:** Adults age 19-64 years old with fever ( $\geq 38.0^{\circ}\text{C}$  [ $\geq 100.4^{\circ}\text{F}$ ]), at least two moderate to severe influenza symptoms, and were positive for influenza A by rapid diagnostic test at screening were randomized to a single dose of CT-P27 90mg/kg (N=88), 45mg/kg (N=90), or Placebo (N=50) and were followed up to Day 110. Subjects had symptoms and body temperature collected two times a day by diary for 8 days and nasopharyngeal swabs collected on baseline and days 2, 3, 5 and 8. Subjects were closely monitored for safety and suspicious ADE (defined as symptoms did not resolve or worsened, or secondary influenza like illness developed). Efficacy endpoint was time to resolution of symptoms and fevers (TTRSF).

**Results:** 221/228 subjects enrolled had laboratory-confirmed influenza. TTRSF was significantly shorter in CT-P27 groups compared to placebo group (1.98-2.03 days shorter vs. placebo, Table 1). There was more rapid reduction in cell culture viral titer in CT-P27 subjects compared to placebo subjects (Table 2 and Figure 1). Treatment emergent-adverse events (TEAE) were reported for CT-P27 90mg/kg; 33.0% (29/88), 45mg/kg; 38.9% (35/90), and Placebo; 42.0% (21/50) in safety population. The most commonly reported TEAE was diarrhea in CT-P27 90mg/kg; 3.4% (3/88), 45mg/kg; 7.8% (7/90), and Placebo; 4.0% (2/50). Suspicious ADE was reported for 2 placebo subjects.

**Conclusions:** CT-P27 resulted in more rapid resolution of symptoms, fever and virus compared to placebo in subjects infected with acute, uncomplicated influenza A. CT-P27 was safe with fewer TEAE and no episodes of suspected ADE. This study supports further development of CT-P27 for the treatment of influenza A.

*Keywords: CT-P27, Monoclonal antibody, Influenza A, Antiviral, Placebo-controlled study*

Topic: Clinical Sciences: Clinical Trials – Study Designs, Clinical Endpoints  
Abstract No: 10536

## DEVELOPMENT OF AN ORDINAL SCALE TREATMENT ENDPOINT IN ADULTS HOSPITALIZED WITH INFLUENZA

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### INTRODUCTION

Development of influenza therapeutics is hampered by the lack of validated clinical endpoints, especially among hospitalized patients. Use of single time-point, discrete outcome variables could be limited by diverse clinical trajectories of patients and low event rates.

### METHODS

We proposed a 6-level ordinal scale (**1**=discharged; **2**=convalescent hospital; **3**=acute hospital, no respiratory failure; **4**=acute hospital, with respiratory failure; **5**=ICU admission; **6**=died) to study clinical outcomes of adults hospitalized with influenza. Individual Patient Data (IPD) from North American (Alberta, Canada) and Asian (Hong Kong) active surveillance cohorts was used for evaluation (PCR-confirmed influenza: 2015/16, 2016/17, 2017/18). Longitudinal ordinal outcomes changes over 30 days, and the impact of neuraminidase inhibitor (NAI) treatment, was analyzed using group-based trajectory models and mixed ordinal logistic regression.

### RESULTS

Altogether, 1226 IPD were analyzed: age (mean±SD, 68.0±18.9 years), male (49.1%), virus subtype (A 78.1%, B 21.9%); received NAI treatment ≤5 days of illness (69.2%). Different clinical trajectories were observed (**Figure-1a**): at baseline, respiratory failure requiring supplemental oxygen (57.2%), ICU-admission (10.4%); at day 30: discharged (75.2%), convalescent hospital (13.7%), acute hospital (4.5%), died (6.6%). Patients' baseline status strongly predicted subsequent trajectories [OR 9.6, 95%CI 7.9–11.8, P<0.001]. NAI treatment was shown to significantly impact on ordinal outcome changes over time [OR 0.5, 95%CI 0.4-0.7, P<0.001] (**Figure-1b**). Results were adjusted for age, comorbidity, viral loads and vaccination status (OR 0.6, 95%CI 0.4-0.9, P=0.004). Findings were evaluated against discrete outcomes (e.g. 30-day mortality alone).

### CONCLUSION

The proposed 6-level ordinal scale is a potentially useful, composite clinical endpoint for influenza therapeutic trials. Considerations on interval analyses, sample size estimation, and variability among healthcare settings will be discussed.

*Keywords: ordinal scale, clinical endpoint, antiviral,*

Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention  
Abstract No: 10770

## Immunogenicity of quadrivalent influenza vaccine for patients with inflammatory bowel disease undergoing immunosuppressive therapy

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**Introduction and Objectives:** Several guidelines recommend influenza vaccination for patients receiving immunosuppressive therapy. The purpose of this study was to evaluate immunogenicity of the quadrivalent influenza vaccine (QIV) in the 2015/2016 season in patients with inflammatory bowel disease (IBD), who receives immunosuppressive therapy, and examined whether booster dose and the blood concentration of Infliximab (IFX) affect the immunogenicity.

**Methods:** IBD patients were assigned to a single vaccination group when the patients' birthday were on even days, or booster group when those were on odd days, and a QIV was administered subcutaneously. Serum samples were collected at 3 points (before vaccination, 4 weeks after vaccination and after the end of influenza season) in the single group and 4 points in the booster group (before vaccination, 4 weeks after the first vaccination, 4 weeks after the second vaccination and after the end of the influenza season). Hemagglutination inhibition antibody (HAI) titer were measured, and geometric mean titer ratio (GMTR), seroprotection rate (SP%), and seroconversion rate (SC%) were calculated.

**Results:** A total of 132 patients with IBD were assigned to single vaccination (n=83) and booster vaccination (n=49) groups. Among them, 27 patients received IFX, and 105 received 5-aminosalicylic acid (5-ASA). No significant difference between the single vaccination group and booster group was observed (geometric mean titers: H1N1: p = 0.81; H3N2: p = 0.79; B / Phuket: p = 0.82; B / Texas: p = 0.84). In patients treated with IFX, SP% and SC% tended to be lower in influenza A strains in patients who maintained blood concentrations  $\geq 0.1 \mu\text{g/ml}$  (SP%: H1N1: OR 0.37 (0.11-1.21); H3N2: OR 0.22 (0.07-0.68), SC%: H1N1: 0.23 (0.06-0.91); H3N2: 0.19 (0.06-0.56)).

**Conclusion:** Single dose QIV showed sufficient immunogenicity in IBD patients, and booster immunogenicity was not obtained. Additionally, immunogenicity was low in patients receiving IFX therapy.

Topic: Clinical Sciences: Clinical Trials – Vaccines

Abstract No: 10743

## MF59-ADJUVANTED QUADRIVALENT INFLUENZA VACCINE PROVIDES CONSISTENT BENEFIT UPON REVACCINATION IN YOUNG CHILDREN

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### Introduction and Objective

MF59-adjuvanted vaccines have demonstrated enhanced immunogenicity and acceptable safety profile after primary vaccination in children 6-72 months of age (Vesikari 2018, Nolan 2012). This study evaluates the immunogenicity and safety of revaccination with an adjuvanted quadrivalent influenza vaccine (aQIV) in previously vaccinated children.

### Methods

We conducted two revaccination studies in children who participated in a parent efficacy study (NCT01964989) and received a primary influenza vaccination with aQIV or non-adjuvanted vaccine. For Study-1, 607 subjects from the US and Finland, from the first season of the parent study, were revaccinated with the same vaccine as the prior year (aQIV/aQIV or TIV/QIV). In Study-2, 1601 subjects from Finland, Thailand, and the Philippines, from the second season of the parent study, were re-randomized to receive the same or the alternative vaccine (aQIV/aQIV; aQIV/QIV; QIV/aQIV; QIV/QIV). Immunogenicity (Days 1, 22, 181), reactogenicity (Days 1-7), and safety were assessed.

### Results

In Study-1, GMT ratio (aQIV/aQIV vs TIV/QIV) on Day 22 ranged from 1.3 to 1.8 for the 4 vaccine strains. Study-2 also demonstrated a superior immune response for aQIV/aQIV vs conventional QIV/QIV in 3 of 4 strains. Notably, subjects primed with either conventional vaccine or aQIV demonstrated a more robust immune response after revaccination with aQIV vs QIV for 3 of 4 strains. Consistent with Day 22 results, antibody titers at 6 months post vaccination were higher with aQIV vs conventional vaccine. Immune response against heterologous influenza strains (A/H3N2 and B/Victoria) also appeared to be higher after repeat aQIV vaccine in both studies. Except for a mild increase in reactogenicity, the safety profile of aQIV was generally similar to nonadjuvanted comparator. Moreover, the safety and reactogenicity profile of aQIV was similar to results from the parent study.

### Conclusion

Overall, these data support annual revaccination with aQIV in young children.

*Keywords: MF-59-Adjuvanted, Influenza, Revaccination, Children, aQIV*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 11123

## PRECLINICAL EVALUATION OF H5N8 VACCINE CANDIDATE (IDCDC-RG43A) IN MOUSE AND FERRET MODELS

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### Introduction

Because H5N1 influenza viruses continuously threaten the public health, the WHO has prepared various clades of H5N1 mock-up vaccines as one of the measures for pandemic preparedness. The recent worldwide outbreak of H5Nx virus which belongs to clade 2.3.4.4 and of which H5N6 subtype belongs and already caused human infection also increases the need of pandemic vaccine for such novel emerging viruses.

### Objectives

We evaluated the protective efficacy and immunogenicity of an egg-based and inactivated whole-virus H5N8 (IDCDC-RG43A) developed by CDC containing HA and NA gene of the parent virus A/gyrfalcon/Washington/41088-6/2014.

### Methods

Mice vaccinated two times elicited low to moderate antibody titer in varying amount of antigen doses against the homologous H5N8 vaccine virus and heterologous intra-clade 2.3.4.4 H5N6 (A/Sichuan/26221/2014) virus.

### Results and Conclusion

Mice immunized with at least 3.0 µg/dose of IDCDC-RG43A with aluminum hydroxide adjuvant were completely protected from lethal challenge with the mouse-adapted H5N8 (A/Environment/Korea/ma468/2015, maH5N8) as well as cleared the viral replication in tissues including lung, brain, spleen, and kidney. Vaccinated ferrets induced high antibody titers against clade 2.3.4.4 H5N8/H5N6 viruses and the antibody showed high cross-reactivity to clade 2.2 H5N1 but not to clade 1 and 2.3.4 viruses as measured by hemagglutinin inhibition and serum neutralization assays. Furthermore, administration of the vaccine in ferrets resulted to attenuation of clinical disease signs and virus spread to peripheral organs including lung, spleen, and kidney from high dose challenge with maH5N8 virus. The protective and immunogenic characteristic of the candidate vaccine are essential attributes to be considered for further clinical trials as a pre-pandemic vaccine for a potential pandemic virus.

*Keywords: H5N8 pre-pandemic vaccine; preclinical evaluation; immunogenicity; protective efficacy;*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 11263

## Immune response after pandemic and seasonal influenza vaccination in healthcare workers

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**Introduction and Objectives:** Vaccination is the most effective strategy for prevention and control of influenza. Healthcare workers (HCW) are often recommended for annual seasonal vaccination due to high-risk of influenza exposure. During the 2009 H1N1 pandemic (H1N1pdm09), HCW were one of the main priorities for pandemic vaccination. The adjuvanted pandemic vaccine was used in Norway, which was reported to generate good humoral and T-cell responses for up to one year. However, the long-term immune response after adjuvanted pandemic vaccination has not been studied. In addition, conflicting results on the impact of repeated annual vaccination on antibody responses have been reported, while the impact on T-cell immunity has not been extensively studied. The H1N1pdm09 strain was subsequently included in seasonal vaccines from 2010/11 to 2016/17 seasons, providing an opportunity to investigate the impact of repeated vaccination against this same strain on immune response over multiple seasons.

**Methods:** We conducted a 5-year follow-up study after adjuvanted pandemic vaccination in HCW (N=250) to investigate the long-term antibody responses elicited by this vaccine and further evaluate the impact of repeated annual vaccination on both humoral and T-cell responses in the post-pandemic seasons.

**Results:** We demonstrated that a single adjuvanted pandemic vaccination induced durable antibodies, supporting the use of an adjuvant in future influenza vaccines. Without seasonal vaccination, HCW with non-seroprotective antibodies were more likely to be infected with the circulating viruses. Therefore, repeated vaccination is required to improve protection in HCW. Repeated annual vaccination increased the H1N1pdm09-specific T cells and memory B cells, improved the quality of CD4<sup>+</sup>T cells, and importantly, maintained the cross-reactive IFN- $\gamma$ -secreting CD4<sup>+</sup> and CD8<sup>+</sup>T cells, which potentially provide protection against severe influenza illness.

**Conclusion:** Our study emphasizes the broad impact of repeated annual vaccination on immune response and supports the current recommendation of annual influenza vaccination in HCW.

Topic: Clinical Sciences: Diagnostics and Disease Markers  
Abstract No: 10564

**THE IMPACT ON HEALTHCARE RESOURCE UTILIZATION AND COST OF ROUTINE MOLECULAR POINT-OF-CARE TESTING FOR RESPIRATORY VIRUSES IN ADULTS HOSPITALISED WITH ACUTE RESPIRATORY ILLNESS: FURTHER ANALYSIS FROM A PRAGMATIC RANDOMISED CONTROLLED TRIAL (RESPOC)**

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**Introduction:**

The ResPOC trial demonstrated that syndromic point-of-care testing (POCT) for respiratory viruses was associated with a number of clinical benefits compared to routine clinical care and laboratory-based PCR testing. However, the cost effectiveness of such a strategy is unknown.

**Methods:**

The ResPOC trial was a pragmatic randomised controlled trial that enrolled 720 patients over two winter seasons in a large UK teaching hospital. Participants were randomised 1:1 to POCT using the FilmArray Respiratory Panel or routine clinical care. Data were collected on mortality, healthcare events and resource utilisation. Costs were calculated for healthcare resource utilisation using UK national costs. Generalised linear models were built using step-wise methods to adjust for influential patient characteristics on costs and patient mortality. Models were assessed for fit and chosen using the Bayesian Information Criterion. The cost model used the gamma family with a log link; the mortality model used the binomial family with a log link. A cost-consequence analysis was conducted with unadjusted and adjusted models with subgroup analysis by diagnosis.

**Results:**

The mean unadjusted costs for usual care were £2845 (95%CI £2358 to £3332), £117 more expensive than the POCT arm (£2668, 95%CI £2263 to £3073). Adjusting costs for patient characteristics, the mean cost for usual care was £2826 (95% CI £2654 to £2998), whilst POCT was £2763 (95% CI £2595 to £2931). Although there was a substantial difference in deaths in the unadjusted analysis, with patient characteristics adjustment, 3.6% of the usual care arm and 3.4% of the POCT arm died within 30 days of hospital admission.

**Conclusions:**

Routine molecular POCT using the FilmArray Respiratory Panel appears to be cost-saving, compared to usual care with laboratory-based testing. There remains some uncertainty in cost-savings due to imprecision in effect measures and further real-world effectiveness trials should be conducted to confirm these results.

*Keywords: point-of-care testing; influenza; cost effectiveness; health economic analysis*

Topic: Clinical Sciences: Therapeutics – Antivirals  
 Abstract No: 10718

## Low frequency of reduced neuraminidase inhibitor susceptibility in twelve EU/EEA countries, 2008-2018

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Influenza viruses cause respiratory morbidity and mortality and neuraminidase inhibitors (NAIs) are used as prophylaxis and treatment against influenza. We analysed the frequency of reduced (RI) or highly reduced (HRI) NAI susceptibility in specimens received through influenza surveillance in the European Union/European Economic Area (EU/EEA) countries in order to inform the treatment guidelines.

Pheno- and genotypic RI and HRI to oseltamivir and zanamivir in 12 EU/EEA countries who submitted influenza antiviral susceptibility data to The European Surveillance System for at least five influenza seasons with minimum of ten influenza viruses per season during 2008/09-2017/18 were included. Comparison of means was performed by chi-square test for categorical variables and Dunn's test with Bonferroni correction for multiple comparisons (significance,  $p < 0.05$ ).

The overall prevalence of RI/HRI to NAIs was 1.0% in the 27407 analysed influenza viruses. The frequency of RI/HRI varied by season, (sub)type, country, hospitalisation and treatment status, while vaccination status did not affect the susceptibility to NAIs. The highest frequency of RI/HRI (2.8%) was detected in season 2010/11. A(H1N1) viruses showed 74%, A(H1N1)pdm09 1.3%, A(H3N2) 0.3% and B viruses 0.2% RI/HRI to oseltamivir and 0%, 0.1%, 0.3% and 0.2% to zanamivir, respectively. Across the seasons, the proportion of RI/HRI of A(H1N1)pdm09 for oseltamivir was highest in France (3.7%,  $p < 0.0001$ ). Of the hospitalised patients, 113 (3.0%;  $p < 0.0001$ ) had a virus showing RI/HRI to oseltamivir compared with the 37 (0.5%) of the outpatients. Of the 179 patients with known treatment status with RI/HRI to oseltamivir, 63 (35%;  $p < 0.0001$ ) had not been treated with NAIs. Amino acid substitutions associated with RI/HRI were mainly H275Y and Y155H in A(H1N1)pdm09 and E119V and R292K in A(H3N2) viruses.

The low frequency of reduced NA susceptibility supports the use of NAIs against severe influenza. Continuous monitoring of susceptibility is crucial in patients treated and not treated with NAIs.



Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 11026

## Clinical effectiveness of baloxavir marboxil compared to oseltamivir - appearance of mutated viruses at position 38 in PA protein for influenza A/H1N1pdm09 and A/H3N2

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**Introduction and Objectives:** Baloxavir marboxil (BA) is a newly approved anti-influenza drug and potent inhibitor of viral replication. However the emergence of reduced susceptibility viruses possessing substitutions at position 38 in PA protein was reported. We compared fever duration between BA and oseltamivir (OS) in Japanese children and evaluated clinical course of mutated viruses.

**Methods:** We conducted an observational study of influenza A/H1N1pdm09 or A/H3N2 in infected adolescents under the age of 20 in Japan during 2018-2019 season. After informed consent, either BA or OS was given to the patients with positive rapid test results. Body temperature was recorded for eight days and second visit samples were taken after 4-5 days from first visit. Mutations in PA were checked by genetic sequencing and subsequent sensitivity assay for BA was done using ViroSpot microneutralization assay. Fever duration from the start of therapy until below 37.5°C was tested by univariate analyses

**Results:** A total of 123 patients were eligible for analysis. BA was given to 82 (31 of A/H1pdm, and 51 of A/H3) and OS was 41 patients (17 of A/H1pdm, and 24 of A/H3). Average age was higher in BA than OS group (10.4±2.6 versus 6.8±3.3 years old, p<0.01). Fever duration of A/H1pdm patients did not differ between BA and OS (1.1±0.9 versus 1.3±0.9 days), but that of A/H3N2 was shorter for BA than OS (0.6±0.8 versus 0.9±0.7 days, p=0.04). At least 4 cases possessed PA mutation at position 38, two I38T and one I38M for A/H3 and one I38S for A/H1pdm. All cases showed defervescence within 48 hours after start of BA. Further genetic and phenotypic assays are underway.

**Conclusion:** BA showed similar clinical effectiveness compared to OS for influenza A infections. No prolongation of fever was observed with PA mutated virus infections but further analysis are needed to confirm these results.

**Keywords:** Baloxavir Marboxil; Oseltamivir; Antiviral Resistance; Clinical course; I38T mutation

Topic: Clinical Sciences: Therapeutics – Antivirals

Abstract No: 11292

## IN VITRO COMBINATION EXPERIMENTS WITH PIMODIVIR AND OTHER INFLUENZA ANTIVIRAL DRUGS

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**INTRODUCTION:** Pimodivir is a PB2-subunit inhibitor of the influenza A polymerase complex in Phase 3 clinical development for the treatment of patients at risk of influenza-related complications, including hospitalized patients.

**METHODS:** In vitro combination experiments, using MDCK cells infected with A/PR/8/34 (H1N1), were performed to determine the synergistic, additive, or antagonistic antiviral activity of pimodivir with neuraminidase inhibitors oseltamivir or zanamivir, polymerase inhibitor favipiravir, and PA endonuclease inhibitor JNJ-64155806. Results were analyzed using the Bliss independence method (MacSynergy, synergy volumes >100 indicate strong synergy) or the Loewe additivity method (combination indices <1 or <0.8 indicate synergy or strong synergy, respectively). The combination of pimodivir and oseltamivir was also assessed in a lethal mouse influenza model using A/PR/8/34. In this model, treatment was started 48h post-infection. Mortality and morbidity, using prevention of body weight loss and pulmonary dysfunction measured by whole body plethysmography (WBP) were assessed.

**RESULTS:** The Bliss independence method resulted in synergy volumes of 312, 268, 296, and 317 for pimodivir with oseltamivir, zanamivir, JNJ-64155806, and favipiravir, respectively. Similarly, the Loewe additivity method resulted in combination indices of 0.58, 0.64, and 0.89 for pimodivir with oseltamivir, zanamivir, and favipiravir, respectively. In the mouse model, pimodivir alone at 1, 3, or 10 mg/kg BID provided complete protection from mortality and restored body weight loss and pulmonary dysfunction. Pimodivir alone, at 0.1 or 0.3 mg/kg, or oseltamivir alone at 10mg/kg did not generate such results. The combination of pimodivir at 0.3 mg/kg and oseltamivir at 10mg/kg provided complete protection from mortality, restored body weight loss and provided lung function benefit.

**CONCLUSIONS:** Pimodivir showed synergistic antiviral activity in vitro with oseltamivir, zanamivir, and JNJ-64155806 and synergistic/additive antiviral activity in vitro with favipiravir. These data will enable to explore potential combinations of antivirals in patients with influenza infection.

*Keywords: pimodivir oseltamivir combination synergy*

Topic: Co-Infection: Bacterial Co-infections with Influenza  
Abstract No: 10850

## Knock out of PB1-F2 for Enhanced Safety of Cold-adapted Live Attenuated Influenza Vaccine

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### Introduction and Objectives:

The PB1-F2 of an influenza A virus has been defined as a virulence factor involved in the secondary bacterial infection by *Streptococcus pneumoniae* (SP) with increased pathogenesis and cytokine dysregulation. Although a cold-adapted live attenuated influenza vaccine (CAIV) has been proven effective for the prevention of influenza infection, the safety issue has long been debated for the potential of gaining virulence. The currently available CAIVs, despite attenuation of virulence, still carries PB1-F2 as potential virulence factor that could increase the susceptibility to the secondary bacterial infection.

### Methods:

Using the CAIV donor strain X-31ca(H3N2) as a model, we generated a PB1-F2 knockout virus (delF2 X-31ca). Using a live imaging system for tracking the bioluminescent SP infection as an model of pneumonia, we compared the disease symptoms after the SP infection in the murine model with prior immunization with either the X-31ca or delF2 X-31ca.

### Results:

Deletion of the PB1-F2 rarely affected the growth abilities of the X-31ca in vitro or in ovo but resulted in further attenuation of virulence in a mouse model. Following the secondary infection with a high doses of SP, the delF2 X-31ca-immunized mice demonstrated lower SP replication in the lower respiratory tract, less mortality, and decreased levels of inflammatory cytokines, as compared to the X-31ca-immunized mice. In case of a low dose infection of SP, however, delF2 X-31ca significantly reduced the duration of SP infection, suggesting that the innate immune response of CAIV protects from bacterial super-infection.

### Conclusion:

These results show that, i) although CAIV provides non-specific protection from bacterial super-infection through innate immune responses, ii) PB1-F2 in CAIV could pose a risk factor for vaccine associated secondary bacterial infection especially with high dose of infection. We suggest that knock-out of PB1-F2 as a useful strategy for further increasing the safety of CAIVs.

**Keywords:** Influenza; super infection; PB1-F2;

Topic: Co-Infection: Bacterial Co-infections with Influenza  
Abstract No: 11140

## Antibody treatment against angiotensin-like 4 (ANGPTL4) reduces pulmonary edema and injury in secondary pneumococcal pneumonia

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### Introduction and Objectives:

Secondary bacterial lung infection by *Streptococcus pneumoniae* (*S. pneumoniae*) poses a serious health concern, especially in developing countries. We posit that the emergence of multi-antibiotic-resistant strains will jeopardize current treatments in these regions. Deaths arising from secondary infections are more often associated with acute lung injury, a common consequence of hypercytokinemia, than with the infection *per se*. Given that secondary bacterial pneumonia often has a poor prognosis, newer approaches to improve treatment outcomes are urgently needed to reduce the high levels of morbidity and mortality.

### Methods:

Mouse models of secondary bacterial infection were employed to explore the role of angiotensin-like 4 c-isoform (cANGPTL4) in pulmonary pathogenesis and evaluate the effect of anti-cANGPTL4 monoclonal antibody treatment. RNA sequencing, electron microscopy, flow cytometry analysis for immune cells and other molecular biology analysis were performed.

### Results:

Using a sequential dual infection mouse model of secondary bacterial lung infection, we showed that host-directed therapy via immunoneutralization of cANGPTL4 reduced pulmonary edema and damage in infected mice. RNA sequencing analysis revealed that anti-cANGPTL4 treatment improved immune and coagulation functions, and reduced internal bleeding and edema. Importantly, anti-cANGPTL4 antibody, when used concurrently with either conventional antibiotics or anti-pneumolysin antibody, prolonged the median survival of mice compared to monotherapy. Anti-cANGPTL4 treatment enhanced immune cell phagocytosis of bacteria, while restricting excessive inflammation.

### Conclusion:

Anti-cANGPTL4 treatment improved the disease outcomes of secondary pneumococcal pneumonia. Taken together, our study emphasizes that host-directed therapeutic strategies are viable adjuncts to standard antimicrobial treatments.

Topic: Co-Infection: Bacterial Co-infections with Influenza  
Abstract No: 11239

## IDENTIFYING INFLUENZA AND ITS CO-INFECTIONS FROM MERS-COV ASSAY-NEGATIVE TRAVELLING PATIENTS FROM THE MIDDLE EAST TO THE PHILIPPINES

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### Introduction and Objectives

Since 2014, only two cases of MERS-CoV have been confirmed out of around 2,300 suspected Philippine cases. Because no further differential testing was done for most of the MERS-CoV-negative specimens, we aimed to determine the causal pathogens for the rest of these individuals considering the potential impact of imported novel microorganisms from travelling patients.

### Methods

Archived respiratory samples (n=203) collected from 163 patients with severe acute respiratory infections from 2014 to 2016 that tested negative by MERS-CoV RT-qPCR were tested using a multiplex PCR respiratory panel. Untargeted metagenomic shotgun sequencing (MSS) was performed on samples that were negative for the multiplex panel to further check for pathogens. Preprocessed reads were analyzed by Centrifuge to taxonomically classify metagenomic reads, and Pavian was used for post-analysis of initial taxonomic assignments.

### Results

Around 90% of the samples were found to be positive for at least one respiratory pathogen in the multiplex PCR panel. Co-infections of 2-6 pathogens (21.67%) with either Influenza A and/or B were mostly bacterial such as *Klebsiella pneumoniae*, and *Haemophilus influenzae*, while viral co-infections observed were Adenovirus, and Rhinovirus. On the other hand, only 2 out of 4 MSS samples were found to be positive for *Pseudomonas stutzeri*, *P. aeruginosa*, and *Prevotella melaninogenica* based on each hit's calculated z-score.

### Conclusions

Influenza virus along with other respiratory pathogens are important causes of acute respiratory infections in travellers. Several of these combinations were found in patients travelling from the Middle East. The metagenomic analysis was able to narrow down the potential causative pathogen; however, laboratory confirmation of the MSS results is important along with the clinical data to facilitate the determination of the primary cause of the infection. Furthermore, identification of imported respiratory infections can enable early mitigation of possible outbreaks in the country.

Keywords: SARI, MERS-CoV, Influenza, Co-infection

Topic: Co-Infection: Bacterial Co-infections with Influenza  
Abstract No: 11307

## TIV VACCINATION MODULATES HOST RESPONSES TO INFLUENZA INFECTION THAT CORRELATE WITH PROTECTION AGAINST BACTERIAL SUPERINFECTION

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### Introduction/objectives:

Influenza virus infection can predispose humans to life-threatening secondary bacterial pneumonia. Vaccination is by far the best method to prevent influenza-related disease. Currently licensed influenza vaccines can be less effective if the induction of neutralizing antibodies is low and/or the influenza virus changes its antigenic surface to escape neutralization. We wanted to investigate the effect of vaccination on the outcome of bacterial superinfection in the absence of virus-neutralizing titers.

### Methods:

We have established a mouse vaccination model that allows to control disease severity after influenza infection in the absence of virus neutralization. With this model, we investigated the effect of vaccination on host immune responses and the outcome of bacterial superinfection with *Staphylococcus aureus*.

### Results:

Balb/c mice that were vaccinated with a trivalent inactivated virus vaccine (TIV) had reduced morbidity after influenza infection but did not completely prevent virus replication. Despite the poor induction of influenza-specific antibodies, animals that were TIV vaccinated and infected with influenza virus were protected from mortality after bacterial superinfection. Vaccination limited loss of alveolar macrophages and did not increase the levels of circulating and lung infiltrating monocyte-like immune cells after infection. Interestingly, influenza infection of TIV vaccinated animals resulted in enhanced levels of eosinophils when compared to non-vaccinated infected animals. As a result of bacterial superinfection, recruitment of neutrophils into the lungs was observed, and TIV vaccinated animals had more than three-fold higher neutrophil counts compared to non-vaccinated controls. TIV vaccination also resulted in high levels of neutrophils in the mediastinal lymph nodes after bacterial superinfection, which was not observed in non-vaccinated mice.

### Discussion:

These observations highlight the importance of disease modulation by influenza vaccination in the absence of virus neutralization and suggest that vaccination is still beneficial to prevent bacterial superinfection even in the absence of virus neutralization.

A.C. and I.C. contributed equally

*Keywords: TIV, bacterial superinfection, Staphylococcus aureus, host immune response*

Topic: Co-Infection: Bacterial Co-infections with Influenza  
 Abstract No: 11317

## Off-target effects of immunity conferred by insect cell-expressed influenza HA-VLPs on secondary bacterial infections

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**Introduction:** Clinical and historical data underscore the ability of influenza viruses to ally with certain bacterial species and predispose the host for secondary bacterial pneumonia, which is a leading cause of influenza-associated mortality. Vaccines against the bacterial species most frequently associated with secondary bacterial infections (SBIs) either lack of comprehensive serotype coverage (*S.pneumoniae*) or are not available (*S.aureus*). This leaves the control of influenza as most promising measure to prevent secondary bacterial complications. In the present work we assessed the protective efficacy of a recombinant influenza vaccine in two murine models of postinfluenza bacterial infection using a vaccine-matched versus non-matched viral challenge strain.

**Methods:** Influenza HA-Gag VLPs composed of the HA of influenza A/PR/8/34 (H1N1) were expressed in *Tnms42* insect cells using baculovirus infection. Different antigen doses (0.02-0.1 µg HA/mouse) and immunization regimens were evaluated in BALB/c mice infected with a homologous or heterologous influenza virus followed by sublethal bacterial *S.pneumoniae* or *S. aureus* challenge. Vaccine-induced protection from pathogen replication in the lung as well as weight loss and mortality was assessed.

**Results:** A single low antigen dose (0.05 µg HA/mouse) of insect cell-expressed VLPs was highly effective in limiting pathogen replication in the lungs of vaccinated mice and fully protected from mortality and SBI-mediated enhancement of disease when matched with the viral challenge strain. In the absence of HI-active neutralizing antibodies we observed a less pronounced but nevertheless significant degree of protection from mortality in comparison to mock-vaccinated mice.

**Conclusions:** Preceding influenza virus infection is known to predispose the host for secondary bacterial infections. We investigated the off-target effects of recombinant influenza VLP vaccine-induced immunity in the context of secondary bacterial pathogens. Anti-influenza HA immunity conferred by a virus-matched vaccine is not only highly effective in protecting from influenza but also from severe secondary bacterial infections.

**Keywords:** Influenza VLP vaccine - Secondary bacterial infection - *S.aureus* - *S.pneumoniae* - Vaccine mismatch

Topic: Co-Infection: Viral Coinfections with Influenza

Abstract No: 10530

## DEVELOPMENT OF A BIVALENT LIVE VIRAL VECTORED VACCINE AGAINST INFLUENZA AND HUMAN METAPNEUMOVIRUS INFECTIONS

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**Introduction and objectives:** Human metapneumovirus (hMPV) infection is one of the major causes of complicated respiratory infections in children, elderly and risk group patients. There is no licensed vaccine against hMPV infection yet. Live attenuated influenza vaccine (LAIV) virus can serve as a viral vector to deliver immunogenic epitopes of hMPV to respiratory cells, thus inducing protective immunity against both infections.

**Methods:** Search of immunogenic fragments of hMPV proteins was performed using Immune Epitope Database tools. Promising fragments were combined into cassettes and properties of these constructs were assessed by computational modelling (i-TASSER, Procheck, ExPasyProtParam, Chimera, and GROMACS). The DNA fragment encoding the hMPV cassette was chemically synthesized and inserted into HA gene of H7N9 LAIV strain. LAIV strain expressing chimeric HA was generated by the means of reverse genetics. Replicative properties of the LAIV-hMPV virus were assessed in eggs and MDCK cells. Immunogenicity and protective efficacy of the chimeric virus were studied in BALB/c mice.

**Results:** The selected hMPV cassette comprises two immunogenic fragments of hMPV, connected with linker: (1) fragment 222-256 of hMPV fusion protein (F) (corresponds to antigenic site IV and experimental neutralizing epitopes); (2) fragment 370-442 of hMPV F (corresponds to antigenic sites V и VI and experimental T-cell epitope). Cassette was linked to N-terminus of H7N9 HA1 subunit via flexible linker (figure). The LAIV-hMPV virus retained the ability to replicate in eggs and MDCK cells and expressed temperature-sensitive and cold-adapted phenotypes similar to the H7N9 LAIV strain. The LAIV-hMPV was safe, immunogenic and protected BALB/c mice from the disease caused by both infections.

**Conclusion:** The insertion of hMPV cassette into LAIV strain did not affect main LAIV characteristics. The LAIV viruses represent a promising platform for the development of multivalent vaccines against influenza and other respiratory viruses.

This study was supported by RSF grant 17-75-20054.

*Keywords: influenza, LAIV, viral vectored vaccine, metapneumovirus*



Topic: Co-Infection: Viral Coinfections with Influenza  
Abstract No: 10846

## Development of a bivalent vaccine against influenza and human adenovirus infections.

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Introduction and objectives: Acute respiratory viral infections are the most widespread group of human infectious diseases. Currently, only influenza vaccines are licensed, whereas other common pathogens, such as adenoviruses, stay out of control. In this study, we examined new bivalent vaccine against influenza and adenovirus infections using live attenuated influenza vaccine (LAIV) as viral vector.

Methods. A cassette containing conservative T-cell epitopes of adenoviral hexon and DNA binding protein was inserted into NA and truncated to 126 residues NS1 genes of H7N9 LAIV virus using the P2A self-cleavage site. The recombinant LAIV-AdV viruses were generated by the means of reverse genetics. Immunogenicity and protective efficacy against influenza and human AdV-5 were determined in BALB/c mice. AdV-specific CD4 and CD8 T-cell responses were evaluated by intracellular cytokine staining and CTL *in vivo* assay. AdV-5 viral titers in mouse lungs were determined by immune plaque assay and real-time PCR using virus-specific primers.

Results. The chimeric LAIV+AdV/NA and LAIV+AdV/NS1 viruses efficiently replicated in eggs, were temperature sensitive and possessed attenuated phenotype typical for LAIVs: the viruses replicated in mouse nasal turbinates, whereas no virus was detected in lungs. Two-dose immunization schedule raised anti-influenza antibody in LAIV-AdV-immunized mice at the same level as H7N9 LAIV control virus. Following Adv-5 challenge, the levels of AdV epitope-specific CD4+TNF+, CD8+TNF+, CD8+IFN $\gamma$ + T-cell subsets were significant higher in LAIV-AdV group, compared to the H7N9 LAIV group. The LAIV-AdV and H7N9 LAIV vaccines fully protected mice from virulent H7N9 influenza virus infection, whereas only LAIV-AdV groups showed protective effect against human AdV-5 infection.

Conclusion. This is the first attempt to design a bivalent vaccine against influenza and human adenoviruses using LAIV viral vector delivery system. The designed LAIV-AdV vaccines warrant further preclinical and clinical trials.

This study was supported by the Grant of the Russian Science Fund 17-75-20054.

*Keywords: LAIV; bivalent vaccine, human adenoviruses, CTL in vivo.*

Topic: Co-Infection: Viral Coinfections with Influenza  
Abstract No: 11013

## COINFECTIONS WITH INFLUENZA AND OTHER RESPIRATORY VIRUSES: OREGON CHILD ABSENTEEISM DUE TO RESPIRATORY DISEASE STUDY (ORCHARDS), WISCONSIN, USA, 2015-2019

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**Introduction and Objectives:** We analyzed coinfections involving influenza and influenza-like illness (ILI)-related viruses among children participating in ORCHARDS, a community-based study evaluating relationships between ILI-associated school absenteeism and medically-attended influenza in the surrounding community.

**Methods:** Parents of children with ILI symptoms voluntarily called a study hotline, and eligible students were visited at home for data and respiratory specimen collection. Naso/oropharyngeal samples were tested for influenza by rRT-PCR (IVD CDC Human Influenza Virus RT-PCR Diagnostic Panel) and other respiratory pathogens (Luminex NxTAG Respiratory Pathogen Panel) at the Wisconsin State Laboratory of Hygiene. Influenza coinfection was defined as detection of influenza plus another virus in eligible children. Severity of illness was rated on a 3-point scale (mild, moderate, severe) by research staff conducting the home visit.

**Results:** Overall, 1,514 home visits were conducted from January 5, 2015, through March 15, 2019. A total of 970 (64.1%) specimens were positive for at least one respiratory pathogen. Overall, there were 342 (35%) specimens with influenza detected, 24 (7.0%) of which revealed coinfections, including one triple infection (with rhinovirus/enterovirus and coronavirus NL63) (Table). Of coinfecting cases, 17 (70.8%) were in children with influenza A(H3N2). The prevalence of coinfection with influenza A(H3N2) was not higher than for non-H3N2 influenza viruses ( $X^2=3.114$ ;  $p=0.078$ ), nor was the illness severity higher with A(H3N2) (ANOVA:  $F=1.12$ ;  $p=0.302$ ). Children with coinfection were similar in age to children with influenza without coinfection (10.3 vs 9.6 years; ANOVA  $F=1.12$ ;  $p=0.290$ ) and there were no differences in terms of severity of illness with and without coinfection ( $X^2=0.687$ ;  $p=0.709$ ).

**Conclusion:** In a community sample of children with laboratory-confirmed influenza, coinfections were common, representing 7% of all influenza infections. No significant differences in age or severity of illness were detected. Influenza coinfections involving a variety of other viruses are commonly observed in school-aged children

*Keywords: influenza; viral; coinfection; severity; community*

Topic: Co-Infection: Viral Coinfections with Influenza  
 Abstract No: 11183

## Pathogenicity of influenza D virus (IDV) and influenza A virus (IAV) co-infection in pigs

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**Introduction:** Disease severity of IAVs in pigs may worsen when co-infecting with bacteria or other viruses. IDV was first identified in pigs with respiratory diseases. In the US feral swine population, the IDV seroprevalence is ~19%; however, ~43% of IAV seropositive feral swine (n=96) have antibodies to IDV, suggesting pigs are more likely to be exposed to IDV when infected with IAV. This study aims to evaluate whether co-infection of IDV and IAV increases pathogenesis in swine compared to infection of either IDV or IAV alone.

**Methods:** We intranasally inoculated IAV/IDV seronegative domestic swine (n=25) with 1) 10<sup>6</sup> TCID<sub>50</sub> of IAV [A/swine/Texas/A01104013/2012(H3N2)], 2) 10<sup>6</sup> TCID<sub>50</sub> of IDV (D/bovine/C00046N/Mississippi/2014), 3) 10<sup>6</sup> TCID<sub>50</sub> of IAV and 10<sup>6</sup> TCID<sub>50</sub> of IDV, or 4) PBS. We collected nasal swabs daily and euthanized all pigs at 5 dpi, collecting 14 tissues across respiratory tract tree for viral titration and immunohistochemical analyses.

**Results:** Nasal shedding results showed that in single infections, 5/5 and 6/7 pigs shed IAV or IDV at 3, 4 and/or 5 dpi, respectively. In the co-infection group 7/7 pigs shed IAV but only 1/7 shed IDV at 3, 4 and/or 5 dpi. In all tissues, the single infection and coinfection pigs were IAV positive. In the IDV group 72% of tissues were positive but only 51% of tissues in the coinfection group were positive for IDV. H&E staining showed considerable apoptosis in the absence of acute inflammation primarily in the co-infection group. Cleaved caspase-3 and Ki-67 staining were present in all treatment groups.

**Conclusion:** Viral tissue load and nasal shedding data suggest same exposure co-infection may competitively favor IAV by interfering or inhibiting IDV. However, IHC results showed apoptosis most pronounced in the co-infection group suggestive of a synergistic interaction, although additional semi-quantitative IHC did not support a difference between groups.

*Keywords: co-infection; swine; IDV*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10715

## ROLE OF PB2 AND PA-X IN AVIAN INFLUENZA VIRUS REPLICATION

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Influenza PB2 is a well-established player in influenza virus host adaptation. In particular, mammalian viruses are associated with a lysine (K) at residue 627 while avian viruses generally have glutamic acid (E) at this position. More recently, PA-X, an alternative frameshift product of the PA gene, has been shown to modulate host responses and possibly host adaptation. We are therefore interested in assessing the intersection of these two factors and its effect on viral replication in mammalian and avian host cells.

Reverse genetics plasmids for the mammalian A/Puerto Rico/8/32 (H1N1) (PR8) and A/Uruguay/716/2007 (H3N2) and the avian A/duck/Suphanburi/AI157/2005 (H6N1) and A/duck/Hong Kong/365/1978 (H4N6) were generated. E/K mutations were introduced to PB2 at residue 627 and PA-X frameshift motif mutations (FS) to PA to reduce frameshifting into PA-X. We tested the impact of these mutations on viral polymerase activity through mini-genome assays in mammalian and avian cells. As the PA-X open reading frame (ORF) has been shown to down-regulate protein expression, we also checked expression of the polymerase components.

Avian mini-genomes exhibited extremely low polymerase activity in both mammalian and avian cells, likely due to PA-X-mediated suppression of the transfected viral polymerase components. PA FS dramatically increased H6N1 polymerase activity. Interestingly, this impact was greater than that observed with the E627K mutation. In mammalian cells, strong synergy as seen with PB2 K627 for both H4N6 and H6N1, resulting in polymerase activity comparable to PR8. In avian cells, however, the PA sequence was the most significant variable. For the mammalian viruses, neither the PA FS nor PB2 had much impact on polymerase activity in any context.

PA-X ORFs of mammalian and avian influenza viruses appear to differ in their impact on viral polymerase activity. Loss of the PA-X ORF along with PB2 K627 synergistically restores avian polymerase activity to mammalian virus-like magnitudes.

*Keywords: influenza, polymerase, PB2, PA, PA-X*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 10659

## DETECTION OF HIGHLY PATHOGENIC AVIAN INFLUENZA A(H5N6) VIRUSES IN WATERFOWL IN BANGLADESH

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### Introduction

Highly pathogenic avian influenza (HPAI) A(H5N6) and A(H5N8) viruses belonging to clade 2.3.4.4 have spread widely in Europe, Africa, the Middle East, and Asia in recent years. A(H5N6) viruses, in particular, have caused severe and fatal human infections in China. A surveillance platform was established in Bangladesh in 2007 in live bird markets (LBMs) to identify emerging HPAI viruses in poultry that pose a potential threat to human and animal health.

### Methods

Surveillance was conducted in 11 peri-urban and 19 urban LBMs in five divisions of the country from June 2016 to October 2016. The number of LBMs under surveillance were decreased to 10 from November 2016 to June 2017. We collected monthly cloacal swab samples from waterfowl, commercial chickens and backyard chickens. We also collected pooled environmental samples. Samples were initially screened for H5, H7 and H9 subtypes using rRT-PCR. All influenza A-positive samples were subjected to full genome sequencing analysis using IlluminaMiSeq technology.

### Results

Influenza A viruses were detected in 299 of 2,600 samples (11.5%). Of the positive samples, 15 subtypes were identified. Nine waterfowl, which appeared healthy at the time of sample collection, tested positive for HPAI A(H5N6) virus. One environmental sample was positive for A(H5N6) viral RNA. Sequencing of the HA gene segments of the ten A(H5N6) viruses confirmed that they belonged to clade 2.3.4.4. The viruses had several molecular markers associated with potential human infection. Molecular clock analysis of the A(H5N6) viruses estimated that the HA gene segments of these viruses emerged in Bangladesh in September 2016 and were closely related to other clade 2.3.4.4 HA gene segments detected in the Middle East and Africa around the same time period.

### Conclusions

Vigilant surveillance in LBMs, an important animal-human interface, is essential to identify emerging influenza viruses with the potential to threaten public and animal health.

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10515

## HUMAN MONOCLONAL IGA ANTIBODIES ELICITED BY SEASONAL INFLUENZA VIRUS VACCINATION EXHIBIT DISTINCT Fc-EFFECTOR ACTIVITY DEPENDENT ON EPITOPE-SPECIFICITY

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**INTRODUCTION:** The primary prophylaxis against influenza viruses is seasonal vaccination which prompts the production of antibody responses to the viral glycoproteins. The antibody repertoire produced is diverse and displays a large range of functional activities. The IgG isotype has been well characterized in terms of mechanisms utilized to neutralize influenza virus, but the IgA isotype has not been equally explored at the monoclonal level after vaccination. IgA1 antibodies may be more relevant for protection from influenza virus infection due to their abundance at respiratory mucosal surfaces, the virus' primary site of entry.

**METHODS:** A panel of eighty human IgA monoclonal antibodies (seventy-nine IgA1 and one IgA2) was isolated from eight donors seven days after vaccination with a quadrivalent inactivated influenza virus vaccine (QIV). These antibodies have been recombinantly expressed and characterized to determine binding specificity, activity in classical hemagglutination inhibition and microneutralization assays, and Fc-receptor engagement in a reporter-based system.

**RESULTS:** In this cohort, vaccination with QIV primarily elicited IgA antibodies against the hemagglutinin (HA) protein of the H1N1 component of the vaccine, though antibodies against the H3 and influenza B HA proteins were also observed. A subset of H1-specific IgA antibodies targeting distinct epitopes was expressed as monomeric, dimeric, and secretory IgA, as well as in an IgG backbone. In a neutrophil-based assay measuring Fc-effector activity utilizing primary human cells, all antibodies elicited responses in an IgG backbone. However, only antibodies targeting the stalk domain showed activity when expressed as IgAs.

**CONCLUSIONS:** IgA1 antibodies with diverse properties are elicited after vaccination with the seasonal influenza virus vaccine. This detailed functional analysis of the monoclonal IgA response to vaccination improves our understanding of the protective response against influenza virus and could aid future vaccine design.

*Keywords: Vaccination; IgA; Fc-effector; Human; Stalk-specific*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10668

## CHANGES IN CROSS-PROTECTIVE IMMUNITY DURING MATURATION OF THE ANTIBODY REPERTOIRE AGAINST INFLUENZA

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**Introduction and objective.** Naïve B cells, upon selection for antigen recognition, increase their binding affinity for antigen through the process of affinity maturation through somatic hypermutation. These mutations tend to congregate in the CDR regions of the antibody IgH and IgL chains, with IgH CDR3 generally believed to be particularly important for antigen specificity and binding avidity.

**Methods.** We have previously cloned a number of high-affinity antibodies (recombinant monoclonal antibodies; rmAb) targeting the hemagglutinin of A(H1N1)pdm09 influenza viruses. We reverted a number of these rmAb to their respective germ-line ("GL") sequences to assess the antigen affinity and biological functions of germ-line versions, compared to the matured versions that had undergone affinity maturation. We also made a series of revertants of an rmAb (145-C09) that binds across two antigenic sites, Ca and Sb, by reverting each IgH CDR to its GL sequence independently.

**Results.** Each GL rmAb had detectable, although reduced, ability to bind the HA of A(H1N1)pdm09, and most maintained *in vitro* neutralization and ADCC activity. For five of the eight rmAbs, broader cross-reactivity was seen for the GL revertant than for the mature version. For the 145-C09 rmAb, a single amino acid change in the IgH CDR2 region ablated all binding activity, while reversion of the IgH CDR3 mutation did not affect binding affinity or functional activity, indicating that the CRD3 region is dispensable for this particular antibody. Somatic mutations in the IgH CDR1 were critical for HI and MN activities.

**Conclusions.** Affinity maturation increases the effectiveness of anti-influenza antibodies, but in many cases reduces cross-reactivity, so that germ-line antibodies are more likely to be cross-reactive but of lower affinity than fully matured versions.

*Keywords: antibody affinity maturation*

Topic: Virology and Pathogenesis: Influenza Glycobiology

Abstract No: 11046

## GENETIC DIVERSITY AND RECEPTOR SPECIFICITY OF HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 DURING HUMAN INFECTION

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**Background:** Highly pathogenic influenza viruses (HPAI) are endemic in wild birds and poultry and continue to cause human infections with high mortality. To date, more than 850 confirmed human H5N1 virus infections have been reported, of which ~60% were fatal. Global concern persists that these avian influenza viruses will evolve into viruses that can efficiently transmit between humans, causing a severe influenza pandemic. Receptor specificity is a hallmark for human adaptation and evolution towards a transmissible virus. Avian viruses preferentially bind to  $\alpha$ 2,3-linked sialic acids, while human viruses bind to  $\alpha$ 2,6-linked sialic acids. Several key residues within the receptor binding site (RBS) of hemagglutinin have been identified that are important for the receptor switch.

**Objective:** We aimed to identify genetic diversity within the RBS of HPAI H5N1 during human infection using next generation sequencing and to characterize the effect on receptor specificity.

**Methods:** We performed whole genome deep sequencing of respiratory specimens from 44 H5N1-infected individuals from Indonesia and found substantial viral diversity within the RBS. Based on the genetic variants observed in the NGS data, we rescued mutant viruses, and performed virus replication, entry and stability assays to characterize virus fitness. In addition, we determined receptor specificity of each mutant.

**Results:** We rescued 37 mutant viruses and most substitutions in the RBS resulted in viable virus. However, virus replication, entry, and stability were often impeded. However, none of the tested substitutions resulted in a clear switch in receptor preference.

**Discussion:** Despite the presence of mutations at key residues involved in receptor specificity during human infection with HPAI H5N1 viruses, no substitutions were identified that caused a switch to human receptor usage and many of these changes resulted in less fit virus. Ongoing experiments suggest that a combination of multiple substitutions is required to result in binding to the human receptor.



Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10550

## CONSERVED EPITOPES OF INFLUENZA VIRUS NEURAMINIDASE AS A TARGET FOR UNIVERSAL INFLUENZA VACCINE DESIGN

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### Introduction and objectives:

Current influenza vaccine strategies mostly focus on developing antibody response against hemagglutinin (HA), whereas neuraminidase (NA) – a major target for influenza antivirals – is being largely ignored in vaccine development. Although current inactivated influenza vaccines contain neuraminidase, its quantity, quality is not standardized. It is known that influenza viruses easily escape from HA-specific antibody immunity due to the HA variable nature. In contrast, NA is a more conserved antigen and some NA-specific antibody can inhibit NA activity of any influenza A virus subtype (Doyle et al. 2013). Here, we extended this research by predicting new highly conserved NA epitopes of influenza A viruses, as an attempt to design broadly-reactive influenza vaccine.

### Methods:

NA amino acid sequences of different influenza A virus subtypes were taken from Influenza Virus Database. Multiple sequence alignment was performed by UGENE software. BepiPred, ElliPro, ABCPred, EpiToPIA, AAPPred, COBEpro programs were used for prediction of linear B-cell epitopes. Selected epitopes were chemically synthesized with purity >95% which confirmed by analytical electrophoresis, HPLC and MALDI TOF. The peptides were conjugated with BSA for immunological studies. Mice were immunized intraperitoneally with three doses of NA peptides two weeks apart and serum samples were used to measure antibody cross-reactivity to different influenza A viruses by ELISA.

### Results:

We described eight new highly conserved NA epitopes and showed that three of them could provide partial protection against a panel of virulent influenza A viruses. Mice sera were able to react with divergent influenza strains in ELISA (H1N1, H5N1, H2N2, H9N2, H7N3, H7N9).

### Conclusion:

The designed NA epitopes are immunogenic, though not sufficient for full protection against virus challenge. However, their addition can enhance the cross-protection of existing influenza vaccines. These conservative NA epitopes can be studied for design of broadly protective influenza vaccines in the future.

*Keywords: influenza neuraminidase, B-cell epitopes, vaccines, humoral immunity*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11342

## UNIVERSAL INFLUENZA DNA VACCINE INDUCES BROADLY SPECIFIC ANTIBODY AND T CELL RESPONSES IN MICE AND NONHUMAN PRIMATES AND AFFORDS CROSS-PROTECTION FROM INFLUENZA A CHALLENGES

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A universal influenza vaccine should induce strong immune responses that target multiple conserved viral regions. We developed a Universal Influenza (UFluA) DNA vaccine composed of four conserved influenza A antigens: The stem region of hemagglutinin, the matrix 2 protein ectodomain (M2e), the nucleoprotein (NP), and the matrix 1 protein (M1). To maximize immunogenicity, modulate responses toward Th1 phenotype and induce mucosal immunity, we also developed a genetic adjuvant that co-expresses the potent adjuvants IL-12 and RALDH2 or the mucosal adjuvant, heat-labile enterotoxin for *E. coli* (LT). We also employed a newly optimized gene gun that achieves direct intracellular delivery of the DNA into antigen presenting cells of epidermis and maximizes target size and cell viability. In mice, UFluA induced robust NP-specific T cell responses in the blood and lung mucosa as well as broadly specific, non-neutralizing M1, M2e and stem-specific antibody that bind antigen expressed on mammalian cells and afforded protection from heterologous H1N1 and H3N2 viruses. In nonhuman primates, gene gun delivery of only 16 mg of the DNA vaccine induced robust antibody along with NP-specific CD8+ T cell responses that localized to the lung and correlated with protection from a heterologous pandemic H1N1 challenge. In a second ongoing study in nonhuman primates, we show that 2-3 doses of the UFluA DNA vaccine induced strong stem, M1 and M2e-specific mucosal and systemic antibody and broadly specific UFlu-specific T cells expressing mucosal homing markers. Together, these results show considerable promise for the ability of our UFluA DNA vaccine to induce broadly specific immune responses targeting multiple conserved antigens, protection from heterologous challenges in mice and a preclinical nonhuman primate model and an important role for mucosal responses in protection. These results support further development of UFlu DNA vaccines for protection from circulating and emerging strains of influenza.

*Keywords: universal influenza vaccine, DNA vaccine, gene gun, nonhuman primate model*

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 10555

## IMPROVED DETECTION OF INFLUENZA A VIRUS IN WILD DUCKS BY SEQUENCING DIRECTLY FROM SWAB MATERIAL.

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**Introduction:** The greatest diversity of influenza A virus (IAV) is found in wild aquatic birds of the orders *Anseriformes* and *Charadriiformes*. In these birds, IAV replication occurs mostly in the intestinal tract with occasional involvement of the respiratory tract. Fecal, cloacal and/or tracheal swabs are typically collected and tested by real-time RT-PCR (qRT-PCR) and/or by virus isolation in embryonated chicken eggs in order to determine the presence of IAV. Virus isolation may impose bottlenecks that select variant populations that are different from those circulating in nature, and such bottlenecks may result in artifactual representation of subtype diversity and/or underrepresented mixed infections. The advent of next generation sequencing (NGS) technologies provides an opportunity to explore to what extent IAV subtype diversity is affected by virus isolation in eggs.

**Objective:** In the present work, we evaluated the advantage of sequencing by NGS directly from swab material of IAV rRT-PCR positive swabs collected during the 2013-14 surveillance season in Guatemala and compared to results from virus isolation.

**Methods:** Samples were collected from hunter-killed ducks during the winter migration season 2013-2014 in the villages of El Pumpo, in the department of Santa Rosa; Pasaco, in the department of Jutiapa and La Gomera, in the department of Escuintla, Guatemala. Samples were processed for virus isolation or subjected directly to NGS.

**Results:** Sequencing IAV genomes directly from swabs led to improved detection of subtype diversity and of alternative amino acid motifs that were not captured by virus isolation. The NGS sequencing data from swabs revealed reduced presence of defective interfering particles compared to virus isolates.

**Conclusions:** We propose an alternative workflow in which original swab samples positive for IAV by rRT-PCR are first subjected to NGS before attempting viral isolation. This approach should speed the processing of samples and better capture natural IAV diversity.

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 11275

## ACTIVE SURVEILLANCE FOR AVIAN INFLUENZA VIRUS IN POULTRY IN VIETNAM, 2017 – 2018

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Vietnam borders China, Laos and Cambodia, which have endemic avian influenza (AI) viruses including H5N1, H5N6 and H7N9. Humans and poultry cross the borders daily and illegally and represent a risk of introduction of AI into Vietnam.

During February 2017 to November 2018, we conducted active surveillance for AI viruses in poultry. Oropharyngeal swabs of healthy appearing poultry (chickens, ducks and Muscovy ducks) and environmental samples (feces and water) were taken from 160 live bird markets (LBM) and culling areas for smuggled poultry in 15 high-risk provinces selected purposely based on history of AI outbreaks, sharing borders with China, high demand of poultry consumption. Samples of five individual birds were pooled and tested for the influenza A matrix (M) gene; positives were tested for H5N1, H5N6 and H7N9 HA and NA subtypes using real-time RT-PCR. U.S. CDC in Atlanta generated complete genome sequence for selected viruses.

A total of 7,487 pooled samples comprised of 6,193 pooled poultry samples from 30,965 individual birds and 1,294 pooled environmental samples were collected and tested; 2,311 (31%) samples were positive for influenza A virus RNA and 177 (7.7%) were positive for H5, 98 for H5N1 (4.2%), 50 for H5N6 (2.2%) and 21 (0.9%) for H7. None of the H7 samples were positive for H7N9 virus and sequence analysis confirmed these were not of the A/Anhui/1/2013-lineage. Most viruses were closely related to previously described viruses and CVVs A/Hubei/29578/2016, A/duck/Hyogo/1/2016 and A/chicken/Vietnam/NCVD-15A59/2015.

Approximately 8% of samples were positive for H5 (H5N1, H5N6) and 0.9% were positive for H7 virus. Active surveillance detected H5Nx viruses and demonstrates the risk of continued poultry outbreaks. Therefore, continued surveillance for AI virus in Vietnam is critical for timely detection and response.

Topic: Virology and Pathogenesis : Viral Replication

Abstract No: 10911

## A class of influenza virus fusion inhibitors with nanomolar activity and the same HA binding pocket as arbidol

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**Introduction and objectives.** Influenza virus hemagglutinin (HA)-mediated fusion of the viral and endosomal membranes requires a drastic and low pH-induced conformational change in HA. This refolding can be prevented by small molecule inhibitors like tert-butylhydroquinone (TBHQ) and arbidol, a drug undergoing phase 3 evaluation. Cocystallographic data showed that TBHQ and arbidol have an overlapping binding pocket in the H3 HA stem. In this study, we explored the mechanism of action of a novel and remarkably potent class of H3 HA-specific fusion inhibitors.

**Methods.** A series of indole-substituted spirothiazolidinone compounds was chemically synthesized. Their anti-influenza virus activity was determined by cytopathic effect reduction assays combined with cytotoxicity testing. Inhibition of HA-mediated fusion was measured in a polykaryon assay in HeLa cells expressing H3 HA in wild-type or mutant form (E57K and D112N; both situated in the HA2 subunit). For compound docking, we used the published cocystal structures of H3 HA complexed to TBHQ or arbidol.

**Results.** We identified a class of fusion inhibitors with unprecedented nanomolar activity against influenza A/H3N2 virus. The best analogue **5f** displayed an antiviral EC<sub>50</sub> value of 0.8 nM and selectivity index of almost 2000, which makes it the strongest influenza virus fusion inhibitor ever reported. It produced full inhibition in the H3 HA polykaryon assay with an EC<sub>50</sub> value of 0.9 μM. This pronounced potency shift between the antiviral *versus* polykaryon assay indicates that **5f** possesses the capacity for endosomal accumulation, a favorable characteristic not recognized with fusion inhibitors before. **5f** was inactive against E57<sub>2</sub>K-mutant H3 HA protein, consistent with *in silico* models of its HA binding mode. The E57<sub>2</sub>K mutant was resistant to arbidol but not TBHQ.

**Conclusion.** While having an overlapping binding pocket with the known fusion inhibitors TBHQ and arbidol, our inhibitors have far higher activity with obvious relevance for antiviral drug development.

**Keywords:** antiviral, arbidol, hemagglutinin, fusion, H3N2

Topic: Virology and Pathogenesis : Viral Replication  
Abstract No: 11177

## Characterization of three human-like neutralizing H5N6 monoclonal antibodies obtained using B cells from vaccinated macaques

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### Introduction and Objective:

Since the first human infection of H5N1 influenza A virus in 1997, the virus has evolved and reassorted to give rise to different clades of H5N1 as well as H5Nx viruses. In May 2014, the first human case of H5N6 infection was identified in China and subsequently, ~20 people were confirmed to have been infected by H5N6. Recently, another 3 cases of human H5N6 infections were detected in China between September 2018 and February 2019. The objective is to use B cell sorting to isolate monoclonal antibodies (mAb) from vaccinated macaques.

### Methods and Results:

Several mAbs have been isolated. Based on ELISA, three mAbs were found to bind to hemagglutinin (HA) *proteins of H5N1, H5N2, H5N6 and H5N8 viruses. Furthermore, they were found to be positive in microneutralization assay performed with H5N6 virus generated using reverse genetics (rg). Interestingly, only two of them have hemagglutination inhibition activities* suggesting that they are binding to the head domain in HA. To determine their protective effects *in vivo*, rgH5N6 virus containing the HA segment of H5N6 and 7 other segments from A/PR/8/1934(H1N1) was used to infect Balb/c mice at 100, 1,000 or 10,000 plaque-forming unit (PFU). From the weight loss, it was observed that rgH5N6 is highly pathogenic and all the mice lost > 25% of its original body weight by day 6. High levels of virus were detected in the lungs indicating successful infection and replication of rgH5N6 in mice.

### Conclusion:

By using this mouse model, the protective effects of the 3 macaque-derived mAbs against H5N6 infection were determined and compared.

*Keywords: H5N6 monoclonal antibodies; vaccinated macaques;*

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 11201

## INTERACTION BETWEEN DEFINED REGIONS OF PB1 AND NA GENE SEGMENTS DRIVES COSEGREGATION OF THESE GENES DURING INFLUENZA REASSORTMENT

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### Introduction

The segmented nature of the RNA genome of influenza A virus (IAV) allows it to undergo reassortment when two strains co-infect the same cell. This process is a major contributing factor in the emergence of novel pandemic strains.

### Methods

To understand the factors that govern gene selection during reassortment we have used two complementary approaches. A nine-plasmid competitive transfection model, which places one gene segment from either A/Puerto Rico/8/34 (PR8, H1N1) or A/Udorn/307/72 (Udorn, H3N2) in competition to be packaged into progeny virions where the other gene segments are fixed, and sequencing of psoralen crosslinked, ligated, and selected hybrids (SPLASH), that maps RNA interactions between segments by high-throughput sequencing.

### Results

We showed previously that the Udorn NA and PB1 gene segments preferentially co-package into progeny virions, driven by nucleotides 1776-2070 of the central-coding region of the Udorn PB1 gene. Sequence comparison of Udorn NA gene with that of A/Wyoming/3/03 (H3N2), which lacks the determinants for PB1-NA co-selection, subsequently allowed us to pinpoint a stretch of 20 complementary nucleotides in this region sufficient to bias the reassortment outcome. Site-directed mutagenesis of the Wyoming NA gene towards the Udorn sequence restored complementarity and co-selection of the Wyoming PB1 gene. SPLASH analysis, performed independently, identified the exact same interacting regions between the Udorn NA and PB1 genes. To examine the effects of reassortment on the RNA-RNA interaction network, SPLASH was performed on reassortant viruses containing gene segments from Udorn or Wyoming with the remaining segments from PR8. We found that reassortant viruses inherit their interaction network from both parents, suggesting that the RNA interaction network is highly flexible and able to accommodate new gene constellations.

### Conclusions

The redundancy of RNA interactions between the different vRNPs explains how IAVs maintain the potential for reassortment between different strains, while also retaining packaging selectivity.

*Keywords: Influenza virus; Reassortment; Viral packaging; Viral assembly; Viral replication*

Topic: Virology and Pathogenesis : Viral Replication  
Abstract No: 11256

## EXOSOME: AN RNAi MESSENGER IN STEALTH

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### Introduction

In RNAi therapeutics, naked unformulated oligonucleotides are prone to nucleolytic-extracellular environments. The formulations of viral-like particles, liposomes or use of cationic polymers evoke antibody-neutralisation, complement activation or apoptotic cascade. Exosomes are amongst the several delivery systems providing a more substantial benefit to risk ratio than unformulated drugs. In this study exosomes derived from human lung carcinoma cells were used as the vehicle to safely deliver the siRNA and miRNA in host cells to inhibit PB1 expression and further downregulate the viral replication.

### Method

Exosomes were isolated from A549 cells cultured in exosomes-depleted media, and their presence was well-characterised through western blot and TEM. Both, the cells and freshly purified exosomes, were electroporated with target-specific RNAi molecules (against viral PB1 gene and host XO gene) and RT-qPCR validated its efficiency. Successively, post-infection by influenza A virus, infected cells were incubated with pre-purified exosomes carrying RNAi cargo. RT-qPCR verified downregulated mRNA levels after the knockdown.

### Result

An effective downregulation of influenza A virus PB1 and xanthine oxidase (XO) genes was observed concurrently with decreased replication of infectious viral particles. miR-323 was used to downregulate the PB1 gene, binding to the conserved region and had been found effective against multiple strains of influenza virus. The exosomal-RNAi cargo provided the safe traffic without any direct interaction with immunogenic particles which might have degraded the RNAi. Since RNAi molecules were post-transcription regulators, the expression of proteins was further exploited and found downregulated at the proteomic platform.

### Conclusion

In this study, we ought to emphasise on the exosome as the best delivery vehicle for RNAi. Our results demonstrated that exosome-mediated combinatorial antisense therapy against the virus as well as host gene could be a better therapeutic alternative. Since exosomes are of host origin, they pose minimum toxicity to the system, suggesting further *in vivo* study.

*Keywords: Influenza; exosome; delivery; RNAi; antisense*



Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission

Abstract No: 10827

## Fitness and Ferret Transmissibility of Influenza A and B Viruses Harboring Clinically Significant (I38T/F/M) Baloxavir Marboxil Resistance Substitutions

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**Introduction & Objectives:** The endonuclease inhibitor Baloxavir Marboxil (BXM) was approved in 2018 (US, Japan) for influenza treatment. Viruses with PA I38T/F/M substitutions have emerged under treatment and exhibited reduced BXM susceptibility; the impact of these variants on treatment success will likely be determined by their fitness. Here, we investigated fitness and ferret transmissibility of influenza A and B viruses with these clinically relevant substitutions.

**Methods:** We generated recombinant A/California/04/2009(H1N1)pdm09, A/Texas/71/2017(H3N2), and B/Brisbane/60/2008 viruses with wild-type (WT) PA I38 and I38T/F/M variants. Baloxavir acid (BXA) susceptibility was determined by virus yield and plaque reduction. PA endonuclease domain (PA<sub>N</sub>) activity was measured by nucleotide substrate cleavage, or in mini-replicon assays. Multi-step growth curves and competitive infections were conducted in MDCK cells. Donor ferrets were inoculated with each virus ( $1 \times 10^5$  TCID<sub>50</sub>/ml) and housed with naïve direct and aerosol contacts.

**Results:** Viruses with I38T/F/M substitutions exhibited reduced BXA inhibition compared to WT (EC<sub>50</sub>, 2-21 or 40-50 nM for A and B viruses, respectively). PA<sub>N</sub> with I38T/F/M substitutions was impaired in substrate cleavage ( $V_{max}$ , 47-71% of WT) but, surprisingly, this was not observed in mini-replicon assays. Influenza A viruses with I38T/F/M substitutions showed minor differences in replication efficiency in MDCK cells, although, WT influenza A viruses outcompeted I38T variants in the absence of BXA. I38T and I38F, but not I38M, substitutions impaired influenza B virus replication. The pathogenicity and transmissibility of the viruses in ferrets varied by substitution and backbone.

**Conclusions:** The fitness costs of BXM resistance-associated substitutions are apparent in direct protein-substrate processing, but less significant in the context of polymerase complex activity or virus infections. Worryingly, some viruses with resistance-associated substitutions behaved similarly to WT viruses and retained pathogenicity in ferrets with transmissibility to naïve contacts. This raises the risk for potential I38T/F/M emergence in BXM-treated populations.

**Keywords:** Antiviral, PA polymerase, Baloxavir, Ferret, Fitness

Topic: Public Health: Disease Burden & Severity  
Abstract No: 10578

## ESTIMATION OF THE INFLUENZA-ASSOCIATED EXCESS MORTALITY IN REAL-TIME IN HONG KONG, 2012-2018

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**Introduction and Objectives:** Statistical models are commonly employed in the estimation of influenza-associated excess mortality that, due to various reasons, is often underestimated by laboratory-confirmed influenza deaths reported by healthcare facilities. However, methodology for timely and reliable estimation of that impact remains limited because of the delay in mortality data reporting. We explore the potential for using a combination of a statistical model fitted to historical data as well as real-time information on influenza activity to predict the impact of influenza epidemics in real-time before mortality data become available.

**Methods:** We explored real-time estimation of influenza-associated excess mortality by types/subtypes in each year between 2012 and 2018 in Hong Kong using linear regression models fitted to historical mortality and influenza surveillance data. The excess mortality was estimated by subtracting the predicted mortality estimated from the fitted model setting influenza activity to zero from the predicted mortality from the model based on the observed influenza activity.

**Results:** Our real-time influenza-associated excess mortality estimates were robust, compared to the retrospective estimates using the same models fitted to all available mortality and influenza surveillance data. We could predict that during the winter of 2017/2018, there were approximately 634 (95% CI: (190, 1033)) influenza-associated excess all-cause deaths in Hong Kong in adults, compared to 259 reported laboratory-confirmed deaths. We estimated that influenza was associated with substantial excess deaths in older adults, suggesting the implementation of control measures, such as administration of antivirals and vaccination, in that age group.

**Conclusion:** The approach that we developed appears to provide robust real-time estimates of the impact of influenza circulation, and complement surveillance data on laboratory-confirmed deaths. These results improve our understanding of the impact of influenza epidemics and provide a practical approach for a timely estimation of the mortality burden of influenza circulation during an ongoing epidemic.

Topic: Public Health: Disease Burden & Severity  
Abstract No: 10828

## Crowdsourced Genealogy Data Reveals the Mortality Footprints of the 1918-1919 Influenza Pandemic

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**Introduction** – The devastating mortality impact of the 1918-19 influenza pandemic has been well documented in the Americas and Western Europe; however, a globally-representative understanding of this pandemic is lacking.

**Methods** – We analyzed global demographic data from the Familinx genealogy project, which contains 86 million crowd-sourced individual profiles spanning 5 centuries. We estimated the timing, impact and age-specific patterns of monthly all-cause mortality associated with the 1918-19 pandemic in the top 14 countries of Europe, North America, Africa and the Pacific, that contributed most deaths to Familinx.

**Results** – The timing and age profile of 1918-19 pandemic mortality recovered from Familinx in the US and Denmark aligned with past literature. Pandemic mortality peaked in October-November 1918 in most countries but was delayed until July 1919 in Australia, in line with the strict quarantine imposed by this country. Mortality impact ranged 35-fold across countries, with New Zealand and South Africa being hardest hit (excess mortality 172% and 90% over annual baseline, respectively), and Denmark faring the best (relative excess mortality 5%). Adults aged 20- 40 yrs experienced elevated pandemic mortality risk in all countries, although the exact age profile differed between countries. Intriguingly, pandemic mortality in New Zealand, Australia and South Africa was shifted to older ages, relative to other countries.

**Conclusion** – Genealogy databases are valuable tools for characterizing major historical infectious disease events affecting mortality; our analysis reveals important disparities in the global burden of the 1918-19 pandemic. Observed differences in the age profiles of pandemic mortality could be due to sampling bias or reflect regional differences in the history of influenza circulation in the 19<sup>th</sup> Century. Further analyses of genealogy databases could help inform the risk of familial aggregation in influenza-related deaths and test hypotheses involving immune imprinting by birth cohort on a global scale.

*Keywords: 1918 pandemic, genealogy, age profile, excess mortality, imprinting*

Topic: Public Health: Epidemiology & Transmission  
 Abstract No: 10999

## CROSS SECTIONAL SURVEY FOR ASSESSMENT OF AEROSOLIZATION OF INFLUENZA A VIRUSES AND POTENTIAL TRANSMISSION RISK FOR LIVE BIRD MARKET WORKERS AT THE ANIMAL-HUMAN INTERFACE IN BANGLADESH

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### Introduction

Influenza A virus (IAV) subtypes H5N1 and H9N2 are endemic among poultry in Bangladesh and poultry are frequently purchased at live bird markets (LBM). We assessed the presence of aerosolized IAVs at LBMs and potential occupational risk to LBM workers.

### Methods

A cross-sectional study was conducted from January to May 2017 among 702 randomly sampled asymptomatic workers at 42 LBMs (selected probability proportional to market size) in Dhaka, Bangladesh. Nasal and throat swabs from workers and air samples collected at LBMs (using cyclonic and impactor air samplers) were tested for IAVs by rRT-PCR; selected air samples (Ct value <38) were placed into viral culture.

### Results

Most LBMs were retail and three had separate slaughtering areas. None practiced market rest days or banned poultry overnight. 60% (25/42) had air samples positive for Influenza A, 31% were positive for H5 and H9, 23.8% for H9 only, one for H5 only, and one was non-subtypeable. IAVs were isolated from 53% (16/30) of rRT-PCR positive air samples. Selling ducks was associated with influenza A in air samples [aOR 15.8 (95% CI:2.2-115.7, p=0.002), adjusted for season, solid waste disposal and cleaning frequency].

Of 702 workers, 93.3% were involved in slaughtering and 84.3% in defeathering. 10.8% of workers had IAV positive, H1 and H3 negative, respiratory specimens: 4% were positive for H5 and H9, 31.6% H9 positive, and 64.5% were negative for H5, H7, H9. Ducks were associated with IAV positive worker respiratory specimens [aOR 3.1 (95% CI: 1.0-9.5, p=0.044), adjusted for age, season, LBM type, presence of non-poultry birds, and use of defeathering machine].

### Conclusion

Bangladesh LBM workers were exposed to aerosolized viable influenza A viruses and IAV RNA was detected in respiratory specimens of some workers. Ducks were associated with aerosolization of influenza A viruses and influenza A virus exposure of LBM workers.

**Keywords:** Influenza A Virus; Live Bird Market; Bangladesh; Avian Influenza; Pathogen Transmission

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11061

## Multi-annual models of seasonal flu at high spatial resolution

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### Introduction and Objectives

Forecasting the burden of seasonal flu is a topic of global interest. Our existing studies show the presence of non-trivial variability in attack rate of influenza within a large urban area, and illustrate a range of mechanistic drivers of this phenomenon within the context of a pandemic, namely infection-dependent mobility, distribution of age, and travel patterns.

### Methods

In this study, we develop a discrete, iterative final-size model of seasonal flu with heterogeneity in age and space. We offer a general parametrised framework for describing antigenic drift, and for 1 or 2 co-circulating subtypes with cross immunity.

### Results

We show how the interplay between drift and cross immunity can generate patterns of heterogeneity in the burden of seasonal epidemics, identifying areas in parameter space in which our model has predictive power. Heterogeneity due to natural history is quantified by comparison with the single-subtype, pandemic case, and model results are fitted to attack rates estimated using available serological data. We use country-level, longitudinal data to approximate existing immunity and study the effect of delay in seeding between 2 subtypes in a given season.

### Conclusion

Our work offers a predictive within-season model, based on high-resolution attack rates of prior seasons and on our best estimates for parameters describing the natural history of multiple subtypes. We quantify the implications data-driven assumptions regarding the burden of seasonal flu, identifying the boundaries of feasibility in forecasting.

**Keywords:** *Seasonal; urban; spatial; heterogeneity*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11075

## Small and variable influenza epidemics in Australian cities suggest that host contact structure may limit the effects of climate and antigenic evolution on local influenza epidemiology

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### Introduction and objectives

Although influenza viruses circulate globally, prevention and treatment necessarily occur at the level of regions, cities, or small communities. At these scales, seasonal influenza virus epidemics vary substantially in timing, duration, and size, and the underlying causes of this variation are poorly understood. Here we use a 15 year city-level data set consisting of 18,250 laboratory confirmed and antigenically characterised influenza virus infections from Australia to investigate the impact of environmental and virological factors on the timing and magnitude of city-level influenza virus epidemics. We use a mathematical model of epidemiological dynamics to investigate how the effects of climatic and virological factors may be modulated by host contact structure within a population.

### Methods

We analyse the variability of city-level influenza epidemics and compared epidemiological patterns with previously hypotheses concerning environmental and virological drivers.

Using a dynamic weighted contact network model approach, we quantify the relative contributions of population immunity, antigenic change and population structure on local-scale epidemiological dynamics.

### Results

We find that the timing of local epidemics in Australia is not associated with anomalous fluctuations in temperature and humidity. Virus antigenic change does not have an observable effect on the magnitude or timing of epidemics.

Our modelling work shows that the disease spread is heavily influenced by the network properties of the underlying contact structure of a population. Population structure can limit the depletion of susceptible individuals and thus lessen the epidemiological impact from the build-up population immunity and antigenic change.

### Conclusion

These findings suggest that local-scale population immunity may be weaker than expected and that influenza epidemiology in Australia is influenced more by stochastic processes than by easily quantifiable environmental or virological variables. New understandings of local-scale influenza epidemiology and population contact patterns are required before meaningful epidemic forecasting is feasible.

*Keywords: local-scale epidemiology; network structure; climatic factors; antigenic change; population immunity*

Topic: Public Health: Human Sero-Epidemiology Studies  
Abstract No: 10801

## VARIATION BY LINEAGE IN ANTIBODY RESPONSES TO INFLUENZA B VIRUS INFECTIONS

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### Introduction

Influenza B virus infections are responsible for a considerable fraction of all influenza-associated morbidity and mortality. Studies on antibody response against influenza B virus by lineage are limited. We examined changes in antibody titers against influenza B virus lineages among PCR-confirmed cases.

### Method

Data were obtained from a community-based cohort study conducted in Hong Kong since August 2009. Serum specimens were collected from every household member at baseline and at 6-month intervals thereafter for 5 years, where the serum antibody titers were determined by HAI. A pooled nasal and throat swab was collected for lineage determination by PCR if any household member reported 2 or more symptoms of upper respiratory tract infection. We constructed a statistical model to describe the antibody titer dynamics during infection, considering the effect of age, the pre-infection titer, the influenza B lineage, and cross-lineage reaction on the magnitude of titer boosting.

### Result

During the study period, we identified 35 and 27 PCR-confirmed influenza infection with B/Victoria and B/Yamagata lineage respectively. Nearly half of the cases had both pre-infection and post-infection HAI titers <1:10 against the lineage of infection. Among those who showed antibody titer response, infections in children led to mean fold rises of >11 in the lineage titer of infection. In terms of cross-reactions, infections with B/Victoria led to mean fold rises of B/Yamagata titers of 2.0, while infections with B/Yamagata led to mean fold rises of B/Victoria titers of 1.7. The titer boost against infection with B/Yamagata lineage was estimated to have a smaller degree of dependence on the pre-infection titer and a larger magnitude in adult, compared with that against B/Victoria infection.

### Conclusion

The antibody titer response against influenza B infection could be different by lineage, where the titer boost was subject to age, pre-infection titer and cross-lineage antibody response.

*Keywords: influenza B virus infection; influenza antibody; statistical modelling*

Topic: Public Health: Human Sero-Epidemiology Studies  
Abstract No: 10902

## ANTIBODY TITRES ELICITED BY THE 2018 SEASONAL INACTIVATED INFLUENZA VACCINE PERSIST FOR AT LEAST 6 MONTHS.

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### Introduction

In Australia, the seasonal inactivated influenza vaccine is typically offered in April; however, the onset, peak, and end of the influenza season varies and the optimal timing for vaccination is unclear. This study aimed to investigate the pattern of decay of the vaccine-induced antibody response over 6 months in different age groups.

### Methods

We conducted a serosurvey among staff aged 18-50y and volunteers aged 65+ ("ambulatory elderly") at two hospitals and residents aged 65+ ("frail elderly") at two aged care facilities. Participants aged <65 years received the 2018 southern hemisphere quadrivalent vaccine and those aged >65 years received enhanced trivalent vaccine (either high dose or adjuvanted). Serum samples were collected at baseline and 1, 2, 4, and 6 months post-vaccination. Antibody titres against vaccine antigens were measured by haemagglutination inhibition assays. Geometric mean titres (GMTs) were estimated using random effects regression.

### Results

Our final sample consisted of 67 adults, 14 ambulatory elderly and 12 frail elderly. Estimated GMTs increased more than 2-fold for all viruses, peaking 1 month post-vaccination at 52 for A(H1N1)pdm09 (95% prediction interval (PI):42,65), 108 for A(H3N2) (95%PI:85,136), 140 for B/Yamagata (95%PI:114,173), and 85 for B/Victoria (95%PI:68,109; not included in trivalent vaccine). Titres declined by 3 months post-vaccination, but thereafter remained steady, with 6 month post-vaccination GMTs 1.4-, 1.3-, 1.9- and 1.9-fold above baseline for A(H1N1)pdm09, A(H3N2), B/Yamagata and B/Victoria, respectively. Decay trajectories were similar for the 3 groups; the group with the highest baseline titre for each subtype remained highest throughout the study.

### Conclusions

All three vaccines elicited a significant increase in antibody titre that peaked 1 month post-vaccination for all vaccine components. Notably, titres persisted above baseline for 6 months and the pattern of antibody decline was similar among different age groups. Our data suggest that antibody-mediated protection should last at least 6 months.

*Keywords: Antibody; Vaccine; Kinetics*



Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 11284

## Shedding of Respiratory Viruses in Human Exhaled Breath and Efficacy of Face Masks in Reducing Viral Dissemination

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**Introduction and Objectives:** There is little data on the efficacy of facemasks as a source control to prevent respiratory virus transmission. We aimed to examine shedding of different respiratory viruses in human exhaled breath, and to determine the potential benefits of facemasks to prevent respiratory virus transmission.

**Methods:** We recruited outpatients age  $\geq 11$ y within 72 hours of ARI symptom onset and collected their nose/ throat swabs. Exhaled breath particles was collected by a bioaerosol collecting device (G-II) which fractionated the exhaled breath into either large droplets ( $\geq 5\mu\text{m}$ ) or small aerosols ( $< 5\mu\text{m}$ ). Patients were randomly allocated to wear or not to wear a surgical facemask for the 30-minute collection, and invited to provide a second collection of the alternate type. Viral aetiology was determined by testing nose swabs with the xTAG Respiratory Viral Panel, and viral RNA quantified by RT-PCR in nose/ throat swabs and the two exhaled breath fractions.

**Results:** 317 exhaled breath samples were collected from 264 patients. 154/264 (58%) patients had respiratory virus infection as determined by xTAG, mostly enterovirus/ rhinovirus (25%), influenza virus (19%) and coronaviruses (11%). Respiratory virus RNA was detected in the exhaled breath of 19/154 (12%) participants. There was no significant difference in viral RNA detection rates between face mask and control groups in the droplets ( $p=0.10$ ) or aerosols ( $p=0.44$ ).

**Conclusions:** Our study is the largest to date to examine release of respiratory viruses in exhaled breath, and makes an important contribution to our understanding of the infectiousness of different respiratory viruses in respiratory droplets and the efficacy of surgical facemasks in preventing respiratory virus transmission.

Topic: Public Health: Pandemic Preparedness  
Abstract No: 10672

## FROM THE FIELD TO A VACCINE CANDIDATE: A PROOF OF CONCEPT STUDY FOR ADVANCING INFLUENZA PANDEMIC PREPAREDNESS

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### Introduction:

As part of CDC's influenza pandemic preparedness efforts, we are exploring advanced technologies for early detection of zoonotic viruses at the animal-human interface to drive proactive candidate vaccine virus (CVV) generation prior to human transmission events. In this proof-of-concept study, we detected and sequenced influenza viruses at a swine exhibition and created a CVV in advance of multi-state zoonotic infections.

### Methods:

Nasal swabs were collected from exhibition swine and viral genomes were sequenced onsite using Nanopore sequencing technology. Viral sequences were emailed from the field, analyzed by alignment and phylogenetics, and used to design synthetic gene segments for reverse-genetics rescue of CVVs. A CVV in compliance with Good Laboratory Practice (GLP) standards (GLP-CVV) was generated in cGMP Vero cells and propagated in specific-pathogen-free chicken eggs. Multiple assays were performed to characterize the GLP-CVV.

### Results:

Digital sequences of swine influenza A viruses were transmitted to CDC in real time for analysis, and engineering of synthetic hemagglutinin and neuraminidase genes for optimal vaccines. Research-grade H1N2 CVVs were rapidly generated to characterize the viruses and GLP-CVV development was initiated in anticipation of potential human outbreaks of the predominant virus detected at the exhibition. One month after the exhibition, the first human infection by a genetically closely related H1N2v virus was detected and 12 additional cases from multiple states were subsequently reported to CDC within the next month. Proactive development of the CVV significantly accelerated the completion timelines, the initiation of comparison to outbreak viruses, and validated this proof-of-concept approach for pandemic preparedness.

### Conclusion:

Active surveillance of animal influenza virus at a potential animal-human interface enabled development of a synthetic CVV against zoonotic H1N2 swine influenza viruses with pandemic potential before they caused human infections. This strategy will be employed in the future to enhance vaccine preparedness for influenza pandemics.

*Keywords: Candidate Vaccine Virus; GLP; Pandemic Preparedness*

Topic: Public Health: Pandemic Preparedness

Abstract No: 10757

## RISK ASSESSMENT ON LUNG INJURY AND TRANSMISSION POTENTIAL OF INFLUENZA VIRUSES USING IN VITRO AND EX VIVO MODELS OF HUMAN RESPIRATORY TRACT

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**Introduction:** Preparation for influenza pandemics remains a public health challenge and risk assessment of influenza viruses is key to tackle this. Current risk assessment tools evaluate the properties, population and ecology of a virus. While acute lung injury (ALI) has been the main cause of deaths of highly pathogenic avian influenza (HPAI) viruses, like H5N1, there is limited risk assessment tool to evaluate the potential of virus to cause ALI. Different influenza virus strains infect and replicate in human respiratory tract differently to affect the pathogenicity and transmission potential. However, this cannot distinguish the disease severity of different virus strains. Previously, we have used a physiologically relevant in vitro ALI model to distinguish the ability of H5N1 and H1N1 viruses to cause ALI through measuring the rate of alveolar fluid clearance (AFC). We proposed to use this in vitro model to risk assess the ability of different influenza virus subtypes to cause ALI, as well as ex vivo explant culture of human respiratory tract to evaluate virus pathogenicity and transmission potential.

**Method:** Primary culture of human alveolar epithelial cells in a transwell and human lung and bronchus explants were infected with different influenza virus subtypes (including avian surveillance isolates). AFC, virus infectivity and replication of ex vivo explants were measured.

**Result:** We found that H1N1, H3N2, H5N1, H5N6 and H7N9 have differential viral replication in human lungs. HPAI H5N1 and H7N9 induced severe AFC impairment compare to seasonal influenza A and influenza B viruses. High preference of bronchus tropism was found in seasonal and pandemic H1N1 viruses but minimal in HPAI H5N1 viruses.

**Conclusion:** In summary, evaluation of virus-induced lung injury can differentiate the ability of viruses to cause severe disease in humans, which in addition enhances the risk assessment of animal influenza viruses identified from ongoing surveillance.

Topic: Public Health: Pandemic Preparedness  
Abstract No: 11237

## POTENTIAL PUBLIC HEALTH BENEFITS FROM REDUCED DELAY IN THE PRODUCTION OF PANDEMIC INFLUENZA VACCINE

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### Introduction

Non-human strains of influenza pose a constant pandemic threat which is only partially mitigated by vaccine. Existing technology and production capacity is expected to produce 400 million doses after a 180 day lead time.

### Methods

Here, we used a global model of influenza transmission and control to investigate the benefits of faster vaccine production. We simulated a moderately severe pandemic with similar epidemiological characteristics to the 2009 pandemic strain other than infection fatality rate, which was set to be 10x more severe than the 2009 strain (but still 20x less severe than 1918). We considered different weekly distribution strategies of a 70% effective vaccine: current allocation, with vaccines distributed to nations in the the same proportions to current usage of seasonal vaccine; incidence allocation, with vaccines distributed in proportion to infection incidence at that time; and a set of more complex interventions based on functions of population size.

### Results

We estimated that with a production delay of 180 days, for our moderately severe scenario, both current allocation and incidence targeting would avert fewer than 100,000 deaths over a scenario with no vaccine at all. For a delay of 90 days, we estimated that ~400,000 deaths could be averted with either current allocation strategies or with allocation according to incidence. More than 600,000 deaths could be averted if production could be started immediately. Also, it is possible that even higher numbers of deaths could be averted if proportionally more vaccines were deployed to large countries.

### Conclusions

Faster strain-specific influenza vaccine production should continue to be a priority even as universal or broadly-effective vaccines are being developed. Our model likely under-estimates the per-dose effectiveness of early vaccines in large countries. Globally optimal strategies for vaccine allocation will be highly inequitable.

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 10374

## IMPROVING EPIDEMIC FORECASTS WITH BEHAVIOURAL INSIGHTS GAINED FROM COMMUNITY-LEVEL SURVEILLANCE

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### Introduction and Objectives

Changes in healthcare-seeking behaviours and clinical decision-making substantially affect traditional influenza surveillance data. This presents a challenge when using these data in near-real-time. Australia experienced a particularly large and severe influenza season in 2017, and an increased perception of risk in the community may have affected healthcare-seeking behaviour. The objective of this study was to determine whether self-reported participant data collected by the Flutracking surveillance system would allow us to account for changes in these behaviours and improve the performance of our seasonal influenza forecasts.

### Methods

We used weekly Flutracking surveillance data to estimate the probability that a person with influenza-like illness would seek healthcare and have a specimen collected for testing. We then used this estimated probability to calibrate seasonal influenza forecasts (which were generated using weekly counts of laboratory-confirmed influenza case notifications) at each week of the 2017 season. We assessed the impact that accounting for behaviour changes had on forecast performance using Bayes factors.

### Results

While the weekly Flutracking data typically include very few self-reported influenza tests, the data for 2017 revealed a substantial change in healthcare seeking behaviour and clinical decision-making. This change was evident prior to the epidemic peak. By calibrating our forecasts at each week to account for this trend, the forecast performance was greatly improved.

### Conclusion

We have demonstrated a unique value of community-level surveillance systems for interpreting traditional surveillance data, by virtue of providing insights into perceived risk and attitudes to seeking healthcare. Similar surveillance systems operate in other countries, so our methods and findings should apply beyond the Australian context. They are also highly relevant to pandemics, where patient and clinician behaviours are likely to change even more drastically than observed in the 2017 Australian influenza season.

*Keywords: influenza; epidemics; forecasting; surveillance*

Topic: Public Health: Surveillance & Forecasting  
 Abstract No: 11337

## Long-term influenza virus surveillance in the Antarctic Peninsula reveals the dynamic circulation of endemic and introduced strains

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**Introduction and objectives:** Influenza A viruses circulate in nature in diverse avian hosts. Until now scarce information is available regarding the prevalence and diversity of IAV in Antarctica, mainly due to its difficult accessibility. On years 2015-2019 we performed 5 expeditions to the Antarctic Peninsula and sampled avian species in 22 locations covering a large geographic distribution (67°46'S; 68° 54'O to 62°08'S; 58°07'O).

**Methods:** We collected a total 4,318 samples (2,174 cloacal and 1,555 blood samples from penguins, and 589 fecal/environmental samples). Results: We found seropositive penguins in 8 sites, showing seropositivity of 1.25-62%, which varied from season to season and where Chinstrap and Adelie penguins had the highest prevalence. We isolated and sequenced 7 H11N2 IAVS from 5 Chinstrap and 2 Gentoo penguins from 3 distant locations (Cape Shirreff, Hanna Point and Ardley Island). Phylogenetic analyses showed the HA genes clustering together with H11N2 viruses identified in a different location of the peninsula in Adelie penguins in 2013 and a snowy sheathbill in 2014. All internal genes clustered together, and showed limited diversity, confirming that these viruses originate from the same genotype. The isolation of H5N5 viruses in 2015, from 2 sites at the South Shetland Islands demonstrating a recent introduction of this virus through avian migration. Of interest, the NP gene of these H5N5 and the H11N2 viruses clustered together, indicating that a recent reassortment event occurred when the H5N5 was introduced. This suggests that the H11N2 subtype is endemic in the penguin population, and potentially other birds of the Antarctic Peninsula. In contrast, the H5N5 virus appears to have produced an outbreak in 2015 in a localized region of the peninsula.

**Conclusion:** Our studies provide a long-term perspective that contributes to the understanding of the ecology and diversity of IAV on this pristine continent.

**Keywords:** Avian influenza; Antarctic peninsula; zoonosis

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10411

## INFLUENZA VACCINE EFFECTIVENESS IN YOUNG JAPANESE CHILDREN OVER FIVE SEASONS

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**Introduction and Objectives:** Evidence is limited for influenza vaccine effectiveness (VE) against laboratory-confirmed influenza among young children. We aimed to estimate influenza VE among young children in Japan where all approved influenza vaccines are egg-propagated, inactivated formulations (IIV) by using the test-negative design which minimizes confounding by health care-seeking behavior and misclassification of diseases.

**Methods:** For seasons spanning 2013-14 to 2017-18 in Osaka and Fukuoka Prefectures, Japan, we prospectively recruited children <6 years who visited 1 of 9 collaborating pediatric clinics within 7 days of influenza-like illness onset. Nasal aspirates were tested for influenza by real-time reverse transcription polymerase chain reaction (PCR). In order to reduce selection bias, systematic recruitment and testing was employed. Date of vaccination was confirmed by medical records or maternity health record books. Cases and controls were defined as being PCR-positive and -negative, respectively. Conditional logistic regression models were used to calculate VE with adjustment for potential confounders.

**Results:** A total of 4,614 subjects (1,917 cases and 2,697 controls) were analyzed (approximately 800 to 1,000 subjects per season). VEs of IIV with 2 doses approximated 50%, ranging from 41% (95% confidence interval [CI]: 14% to 60%) in 2016-17 season to 63% (95% CI: 45% to 76%) in 2017-18 season. Subtype-specific analyses showed significant VEs for predominant circulating strains every season, irrespective of their antigenic match to vaccine strains (56% and 65% for A[H1N1]pdm, 37% and 50% for A[H3N2], and 60% for B[Yamagata]). Higher VEs were consistently observed in those aged 1-2 years compared with those aged 3-5 years (55% to 80% vs. 13% to 54%) across seasons.

**Conclusion:** IIV provided modest and significant protection against laboratory-confirmed influenza in young Japanese children. Lower VE in older children may indicate that potential influence of pre-existing immunity is important in interpretation of VE.

**Keywords:** Influenza vaccine; effectiveness; test-negative design; children

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10445

## THE IDENTIFICATION AND ESTABLISHMENT OF NEW CORRELATES OF PROTECTION IN RANDOMIZED-CONTROLLED TRIALS

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**Introduction and Objectives:** Correlates of protection (CoPs) are important for the development and evaluation of next-generation influenza vaccines. Although there are three currently established CoPs for inactivated influenza vaccines (IIVs), they do not fully explain IIV-induced protection, and do not predict effectiveness of live-attenuated influenza vaccines. In view of the need for additional CoPs for next-generation influenza vaccines, here we assess study design considerations, including sample size requirements for epidemiological studies to assess the independent associations of two or more CoPs with clinical endpoints and their causal contributions to vaccine-induced protection.

**Methods:** We conducted simulation studies to estimate the statistical power of randomized controlled trials of different sample sizes to establish independent associations of two CoPs with influenza virus infection with multiple logistic regression and detect the causal contribution of each CoP towards vaccine-induced protection with causal mediation analysis. We used the hemagglutination inhibition antibody (HAI) and neuraminidase inhibition antibody (NAI) titers as examples in these analyses.

**Result:** In simulations where unit increases in HAI titer reduces the risk of infection by 20%, consistent with 50% protection for a titer of 40, sample sizes of approximately 2,500 participants are estimated to have 80% power to identify the HAI titer as a CoP. When unit increases in HAI and NAI titers reduces risk by 20% and 15% respectively, our study suggests that a sample size of above 4,000 may be needed for a study to have 80% power to detect independent associations between both HAI and NAI titers with influenza virus infections and their individual causal contribution towards protection.

**Conclusion:** Large sample sizes may be required to assess the association of a new CoP with clinical endpoints and its causal contribution to protection. This indicates the need for independent studies that are specifically designed and adequately powered to achieve this objective.

*Keywords: Correlates of protection, randomized controlled trials, statistical power, association, causal mediation analysis*



Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10685

## COMPARISON OF HUMAN H3N2 ANTIBODY RESPONSES ELICITED BY EGG-BASED, CELL-BASED, AND RECOMBINANT PROTEIN-BASED INFLUENZA VACCINES DURING THE 2017-2018 SEASON

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**Introduction and objectives:** Most influenza vaccines contain antigens that are prepared from viruses grown in fertilized chicken eggs, which is not ideal because egg-adaptive mutations can alter antigenicity. Alternative technologies for producing influenza vaccine antigens include cell-based and recombinant protein-based strategies. Surprisingly, both the egg-based and cell-based vaccine showed relatively low H3N2 vaccine effectiveness during the 2017-2018 season. Here, we studied potential differences in immunogenicity of influenza vaccine antigens prepared in different systems by comparing antibody responses in 85 humans vaccinated with Flublok (recombinant protein-based), Flucelvax (cell-based), Fluzone (egg-based), or Fluzone High-Dose (egg-based) during the 2017-2018 season.

**Methods:** We completed neutralization assays using an egg-adapted H3N2 virus, a cell-based H3N2 virus, wild-type 3c2.A and 3c2.A2 H3N2 viruses, and the H1N1 vaccine strain. We also performed ELISAs with recombinant HA representative for wild-type 3c2.A H3N2 virus to study non-neutralizing antibody responses. Vaccine groups were compared in adjusted analysis.

**Results:** Post-vaccination titers to 3c2.A and 3c2.A2 were higher in Flublok recipients compared to Flucelvax or Fluzone recipients ( $p < 0.001$ ). Post-vaccination titers to 3c2.A and 3c2.A2 were similar in Fluzone High-Dose and Flublok recipients ( $p = 0.370$  and  $p = 0.473$ , respectively); however, seroconversion rates to these viruses were lower in Fluzone-High Dose recipients compared to Flublok recipients ( $p = 0.056$  and  $p = 0.022$ , respectively). Surprisingly, post-vaccination neutralizing antibody titers in Flucelvax recipients were low to all H3N2 viruses tested, including the cell-based H3N2 strain. Flucelvax also elicited low H3-specific ELISA antibody titers, which suggests that the Flucelvax H3N2 component has low overall immunogenicity. Titers to H1N1, which was antigenically similar among the vaccines, were not significantly different between the vaccine groups.

**Conclusion:** Together, these results suggest that H3N2 vaccine antigens prepared in different systems elicit substantially different responses in humans. Ongoing studies are being completed to determine why recombinant protein-based H3N2 antigens elicit superior responses compared to cell-based H3N2 antigens.

*Keywords: hemagglutinin; egg-adaptation; Fluzone; Flublok; Flucelvax*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10701

## RETROSPECTIVE EVALUATION OF ANTIGENIC SIMILARITY BETWEEN EGG-DERIVED VERSUS CELL-DERIVED INFLUENZA VACCINE REFERENCE STRAINS AND CIRCULATING INFLUENZA B-VICTORIA AND YAMAGATA VIRUSES

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**Introduction:** Reduced influenza vaccine effectiveness (VE) can be partially explained by viral adaptation resulting in antigenic mismatch between circulating influenza strains and the vaccine strain. We previously reported that circulating A/H3N2 viruses were consistently more antigenically similar to cell- than egg-derived reference viruses over multiple influenza seasons. Akin to A/H3N2, egg-adapted mutations that alter a key glycosylation site in the hemagglutinin protein in B/Victoria-lineage (BVic) viruses affect antigenicity. Data also suggest that BVic viruses closely resemble A/H3N2 strains in their propensity for antigenic drift and are more likely than the B/Yamagata-lineage (BYam) strains to undergo viral adaptation.

**Methods:** Using publicly available reports from the Worldwide Influenza Centre, London (Crick) for the Northern Hemisphere influenza seasons of 2013–2018, we compiled data on the antigenic similarity of BVic and BYam circulating virus isolates to the reference virus. Antigenic similarity was defined as circulating viruses showing no more than a four-fold reduction in titer to antisera raised against cell- (i.e., Madin-Darby Canine Kidney [MDCK] cells) or egg-propagated versions of each B vaccine reference virus in hemagglutination inhibition (HI) assays.

**Results:** For most seasons, a substantially higher proportion of tested, circulating BVic viruses antigenically matched the cell-propagated reference viruses than the corresponding egg-propagated reference vaccine viruses (Figure 1a). No similar pattern was found for BYam viruses (Figure 1b).

**Conclusions:** Circulating BVic viruses were more frequently antigenically similar to cell- compared with egg-propagated reference viruses over multiple seasons. No such pattern was evident for BYam strains. These data, along with our previous descriptive analysis of H3N2 strains, support the rationale of continuing to utilize cell-derived A/H3N2 and BVic seed virus to manufacture seasonal influenza vaccines, since they more closely match the circulating strains.

*Keywords: Influenza; Egg-derived vaccine; Antigenic mismatch*

# POSTER DISPLAY

Topic: Clinical Sciences: Avian & Zoonotic Influenza - Human Infections  
Abstract No: 10796

## THE FIRST HUMAN CASE OF ZONOTIC INFLUENZA SWINE H3N2 VARIANT IN AUSTRALIA AND ITS ASSOCIATION WITH LOCAL SWINE INFLUENZA VIRUSES

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### Introduction and objective

In Australia, influenza A virus (IAV) was first detected in swine populations in 2009 following the human H1N1 pandemic (H1N1pdm09). Evidence accumulated since then suggests that several IAV lineages co-circulate, including some introduced from humans as early as 1968. Currently, multiple human-derived IAV subtypes (H1N1, H1N2, H3N2, H1N1pdm09) and/or their reassortants are endemic in Australian swine, with antigenic data predicting limited protection from seasonal influenza vaccines. Here we report the first human case of a swine H3N2 variant (H3N2v) virus infection in Australia and identify its origins with reference to other Australian swine IAVs (sIAV).

### Methods

IAV was isolated from nasal swabs from infected human patient and swine samples. Real time RT-PCR was used for initial virus typing; haemagglutination inhibition assay and Next Generation Sequencing, followed by phylogenetic analysis using maximum likelihood and Bayesian methods, were used for virus characterisation.

### Results

In September 2018, Australia's first swine H3N2v virus (A/South Australia/85/2018) was detected in a nasal swab collected from a 15-year old female from a semi-rural area in South Australia, who had a mild, self-limited influenza-like illness. The virus was antigenically and genetically distinct from currently circulating human H3N2 viruses. Whole genome sequencing and phylogenetic analysis of each gene segment revealed that it was a 2:6 reassortant virus with surface glycoprotein genes derived from seasonal H3N2 viruses resembling those that circulated in humans in 1996-1997, while the internal protein genes were derived from H1N1pdm09.

### Conclusion

Highly divergent sIAV pools are present in Australian swine, some can infect humans, especially children and this may pose a pandemic threat. This highlights the importance for more vigilant surveillance on swine farms and for people who work with swine to detect influenza infections at the human–swine interface.

*Keywords: swine influenza, zoonotic, H3N2v*

Topic: Clinical Sciences: Avian & Zoonotic Influenza - Human Infections  
Abstract No: 11090

## H1N1 ENCEPHALITIS – AN UNUSUAL PRESENTATION

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**Introduction:** This presentation highlights an unusual presentation of H1N1 related Encephalitis without respiratory symptoms and considering this diagnosis with present H1N1 endemicity. A 42 years old lady developed intermittent headache and projectile vomiting. She was operated for Pituitary Macroadenoma in 2009 and was on treatment for Ulcerative Colitis and Diabetes Mellitus for the past 04 years. On the third day of fever, had altered sensorium. Examination revealed Temp 103F, Pulse 112/min, normal blood pressure. SpO2 was 95% at room air. CNS examination revealed GCS E1M4V1; pupils 3mm, not reacting; moving limbs to noxious stimuli, bilateral extensor plantars. Other systems unremarkable. Investigations revealed normal haematological/biochemical parameters. Peripheral smear and Paracheck for malaria, NS1Ag, IgG/IgM for dengue negative. Chest X-ray was normal. Started on broad-spectrum IV antibiotics, Acyclovir and Artesunate. CE MRI Brain showed hyperintensities in bilateral posterior basifrontal region, mammillary bodies, hypothalamus, anterior thalamus with diffusion restriction of above areas and no contrast enhancement. Cerebrospinal fluid examination was suggestive of viral meningitis. Her arterial blood gas was normal. Repeat MRI showed increase in size of lesions seen previously with involvement of bilateral basal ganglia and midbrain. Throat swab for H1N1 by RT-PCR was positive (received on day 5). Despite all efforts, she succumbed on day 5 of hospitalization. CSF for Herpes Simplex Virus by DNA-PCR and Japanese Encephalitis virus IgM by ELISA were negative (received post-mortem).

**Results:** Absence of respiratory symptoms at presentation or after admission (not reported in literature yet) and unusual site of brain affliction. Risk of serious H1N1 infection is known in people with underlying medical conditions (diabetes mellitus, immune suppression, extremes of age, chronic ailments) and pregnant women [1,2,3]

**Conclusion:** Influenza associated encephalopathy/encephalitis in adults is a rare complication and remains a diagnostic challenge. Highlights the importance of considering this diagnosis with present H1N1 endemicity.

*Keywords: H1N1 RTPCR, viral meningitis*

Topic: Clinical Sciences: Avian & Zoonotic Influenza - Human Infections

Abstract No: 11142

## Prevalence of antibodies against avian influenza A(H5N1) and A(H7N9) on live poultry market workers in Hanoi, 2017.

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### Introduction

Highly pathogenic avian influenza A/H5N1 (H5N1) is endemic in poultry in Vietnam. The country has experienced the third highest number of human infections with H5N1 in the world. Then, the neighboring country - China first detected human A(H7N9) in 2013, that raised a potential of exposure A(H7N9) in Vietnam.

### Methods:

A cross-sectional seroprevalence survey study was conducted among adult workers at 3 markets selling live poultry in the Hanoi (Dong anh, Thuong tin, Thanh tri), with total of 202 samples.

Using the horse hemagglutination inhibition assays (HHI) and the microneutralization assay (MN) with all three clades of HPAI (H5N1) viruses that have circulated in Vietnam and A(H7N9) (supported by Tottory University ) at Biosafety level 3 laboratories at High-tech center of National Institute of Hygiene and Epidemiology.

### Results:

The overall seroprevalence was 3.0% (95%CI; n=6 subjects). Of those, four subjects were identified as seropositive of clade 2.3.4. and two subjects were positive of clade 2.3.2.1, and no seropositive sample against H7 was detected . We did not find any cases co-infection between clade 2.3.4.4 and clade 2.3.2.1 of H5 viruses.

### Discussion:

Our study focused on PMWs from three live bird markets in Hanoi, where reported as a large number of poultry selling. The continued circulation and evolution of HPAI H5N1 requires a comprehensive surveillance on both human and animal sites throughout country, then follow up and expand study on PMWs is essential work to estimate possible avian-human transmission of avian influenza H5N1 viruses in Vietnam.

Topic: Clinical Sciences: Clinical Trials – Study Designs, Clinical Endpoints  
Abstract No: 11084

## CONTROLLED HUMAN INFECTION MODEL (CHIM) DESIGN FOR INFLUENZA VACCINE DEVELOPMENT

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**Introduction:** BARDA is pursuing improved influenza pandemic preparedness through the advanced development of more effective vaccines. Controlled Human Infection Models (CHIM) allow comparative evaluation of candidates and the identification of potential correlates; both of which are essential for de-risking late stage development. Endpoints for CHIM have included prevention of virologically confirmed infection and infection associated illness. However, the subjective nature of self-reported illness in the model can be a barrier, especially with small sample sizes.

**Method:** BARDA is supporting large (~150 subject) CHIM studies to evaluate new vaccine candidates and identify the contribution of mucosal and CD8 T-cell mediated immunity to influenza vaccine efficacy. Methods to improve CHIM for influenza vaccine development, including the use of transcriptomics and wearable diagnostic devices to objectively assess symptoms, will be described.

**Results:** Based on results of BARDA-supported investigations, we propose: 1) protection can be conferred through different, largely independent mechanisms; 2) a vaccine that induces a combination of serum antibodies and immune effectors that home to mucosal tissues will be the most effective approach; and 3) multiple correlates of protection will be needed to accurately predict the performance conferred by more effective vaccines against novel influenza viruses.

**Conclusion:** Use, and further refinements of, CHIM will play a critical role in the development of improved influenza vaccines.

*Keywords: human challenge model, vaccine, protection, mechanism*

Topic: Clinical Sciences: Clinical Trials – Study Designs, Clinical Endpoints  
Abstract No: 11236

## DETERMINING AN OPTIMAL ENDPOINT FOR SEVERE INFLUENZA REQUIRING HOSPITALIZATION: A LITERATURE REVIEW OF CATEGORICALLY ANALYZED ORDINAL SCALES AS ENDPOINTS IN CLINICAL TRIALS ACROSS ALL THERAPY AREAS

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**Introduction:** Currently, no drugs have been approved for treatment of severe influenza requiring hospitalization. An optimal endpoint to evaluate new treatments for this population has yet to be identified. FDA guidance recommends that a primary composite endpoint should include: clinical signs and symptoms, duration of hospitalization, time to normalization of vital signs and oxygenation, requirements for supplemental oxygen or assisted ventilation, and mortality. Categorically-analyzed ordinal scale endpoints have been reported in influenza, although not from Phase 3 registrational trials. To aid drug development, we examined how ordinal endpoints are analyzed and presented in other therapy areas and assessed if such an endpoint could be utilized in influenza.

**Methods:** Searches were performed using the PubMed literature database for articles published from 2009 to 2019 in the English language, describing Phase 2-4 clinical trials. Published literature was screened to identify articles including an ordinal scale endpoint analyzed as an ordered categorical variable.

**Results:** Seventy-four potentially relevant clinical publications were identified and reviewed, among which 38 used ordinal scales. Of these, 17 were analyzed as ordered categorical variables across various therapeutic areas: stroke (n=4), respiratory conditions (n=3), oncology (n=3), psychology (n=2), neurology (n=2), infectious diseases (n=1), pain (n=1) and diabetes (n=1). Ordinal scales contained 3–7 categories. Data were typically presented as tables or figures (mostly stacked bar charts; examples below), with varying colors to show the change in proportions of categories (i) over time or (ii) across different categories (e.g. toxicity ordinal scale by efficacy score).

**Conclusion:** Ordinal scales with categorical analyses have been used in a broad range of therapy areas as clinically relevant endpoints. This lends support to influenza with the definition of an appropriate categorical ordinal scale that meets the FDA guidance for influenza to show the efficacy of experimental drugs.

*Keywords: influenza, ordinal scale, endpoints,*



Topic: Clinical Sciences: Clinical Trials – Study Designs, Clinical Endpoints  
Abstract No: 11243

## HUMAN VIRAL CHALLENGE MODEL WITH A/PERTH/16/2009: A SYSTEMATIC ANALYSIS FROM FIVE CLINICAL STUDIES

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### Introduction and objective

The human challenge model of infection with influenza has provided a unique opportunity to comprehensively understand the course of viral respiratory disease as most experimental parameters have been fixed. A systematic analysis of a placebo dataset across multiple studies run by one group at a single centre and using the same strain is key to enhance influenza disease research. Our objective was to conduct the most comprehensive analysis for an H3N2 virus in recent times.

### Methods

Good Manufacturing Practice wild-type A/Perth/16/2009 H3N2 influenza virus was administered intranasally to 216 serosuitable and eligible volunteers (5 studies, no treatment involved) who remained in our quarantine facility for a total of 11 days. 10-item symptom diary cards were completed three times daily. Among many samples obtained were nasopharyngeal swabs, blood and mucus. In this analysis, multiple evaluable endpoints (single and composite) were investigated.

### Results

Of the 216 subjects inoculated, 89.8% shed virus (detected in respiratory samples) and/or seroconverted. Peak virus shedding was observed on day 3 post inoculation for most subjects. Upper respiratory tract and systemic symptoms were the most reported Influenza-like illness, with 73% and 54% of subjects respectively. Nasal secretion was greatest on day 4. Multiple correlations have been derived, identified and/or confirmed from this analysis, some of them only reaching significance due to the large sample size.

### Conclusion

These results demonstrate the broad variety of phenotypes of influenza infection and are key to discovering correlates of protection and predictors of outcome of infection. Thus, this novel analysis allows for improved design and powering of human challenge and field studies. Additionally, deep mining of data and samples is pivotal to the discovery of, and the decision-making process for, new therapies.

*Keywords: Human Viral Challenge, Meta-analysis, Correlates, Design*

Topic: Clinical Sciences: Clinical Trials – Study Designs, Clinical Endpoints  
Abstract No: 11257

## REAL-WORLD JOURNEY THROUGH THE HOSPITAL RECOVERY SCALE (HRS): US MEDICARE POPULATION

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**Introduction and objectives:** To date, no antiviral therapy is approved by the US FDA to treat influenza in hospitalized patients, and no optimal endpoint has been accepted for regulatory evaluation. An ongoing Phase 3 trial of pimodivir, a novel antiviral for treatment of influenza A, is using the Hospital Recovery Scale (HRS) as a measure of clinical outcome. The HRS is an ordinal scale consisting of six ordered mutually exclusive categories representing patients' clinical status: (1) Not Hospitalized; (2) Non-ICU Hospitalization, Not Requiring Supplemental Oxygen; (3) Non-ICU Hospitalization, Requiring Supplemental Oxygen; (4) ICU Hospitalization, Not Requiring Invasive Mechanical Ventilation; (5) Requiring Invasive Mechanical Ventilation; (6) Death. Our objective was to better understand how patients currently flow through HRS categories over time in a real-world setting.

**Methods:** Data analyzed were from the 5% Medicare sample covering the 2016-2017 influenza season (1-July-2016 to 30-June-2017). Patients were required to have an influenza ICD-10-CM code in any diagnosis position on an inpatient claim and Medicare Part A and B coverage under fee-for-service. Based on claim billing codes, patients were assigned to an HRS category for 14 consecutive days beginning on the day of hospital admission.

**Results:** Of 4,555 patients included in the analysis, 55% were female and mean age was 77.2 years. On admission to the hospital, 20% were in HRS category (2), 42% in (3), 32% in (4), and 7% in (5). Over time the majority of patients progressed to HRS categories (1) or (6). Mean length of stay was greater for patients entering the hospital in higher HRS categories [3.9 days in (2), 4.9 days in (3), 5.9 days in (4), and 10.2 days in (5)].

**Conclusion:** The HRS may be a valuable tool for measuring changes in clinical status among hospitalized patients with influenza in both clinical trials and real-world settings.

*Keywords: Pimodivir, influenza, hospital recovery scale*

Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention  
Abstract No: 10489

## AFLURIA QUADRIVALENT INFLUENZA VACCINE FOR ADULTS AND PAEDIATRIC USE

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### Introduction

In 2010, increased reports of fever and febrile convulsions were reported with post-marketing use of Fluvax trivalent influenza vaccine (IIV3) in children, predominantly those under 5 years of age, compared to previous years. As a result the indication for Fluvax IIV3 was limited to 5 years and above.

Scientific investigations into fever reports revealed that the lipid-mediated delivery of RNA fragments led to cytokine/chemokine signal *in vitro* assays. Use of higher concentrations of detergent sodium taurodeoxycholate (TDOC) to reduce lipid levels led to reduction of lipid-mediated delivery of RNA fragments and cytokine release. Findings of the scientific investigations led to modifications of the manufacturing process for the quadrivalent influenza vaccine.

### Methods

Immunogenicity and safety of Afluria quadrivalent influenza vaccine (IIV4) made with the modified manufacturing process were investigated in a clinical development program involving elderly, adult and paediatric subjects. Subjects provided blood draws, for immunogenicity measurements by haemagglutinin antibody titres, before and 28 days after vaccination with either Afluria IIV4 or licensed comparator vaccines. Solicited local and systemic adverse events were collected for 7 days, unsolicited adverse events for 28 days and serious adverse events for 6 months after vaccination. Phase 3 pre-licensure clinical studies were conducted over three consecutive northern hemisphere influenza seasons (2014/15, 2015/16 and 2016/17), firstly in elderly and adult subjects and then in paediatric subjects.

### Results

Afluria IIV4 demonstrated non-inferior immune responses and similar local and systemic adverse events including fever rates, when compared to licensed comparator vaccines.

### Conclusion

Immunogenicity and safety data from the clinical studies showed that increased TDOC concentration in the manufacturing process has attenuated the fever response previously observed in young children with Fluvax IIV3. The data has since been submitted to regulatory authorities and Afluria IIV4 is now indicated for use in adults and children from 6 months of age.

*Keywords: Afluria Quadrivalent; Paediatrics; Immunogenicity; Safety;*

Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention

Abstract No: 10739

**A PILOT RANDOMISED CONTROLLED STUDY TO EVALUATE THE  
IMPACT OF TEXT MESSAGE REMINDER ON INFLUENZA VACCINE  
UPTAKE IN CHILDREN WITH CHRONIC LUNG DISEASE:  
FEASIBILITY STUDY**

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**Introduction & Objective:** Children with chronic lung disease (CLDs) are at higher risk of influenza-related complications and death. However, the uptake of the influenza vaccine remains suboptimal. We conducted a pilot randomised controlled trial to determine the feasibility of hospital-based automated text message reminder system in improving influenza vaccine uptake in children with CLDs

**Method:** Children with CLDs attending specialist clinics at Sydney Children's hospital were randomly divided into the intervention and the control arm. During influenza season (April-October in Australia), carers of the children in the intervention arm were sent a short personalised text message twice every month, reminding them to take their children for influenza vaccine. At the end of the influenza season participants in both the arms were asked to complete an online survey to determine vaccine uptake, understand parental attitude towards influenza vaccine and acceptability of the reminder system.

**Results:** We had 23 children with CLDs in each arm. The uptake of influenza vaccine was 91% (21 of 23) in the intervention arm and 61% (14 of 23) in the control arm. Around 50% of children in both arms received the vaccine from primary care providers. A greater proportion of participants in both the arms thought a child can die from influenza (42% in intervention and 71% in control arm). In the control arm, 33% carers of children who did not receive the influenza vaccine reported they did not know that their child has to take the vaccine. In the intervention arm while the text message reminder did not impact the timing of the vaccine, 44% of the carers reported it impacted 'quite a bit' on their decision to get their children vaccinated.

**Conclusion:** Our pilot study has shown that a reminder text sent from the hospital setting can improve influenza vaccine uptake in high-risk children.

*Keywords: chronic lung diseases, influenza vaccine uptake, vaccine reminder system, high-risk paediatric population*

Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention

Abstract No: 10756

## The Safety, Pharmacokinetics, and Clinical and Virological Outcomes of Baloxavir Marboxil 2% Granules for Pediatric Patients Weighing Less Than 20 kg with Influenza in Japan

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### Background:

Single-dose baloxavir marboxil (BXM) rapidly reduces influenza virus titers and symptoms in adults and pediatrics with uncomplicated acute influenza. To develop a preferable formulation for younger pediatrics, a clinical study of granule formulation was conducted during 2017/2018.

### Method:

This was an open-label, multi-center, non-placebo-controlled study that assessed the safety, PK, and clinical outcomes of BXM 2% granules in pediatrics <20kg. Patients <10kg received a single oral dose of 1mg/kg and those ≥10kg received 10mg BXM. ITTI (Intention to treat infected) population was defined as patients with PCR-confirmed influenza infection receiving BXM. Clinical and virological endpoints were time to illness alleviation, fever resolution, and change in viral titer.

### Results:

There were 33 patients enrolled in the ITTI population, ranging from 43 days to 6 years of age (<10kg: n=12, ≥10kg: n=21). The most common virus strain was type B (36.4%), followed by A(H1N1)pdm (33.3%) and A(H3N2) (27.3%). There were no deaths, serious adverse events (SAEs), or AEs leading to discontinuation. AEs were reported in 18 patients. The geometric means of C<sub>24</sub> (BXM active form) were 46.8 (10 mg) and 69.4 ng/mL (1 mg/kg), which were comparable to those in the Ph3 pediatric study during 2016/2017.

The median time to illness alleviation and fever resolution was 45.3 and 34.0 hours. More than 4-log reduction in the mean virus titer was observed on Day2. Temporary elevations of the titer were observed after Day2 in patients with PA/I38X (one A(H1N1)pdm, four A(H3N2)) and type B, accompanying symptom rebound in some cases.

### Conclusion:

Single dose of BXM 2% granules was well tolerated and appeared to be effective in pediatric patients. The 1 mg/kg dose of granule formulation can be considered a suitable treatment option that may benefit younger pediatrics. To explore options to reduce viral titer and symptoms rebound, further studies might be warranted.

*Keywords: Baloxavir marboxil; pediatric; treatment; study; influenza*

Topic: Clinical Sciences: Clinical Trials - Treatment and Prevention  
 Abstract No: 11299

## CENTERSTONE: a global phase IIIb, randomised, double-blind, placebo-controlled clinical efficacy study of baloxavir marboxil for the reduction of direct transmission of influenza from otherwise healthy patients to household contacts

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**Background:** Antivirals are an important adjunct to vaccination for control of influenza. First-in-class baloxavir marboxil stops viral replication by inhibiting cap-dependent endonuclease and rapidly reduces viral shedding in otherwise-healthy and high-risk patients (CAPSTONE-1 & -2). CENTERSTONE investigates if baloxavir's antiviral efficacy reduces transmission from index patients (IPs) to their household contacts (HHCs).

**Methods:** This global phase IIIb, double-blind, placebo-controlled study targets recruitment of 2,030 HHCs starting mid-2019 (EUDRACT #2018-004056-37). Eligible IPs ≥12-≤64 years old must have influenza symptom onset within 48h of screening, be PCR(+), and not have high complications risk. Approximately 1130 IPs will be randomized 1:1 to single dose baloxavir (40 or 80mg for IPs <80 or ≥80kg respectively) or matching placebo. Subsequently, households will be enrolled if all HHCs are PCR(-), ≥2 HHCs have not been vaccinated ≤6m prior to screening ("unvaccinated"), and no HHC <2y, immunocompromised, or pregnant. The highly sensitive cobas® Liat® Influenza A/B nucleic acid point-of-care test will be deployed ideally at all sites, with all samples reconfirmed via central laboratory. Respiratory swabs will be obtained from HHCs on days 5 and 9. CENTERSTONE is powered to detect a 30% relative risk reduction in the secondary transmission rate, with the primary endpoint being the proportion of unvaccinated HHCs PCR(+) by day 5. Secondary endpoints include (1) proportion of unvaccinated HHC PCR(+) by day 5 and developing symptoms at any time during the 9 day study; (2) proportion of household units with ≥1 HHC meeting the primary endpoint; and (3) proportion of unvaccinated HHC PCR(+) by day 9 if ≥1 HHC was PCR(+) by day 5 (capturing high-likelihood within-household secondary and tertiary transmission) or HHC PCR(+) with virus bearing mutations associated with reduced baloxavir susceptibility (capturing transmission of mutant virus).

**Conclusion:** CENTERSTONE will evaluate baloxavir's potential to reduce transmission and inform epidemic and pandemic modelling.

**Keywords:** Baloxavir marboxil; transmission; prevention; study; influenza

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 10099

## Live attenuated influenza vaccine (LAIV) induces functional neutralizing and neuraminidase inhibiting antibody responses after immunization in children and adults

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### Background

The live attenuated influenza vaccine (LAIV) was licensed for use in Europe for children (2-17 years old) in 2012. Vaccine induced antibodies directed against the two major viral surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA), play important roles in limiting virus infection. Antibodies to the HA protein inhibit viral attachment to the host cell receptors. The micro-neutralization assay measures mainly neutralize antibodies against HA. NA inhibiting antibodies also play an important protective role in influenza infection. The aim of this study was; To evaluate humoral immune response in children and adults vaccinated with LAIV.

### Methods

We conducted a clinical study in forty children and thirty-seven adults that were scheduled for elective tonsillectomy. Subjects were intranasal vaccinated with LAIV. Blood samples were collected at various time interval, before vaccination (day 0), 3-14, 28, 56 days post-vaccination. We dissected the overall antibody responses directed towards the HA and NA glycoproteins induced by LAIV against A/H1N1, A/H3N2 and B viruses using micro-neutralization (MN), enzyme-linked immunosorbent assay (ELISA) and quantified the overall binding strength with avidity ELISA. Neuraminidase inhibiting (IN) antibody response were measure by enzyme-linked lectin assay (ELLA).

### Results

LAIV induced significant increases in influenza specific MN antibody responses to H3N2 and B antigens post-vaccinations and increased only slightly to H1N1 antigen in children. LAIV elicits potent IN activity in both children and adults against all three virus strains. Influenza specific antibodies were quantified with ELISA, we saw significant antibody responses against all three strains. The binding assay indicated that high immunoglobulin levels correlate with low avidity after LAIV in children.

### Conclusion

We are currently finishing analysing the results from this proejct, but the initial results shows that LAIV elicits a strong MN response in children and the avidity response inversely correlate with immunoglobulin levels. We will present the final data on the poster.

*Keywords: Influenza vaccine, LAIV, Follicular helper T cell, Serology*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 10473

## Modification of the vaccine manufacturing process improves the pyrogenicity profile of inactivated influenza vaccines in young children

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### Background and Objective

There were increased reports of fevers in young children younger than 9 years receiving the Seqirus/CSL Southern Hemisphere 2010 trivalent inactivated influenza vaccine (IIV3). Modifying the vaccine manufacturing process by increasing the minimum concentration of viral splitting agent (sodium taurodeoxycholate [TDOC]) from 0.5% w/v to 1.5% w/v for all strains resolved this safety issue in young children. The objective of the analysis is to compare any and severe fever rates in two pediatric studies of Seqirus quadrivalent inactivated influenza vaccine (S-IIV4), prepared using the modified manufacturing process, with fever rates in three pediatric studies of historical (pre-2010) IIV3 formulations.

### Methods

Any fever rates and severe fever rates were compared between S-IIV4 development studies and pre-2010 IIV3 studies in three paediatric age groups (6 months to < 3 years, 3 years to < 5 years, and 5 years to < 9 years).

### Results

In all age groups, any fever and severe fever rates were comparable to licensed QIV comparators and substantially lower in S-IIV4 studies compared to the pre-2010 IIV3 studies, despite the addition of a fourth vaccine strain. No febrile seizures were observed in the 7 days after any vaccination in any of the studies.

### Conclusions

S-IIV4, manufactured using a higher TDOC concentration compared with that used in pre-2010 vaccine formulations, is associated with less pyrogenicity than its historical pre-2010 IIV3 formulations. In addition, as these recent clinical studies were conducted over three NH influenza seasons during which A/H3N2 and B strain changes occurred, the findings lend support to the generalizability of the results to further vaccine formulations containing different virus strains.

*Keywords: Fever, Inactivated influenza vaccines, Paediatrics, Quadrivalent influenza vaccine, Safety*



Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 10537

## VACCINATION WITH ADJUVANTED VACCINE INDUCED HIGHER STRAIN CROSS-REACTIVE ANTIBODY RESPONSE THAN NON-ADJUVANTED VACCINE

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Influenza vaccination is the most effective means to reduce the substantial morbidity and mortality caused by influenza infection. The efficacy of the most current influenza vaccines is highly sensitive to antigenic changes. Adjuvants, such as MF59, are effective in improving the vaccine immune response by inducing qualitative and quantitative expansion of the antibody repertoire increasing the protective potential. Adjuvanted vaccines have been shown to increase antibody-mediated cross-reactive immunity, which may provide potential significant benefits in the case of pandemic responses and broader seasonal protection.

A recent clinical trial showed that an MF59-adjuvanted vaccine was more efficacious than a non-adjuvanted comparator in <2-year old subjects, an effect that was not evident in the  $\geq$  2-year old subjects. The trial was conducted during the influenza seasons when the prevalent circulating viruses were from an A/H3N2 antigenically significantly different from the vaccine strain, suggesting that the increase efficacy demonstrated by the adjuvanted vaccine may be mediated by strain cross-reactive antibodies. Multiple variables may have differentially affected the strain specificity of antibody responses in the two populations analyzed, i.e. age-dependent development of the immune system and/or pre-existing immunological experience with influenza virus infection and/or vaccination.

A small minority of subjects who had documented influenza also had blood samples taken for immunological testing. Therefore, a subset of the trial population, representing subjects with distinct age and/or immunological history irrespective of influenza infection, was tested for antibody responses to the H3N2 strain present in the vaccine as well as two H3N2 drifted strains antigenically matching the viruses circulating during the trial seasons. The results showed that adjuvanted vaccine improved the cross reactive antibody response in subjects with lower preexisting antibody titers, regardless of their age or vaccine history, suggesting the potential efficacy benefit by adjuvanted vaccine on subjects with lower preexisting antibody response.

*Keywords: MF59, adjuvant, vaccine, antigenic match*

Topic: Clinical Sciences: Clinical Trials – Vaccines

Abstract No: 10719

## MICRONEUTRALIZATION ASSAY TITERS AS ESTIMATES OF PROTECTIVE EFFECTIVENESS AGAINST INFLUENZA INFECTION IN CHILDREN

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**Introduction and Objectives:** Alternatives to the hemagglutination inhibition (HI) assay are needed, in part due to changes in circulating A/H3N2 strains resulting in lost ability to agglutinate erythrocytes. Our study was conducted to determine the relationship between microneutralization (MN) and HI titers against A/H1N1, A/H3N2 and B strains, and to estimate MN cut-off titers predictive of protective effectiveness based on HI.

**Methods:** Sera were collected from two clinical vaccine trials enrolling children up to 72 months of age with evaluation of HI and MN titers against influenza A/H1N1, A/H3N2 and B strains. Paired MN and HI results were analyzed, and agreement of the two assays based on different measures of serologic response was assessed. Estimates of MN cut-off titers of protective effectiveness were based on HI titers using slope and intercept estimates from Deming regression analyses.

**Results:** Using the CBER seroconversion definition, positive agreement of MN relative to HI ranged from 86.67 to 100% (lower 95% CI: 80.23 to 97.68%) and negative agreement ranged from 14.29 to 100% (lower 95% CI: 6.33 to 69.98%). A significant degree of correlation was observed between HI and MN titers across all strains and vaccines (0.85 to 0.98). Based on these comparisons, an HI titer of 1:40 corresponded to MN titers of 65 to 151 (A/H1N1), 32 to 52 (A/H3N2) and 42 to 119 (B). An HI titer of 1:110, previously associated with protection in children, corresponded to MN titers of 207 to 546 (A/H1N1), 80 to 163 (A/H3N2) and 137 to 459 (B).

**Conclusion:** High positive but substantially lower negative agreement of the two assays based on seroconversion rates against A/H1N1, A/H3N2 and B strains was observed. MN protective titer estimates were higher compared to the 1:40 and 1:110 HI protective titer thresholds.

Topic: Clinical Sciences: Clinical Trials – Vaccines

Abstract No: 10742

## MF59-Adjuvanted Quadrivalent Subunit Influenza Vaccine (aQIV) is Non-Inferior to the Licensed MF59-Adjuvanted Trivalent Vaccine (aTIV) and Well-Tolerated in Elderly Adults

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### Objectives:

To demonstrate that vaccination with aQIV elicits non-inferior strain specific immune responses as compared to both an aTIV containing the same virus strains as the licensed adjuvanted influenza vaccine (Fluad, aTIV-1), and an aTIV containing the alternate B strain (aTIV-2), when administered to adults > 65 years of age; and to assess safety and tolerability.

### Methods:

In a Phase 3, double-blinded, controlled, randomized study, 1778 subjects ≥65 years were randomized as 2:1:1 and received aQIV (n=889), aTIV-1 (n=445) or aTIV-2 (n=444) during the 2017-18 influenza season. Subjects provided serological specimens on Days 1 and 22 for measurement of immune response. Safety and reactogenicity (Days 1-7) were monitored in all subjects.

### Results:

The non-inferiority criteria for the GMT ratio was met for all 4 strains of the aQIV vaccine. The upper bound of the two sided 95% confidence interval (CI) for the GMT ratios did not exceed the non-inferiority margin of 1.5 (A-H1N1=1.27, A-H3N2=1.09, B-Yamagata=1.08 and B-Victoria=1.08). Also the non-inferiority criterion for the seroconversion (SCR) difference was met for all 4 strains of aQIV as the upper bound of the 95% CI of the intergroup difference for SCR was below 10% (A-H1N1=7.76, A-H3N2=4.96, B-Yamagata=3.27, and B-Victoria=2.55). aQIV was shown to be immunologically superior to aTIV1+aTIV2 for the B strain not included in each TIV vaccine as measured by GMT ratio and SCR difference. aQIV and aTIV vaccines were well-tolerated with reactogenicity profiles generally comparable.

### Conclusions:

In older adults, a quadrivalent vaccine comprised by the addition of a second B strain to an MF59-adjuvanted trivalent influenza vaccine had good tolerability and comparable strain specific immunogenicity when compared to vaccines which separately had the same vaccine strains.

*Keywords: MF59-Adjuvanted; aQIV; Influenza; B antigen; Quadrivalent*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 10745

## ANTIBODY RESPONSES AGAINST HETEROLOGOUS H5N1 STRAINS FOR AN MF59-ADJUVANTED CELL CULTURE-DERIVED H5N1 (aH5N1c) INFLUENZA VACCINE IN HEALTHY PEDIATRIC SUBJECTS

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### Objective

To evaluate aH5N1c vaccine antibody responses against heterologous influenza strain(s) as measured by HI and MN assays following a primary analysis of homologous (Turkey) results for meeting CBER and the former CHMP criteria in healthy pediatric subjects.

### Methods

662 subjects 6mo to  $\leq 17$  yrs were equally randomized to receive two full or half doses of the MF59 adjuvanted, cell derived H5N1 vaccine, aH5N1c, three weeks apart, stratified by 3 age cohorts. In a pre-defined exploratory analysis, antibody responses against five heterologous influenza strains (H5N1 Vietnam, Indonesia, Egypt, Hubei, Anhui) were measured by HI and MN assays on Days 1 and 43 for a subset of the full-dose groups.

### Results

Seroconversion and an HI titer  $\geq 1:40$  at Day 43 was achieved by 32% to 72% of subjects, with the CBER criterion for seroconversion being met for the H5N1 Egypt, Hubei and Vietnam strains. Overall the highest heterologous antibody response was observed against the H5N1 Egypt strain. Subjects met all 3 former CHMP criteria against the Egyptstrain, 2 of the 3 criteria (seroconversion and GMR) against the H5N1 Hubei and Vietnam strains and the former CHMP criterion for GMR against the H5N1 Indonesia and Anhui strains. GMTs on Day 43 increased between 8- and 40-fold compared to Day 1. Immune responses against heterologous strains were generally higher when measured using the MN assay compared with the HI assay. A 4-fold increase in MN titers against all 5 heterologous strains at Day 43 was achieved by 83% to 100% of subjects.

### Conclusion

Two full doses of aH5N1c vaccine in children resulted in increased immunogenicity against heterologous H5N1 strains. These findings illustrate the potential for an MF59 adjuvanted, cell-derived H5N1 vaccine to provide cross protection against other H5N1 strains.

*Keywords: aH5N1c; Influenza; Vaccine; Heterologous, MF-59 Adjuvanted*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
 Abstract No: 10747

## IMMUNOGENICITY, LOT-TO-LOT CONSISTENCY, AND SAFETY OF AN MF59-ADJUVANTED CELL CULTURE-DERIVED H5N1 (aH5N1c) INFLUENZA VACCINE IN HEALTHY ADULTS

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### Objective

To evaluate immunogenicity and lot-to-lot consistency of 3 consecutively produced lots of aH5N1c pandemic vaccine by H5N1 HI antibody responses in healthy subjects  $\geq 18$  years of age, and to assess standard safety.

### Methods

3196 subjects were equally randomized to receive one of 3 consecutively produced aH5N1c vaccine lots or saline placebo, as 2 observer-blinded doses (Day 1 and Day 22). Subjects were equally stratified into 2 age groups, 18 to <65 and  $\geq 65$  years of age. Immunogenicity was measured before each vaccination, and on Day 43. Safety was monitored throughout.

### Result

HI antibody responses increased after both vaccine doses and at Day 43 both age-appropriate CBER immunogenicity criteria were met.

Percentage of Subjects with HI $\geq 1:40$ Day 1 and Day 43				
Age Group	18 to <65 Yrs		$\geq 65$ Yrs	
	Active	Pbo	Active	Pbo
<b>Day 1</b>	<b>N=1116</b>	<b>N=372</b>	<b>N=113</b>	<b>N=367</b>
% HI $\geq 1:40$	13.0	15	27.8	24.5
95% CI	(10.7, 15.6)	(11.5, 19.4)	(24.9, 30.9)	(20.1, 29.6)
<b>Day 43</b>	<b>N=1076</b>	<b>N=349</b>	<b>N=1080</b>	<b>N=351</b>
% HI $\geq 1:40$	95.0	8.5	85.7	20.8
95% CI	( <b>93.4</b> , 96.2)	(5.9, 12.1)	( <b>83.3</b> , 87.9)	(16.6, 25.8)

No changes over time were seen in placebo subjects. Lot-to-lot consistency of aH5N1c vaccine was demonstrated, with the 2-sided 95% CIs for the pairwise comparisons of ratios of GMTs being (0.90, 1.13), (0.86,1.08) and (0.87,1.09), within the predefined equivalence ranges of 0.667 and 1.5. AEs were more frequently reported in the active treatment group, but primarily due to solicited local AEs. The majority of solicited local and systemic AEs reported were of mild intensity.

### **Conclusion**

The MF59 adjuvanted, H5N1 influenza vaccine, aH5N1c, manufactured on a cell culture platform, elicited high levels of antibodies and met CBER immunogenicity criteria at Day 43 with a clinically acceptable safety profile. Lot-to-lot manufacturing consistency was demonstrated by equivalence of GMTs.

*Keywords: Influenza; Vaccine; Cell Culture-Derived; H5N1; Pandemic*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 10748

## ANTIBODY RESPONSES AGAINST HETEROLOGOUS H5N1 STRAINS FOR AN MF59-ADJUVANTED CELL CULTURE-DERIVED H5N1 (aH5N1c) INFLUENZA VACCINE IN ADULTS AND THE ELDERLY

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### Objective

To evaluate aH5N1c vaccine antibody responses against heterologous influenza strain(s) as measured by HI and MN assays following a primary analysis of homologous (Turkey) results for meeting CBER and the former CHMP criteria in healthy adult and elderly subjects.

### Methods

In separate but similar studies, a total of 975 subjects 18 to <65 years of age (adults), and 1388 subjects >65 years of age (elderly), were equally randomized to receive two full or half doses of the MF59 adjuvanted, cell derived H5N1 vaccine, aH5N1c, three weeks apart. In a pre-defined exploratory analysis, antibody responses against five heterologous influenza strains (H5N1 Vietnam, Indonesia, Egypt, Hubei, Anhui) were measured by HI and MN assays on Days 1 and 43 for a subset of the full-dose groups.

### Results

For 5 heterologous strains, the GMT on Day 43 in the adult and elderly subjects (full-dose vaccine) significantly increased from baseline. For adults, depending on the heterologous strain, seroconversion (SC) and HI  $\geq 1:40$  was achieved by 28% to 64% of subjects on Day 43. Notably, the Day 43 SC rate for 3 heterologous strains (H5N1 Egypt, Hubei and Vietnam) had a lower bound of 95% CI at  $\geq 40\%$ , which exceeded the corresponding homologous strain criterion. For the elderly, SC and HI  $\geq 1:40$  was achieved by 17% to 57% of subjects on Day 43. Again, notable increases in rates for SC and HI  $\geq 1:40$  were observed for 3 heterologous strains (H5N1 Egypt, Hubei and Vietnam). Immune responses using the MN assay against these heterologous strains were generally higher compared with the HI assay.

### Conclusion

In adults and the elderly, full-dose aH5N1c vaccine demonstrated increased immunogenicity against heterologous H5N1 of five separate genetic clades. These findings illustrate the potential for MF59 adjuvanted, cell-derived H5N1 vaccine to provide cross protection against other H5N1 strains.

*Keywords: Influenza; Vaccine, H5N1, Cell-Derived; Heterologous; Elderly*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
 Abstract No: 10972

## Neutralizing Antibody Responses to Licensed Egg-derived, Cell-derived and Recombinant Seasonal Influenza Vaccines in US Department of Defense Healthcare Beneficiaries

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**Introduction:** Annual influenza vaccines are recommended for preventing influenza disease. However, the effectiveness of influenza vaccines varies annually depending on the virus type and subtype and whether vaccine strains and circulating strains are matched. Currently, egg-derived, cell-derived, and recombinant influenza vaccines are available and considered interchangeable. However, mutations in influenza hemagglutinin (HA) that can emerge during propagation in eggs may change HA antigenicity. Thus, there is concern that egg-adapted mutations may reduce vaccine effectiveness. Data directly comparing the immunogenicity of cell-derived, egg-derived and recombinant influenza vaccine types are limited.

**Methods:** Using an HA-pseudovirus neutralization assay we measured neutralization titers of pre- and post-immunization sera from military healthcare beneficiaries who were immunized with one of three types of influenza vaccines in a randomized, open-label trial during the 2018-2019 influenza season. A total of 136 pre- and post-immunization paired sera were collected, one third of which represents each vaccine type. Neutralization titers against vaccine-matched H1N1 (A/Michigan/45/2015-like) and H3N2 (A/Singapore/INFIMH-16-0019/2016-like) strains and circulating strains were measured.

**Results:** All three vaccines types elicited neutralizing antibody responses to both H1 and H3 vaccines antigens, but titers and seroconversion rates were higher in those immunized with the recombinant influenza vaccine. Egg- and cell-derived influenza vaccines elicited similar responses against both H1 and H3 antigens. In addition, pre-immunization titers against egg-derived H1 and H3 antigens were high, but pre-immunization titers against cell-derived H3 antigen were low. All three vaccines types elicited antibodies against the A/Singapore/NFIMH-16-0019/2016-(H3N2)-like component but reacted poorly with A/Kansas/14/2017 (3C.3a), which is the recommended H3N2 virus strain for 2019-2020 northern hemisphere influenza vaccines.

**Conclusion:** Egg- and cell-derived influenza vaccines elicited similar neutralizing antibody responses to the antigens tested, while the recombinant influenza vaccine elicited the highest responses. Additional studies assessing the immunogenicity and effectiveness of these vaccines across many influenza seasons are needed.

*Keywords: influenza vaccines, antibody responses*



Topic: Clinical Sciences: Clinical Trials – Vaccines

Abstract No: 10982

## ANTIBODY RESPONSE TO 2018-19 INFLUENZA B/VICTORIA-LINEAGE VACCINE IN CHILDREN ORIGINALLY PRIMED TO 2008-09 B/YAMAGATA-LINEAGE VACCINE: LONGITUDINAL ASSESSMENT OF POTENTIAL LINEAGE-SPECIFIC IMPRINTING

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**INTRODUCTION/OBJECTIVES** : In a randomized controlled trial called TITRE I (ClinicalTrials.gov Identifier: NCT00710866), infants and toddlers 6-23 months of age received two priming doses of 2008-09 trivalent inactivated influenza vaccine (TIV) containing B/Yamagata-lineage antigen. In follow-up trials conducted in 2009-10 (TITRE II: NCT01067404) and 2010-11 (TITRE IIB: NCT01235000) sequential subsets of TITRE I children received seasonal doses of TIV instead containing B/Victoria-lineage antigen. These children showed strong antibody recall responses to the original B/Yamagata-lineage priming antigen, but minimal B/Victoria-lineage responses. The recommended B/Victoria-lineage antigen remained unchanged until the 2018-19 season. The goal of the current study, TITRE III (NCT03753347), is to assess whether TITRE I children can now mount a protective antibody response to the updated 2018-19 B/Victoria-lineage antigen ten years after original priming to B/Yamagata.

**METHODS**: Subjects from British Columbia and Quebec, Canada who completed the TITRE I trial in 2008-09 were eligible. Baseline sera were collected prior to receipt of a 0.5mL dose of 2018-19 quadrivalent influenza vaccine (FLUZONE®, Sanofi Pasteur) with post-immunization sera collected 27-45 days later. The primary outcome was the percentage of children with hemagglutination inhibition antibody titres  $\geq 40$  to the current (B/Colorado/06/2017) and prior (B/Brisbane/60/2008) Victoria-lineage antigens.

**RESULTS**: TITRE III enrolment is completed with 55/230 (24%) original TITRE I children participating. Mean age of participants is 10.5 years (range 10-11 years). Immunogenicity testing is currently ongoing and main findings will be presented.

**CONCLUSIONS**: TITRE III is unique in its long-term follow up of children with known influenza B lineage-specific priming exposure. Earlier findings in these children suggested preferential recall of antibody to the influenza B lineage of first childhood imprinting (i.e. original antigenic sin). Given multiple population cohorts have been primed to one or the other influenza B lineage (through infection and/or historic TIV recommendations), the response to both lineages warrants better understanding throughout childhood.

*Keywords: influenza B; lineage; antibody; vaccine; imprinting*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
 Abstract No: 10993

## EVALUATION OF METABOLIC PREDICTORS OF INFLUENZA VACCINE IMMUNE RESPONSE IN SINGAPORE'S ELDERLY POPULATION: THE DYNAMIC TRIAL

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**Introduction and Objectives:** While immunosenescence and inflamm-aging are known to contribute to suboptimal immune responses to influenza vaccines, limited studies have evaluated the role of metabolic co-morbidities despite their rising global incidence. The DYNAMIC Trial, a Phase IV clinical trial aims to investigate metabolic predictors of influenza-vaccine immune response in a multi-ethnic elderly community cohort in Singapore (NCT03399357).

**Methods:** Participants received standard-dose trivalent influenza vaccine (IIV3). Baseline total 25-(OH) D was measured. Primary outcome was haemagglutination inhibition titer (HAI) response to vaccine strains at D28 compared to baseline (D0), analysed via geometric mean titers (GMT), D28/D0 ratio, log<sub>2</sub>(D28/D0) fold change, proportion seroprotected and seroconverted at D28. Robust regression and logistic regression were performed to ascertain the effects of various covariates on HAI response.

**Results:** 234 participants (age ≥ 65) were enrolled during June-Dec 2017. 220 participants completed study visits. 71 (30.3%) and 159 (68%) had medically-stable diabetes mellitus (DM) and hyperlipidemia respectively. Mean BMI was 25.37 kg/m<sup>2</sup> (SD 4.73). Median total 25-(OH) D was 26 ng/ml; IQR 21-29. Figure 1 shows GMT responses. Baseline total 25-(OH)D, BMI, DM, metformin and statin use were not associated with post-vaccination HAI response. 79 participants (35.9%) had received 2016 IIV3, i.e. immediate previous year's vaccine which contained same A/H3N2 and B strain as study year's IIV3. Receipt of 2016 IIV3 was associated with reduced odds of seroconversion to A/HongKong/4801/2014 (H3N2)-like virus (aOR=0.13 (0.07 to 0.26), p<0.01) and B/Brisbane/60/2008-like virus (aOR=0.4 (0.16 to 0.99), p=0.03) adjusting for ethnicity, compared to those who had not received IIV3 in previous 5 years.

**Conclusions:** Well-controlled metabolic conditions did not affect HAI response in this cohort. Effects on cell-mediated immunity needs investigation. Timing of prior IIV3 with regards to seroconversion has implications for timing and frequency of influenza vaccination in older adults and needs further study.

*Keywords: influenza vaccine; immune response; metabolic; older adults*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 11008

## ENHANCED ANNUAL INFLUENZA VACCINATION STRATEGIES GENERATE HIGHER QUALITY IMMUNE RESPONSES IN OLDER ADULTS

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### Introduction and Objectives:

Seasonal inactivated influenza vaccines with enhanced immunogenicity aim to improve protection in older adults. Aside from antibodies targeting the head of the hemagglutinin (HA) protein, other humoral and cellular immune responses were examined to probe the protective capacity of these vaccines.

### Methods:

A randomized controlled trial was conducted in 2017-2018 to investigate whether annual immunization with MF59-adjuvanted, high-dose HA or recombinant HA vaccines may broaden immune responses compared to receipt of standard-dose influenza vaccine in older adults (65-82 years) in Hong Kong. *In vitro* assays at baseline, days 7 and 30 post-vaccination (n=20 per group) were performed to measure serum titers against HA. Antibody avidity, IgG subclass (IgG1, 2, 3, 4) usage and antibody-dependent cellular cytotoxicity (ADCC) were measured to assess antibody quality. Vaccine stimulation of ADCC activity, circulating T follicular helper (Tfh) memory cells, and plasmablasts were correlated with antibody profile.

### Results:

All vaccine recipients had a comparable rise in anti-HA IgG titers post-vaccination but antibody avidity against was higher in the adjuvanted and recombinant-HA vaccines, which correlated with increased HA-specific ADCC activity. Post-vaccination responses were dominated by IgG1 and different vaccines preferentially boosted and utilized IgG1 and/or IgG3 for effector functions. All vaccines enhanced recall of activated memory Tfh cells, which also correlated with increased plasmablast activity and higher avidity antibodies in the adjuvanted vaccine group.

### Conclusion:

Enhanced adjuvanted, high-dose, and recombinant HA vaccines elicit a different spectrum of cellular and antibody functions, avidity and IgG subclass usage which may contribute to greater protective potential. A better understanding for correlates of protective immunity beyond HI antibodies is needed for rational targeting of vaccination strategies in older adults.

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 11070

## Adjuvanted H5N1 vaccine elicits rapid and multifaceted humoral immune responses in human

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### Introduction and Objectives

The highly pathogenic avian influenza (HPAI) H5N1 viruses have caused widespread outbreaks in domestic livestock, and sporadic human infections. There have been 860 confirmed human cases and 454 deaths, from WHO. The high mortality rate of HPAI H5N1 viruses in human poses an enormous threat to public health globally. Undergoing continuous genetic and antigenic evolution, H5N1 viruses can be grouped into 10 clades and dozens of subclades. Vaccination is the most cost-effective measure to combat influenza virus. “Universal” vaccines capable of eliciting broad protective immune responses against HPAI H5N1 viruses are desired and under development and evaluation in animal models and clinical trials.

### Methods

We conducted a dose escalating study of 30µg HA non-adjuvanted, 1.5, 7.5, and 30µg HA with Matrix M adjuvant in healthy volunteers (15 adults each group). Blood samples were collected at multiple time points. We investigated the kinetics of multi-faceted humoral immunity induced by the vaccine using a panel of assays including hemagglutination inhibition (HI) assay, microneutralization (MN) assay, pseudotype-based neutralization (PN) assay, enzyme-linked lectin assay (ELLA), ELISA, and luciferase reporting antibody-dependent cell-mediated cytotoxicity (ADCC) assay. Mice receiving post vaccine human serum transfer were challenged with RG14 virus to assess if vaccine elicited protective antibody responses against HPAI H5N1 virus.

### Results

The high dose (30mg) with adjuvant gave the most robust HA specific antibody responses. Adjuvanted low dose (1.5 and 7.5mg HA) vaccine elicited potently protective and broad antibody responses but delayed kinetics. Matrix M adjuvanted virosomal H5N1 vaccine also elicited high amount of neuraminidase specific antibodies as well as ADCC antibodies, two mechanisms playing key roles in virus clearance.

### Conclusion

Our study shows Matrix M adjuvanted virosomal H5N1 vaccine elicits rapid, robust and broadly protective multi-faceted humoral immune response in human.

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 11092

## SAFETY OF INFLUENZA VACCINE IN ADULT HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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### Background

Influenza (flu) vaccination is recommended for hematopoietic stem cell transplant (HSCT) recipients; however, optimal timing of post-transplant flu vaccine administration is unknown. We assessed post-vaccine symptom severity and reactogenicity in adult HSCT patients receiving high dose (HD) or standard dose (SD) flu vaccine in a clinical trial. We compared outcomes amongst three different time periods post-transplantation.

### Methods

Adult HSCT recipients were enrolled in a multicenter phase II trial evaluating the safety and immunogenicity of HD trivalent flu vaccine versus SD quadrivalent flu vaccine. Participants were 3-23 months post allogeneic HSCT and received two vaccines 28-42 days apart. Participants recorded reactogenicity, graded for pain, tenderness, swelling/induration as none, mild, moderate and severe respectively from days 0-7 after each vaccination. Temperature, swelling/induration size and redness size were also recorded. The study is ongoing and remains blinded as to vaccine type.

### Results

From October 2017 to March 2019, 124 enrollees received at least one influenza vaccine. The mean age was 53 years, mean time post-HSCT was 7.4 months, and 61% were male. Both vaccines were administered to 106/124 enrollees. Table 1 compares reactogenicity by time of vaccination post-HSCT. Reactogenicity was similar between the three groups, though participants more than a year post HSCT documented higher temperatures after the second vaccine (Table 1). No difference in reactogenicity symptoms were noted after the first vaccine compared to the second vaccine (Figure 1). Four participants had severe adverse events, none related to the vaccine.

### Conclusion

Safety of flu vaccine in adult HSCT recipients at 3-5 months post-HSCT is comparable to those at 6-11 or 12-23 months post-HSCT. A second vaccine in the same season was not associated with additional adverse events. Further studies will focus on the comparative immunogenicity of early post-HSCT and repeated vaccination with HD or SD flu vaccine in adult HSCT recipients.

*Keywords: vaccine; transplant; safety*

Topic: Clinical Sciences: Clinical Trials – Vaccines

Abstract No: 11108

## SEASONAL INFLUENZA VACCINATION INDUCES CROSS-REACTIVE ANTIBODIES AGAINST A/H3N2 VIRUSES

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### Introduction

The A/H3N2 strain included in the seasonal influenza vaccine has been updated nearly every year due to rapid antigenic drift of the circulating virus. The H3N2 virus first emerged in 1968 and has since formed distinct antigenic clusters. Previous exposure to H3N2 viruses, either through infection or vaccination, may influence antibody responses. Yet, little is known about the impact of historically encountered H3N2 viruses on human antibody repertoire after vaccination.

### Objectives

In this study, we aimed to explore the breadth of antibody response towards antigenically distinct H3N2 viruses after seasonal influenza vaccination.

### Methods

Healthcare workers (HCWs), immunized with an inactivated seasonal influenza vaccine in either 2010 or 2013, were included in the study. Serum samples were collected pre (D0) and post vaccination (D21, 6M and 12M). Antibody responses against H3N2 viruses of 14 antigenic clusters, including the homologous vaccine strains, were measured utilizing the hemagglutination inhibition (HI) assay.

### Results

Our preliminary results show that seasonal vaccination boosted HI antibodies not only against the vaccine strains, but also towards previously circulating viruses. Interestingly, antibodies generated by vaccination in 2010 or 2013 cross-reacted with the H3N2 strains that circulated in later seasons, which the HCWs had not been exposed to yet.

### Conclusion

We found that an inactivated seasonal influenza vaccine has the potential to induce both homologous and cross-reactive antibodies against H3N2 viruses. Influenza exposure history and antigenically similarities between different strains might influence antibody responses to vaccination and will require further investigation.

*Keywords: Influenza vaccine; H3N2 virus; cross-reactive antibodies; antigenic drift; hemagglutinin*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 11116

## RATIONALLY DESIGNED HETEROLOGOUS PRIME AND BOOST VACCINATION STRATEGY FOR INDUCTION OF CROSS-PROTECTIVE IMMUNITY USING STOCKPILED H5 INFLUENZA VIRUS VACCINES

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### Introduction

In past influenza pandemics, a vaccine exactly matching the antigenic properties of the pandemic virus was not available until several months after emergence. Modeling studies indicate that early immunization is critical to reduce morbidity and mortality in a pandemic. Therefore, strategies for immediate deployment of antigenically-related stockpiled pre-pandemic vaccines are important. Previous studies suggest that heterologous antigen prime and boost vaccination regimens may be superior to homologous prime and boost in generating cross-reactive immune responses to antigenically divergent influenza viruses.

### Methods

A randomized, double-blind, Phase 2 clinical study was conducted to assess the safety and immunogenicity of a homologous or heterologous vaccination series with adjuvanted, inactivated monovalent influenza H5 vaccines stored in the US National Pre-pandemic Influenza Vaccine Stockpile (NPIVS). A panel of H5 vaccines from different clades was chosen based on the relative antigenic distance between the HAs of pre-pandemic vaccine strains and relative to the currently circulating H5 influenza viruses. A two-dose or three-dose regimen of adjuvanted vaccine utilized various HA antigens from the NPIVS.

### Results

We will show 1) the antigenic characteristics of 2 antigens used for prime and boost and 2) how the sequence in which the priming and boosting antigens are administered may be important for optimal elicitation of immune responses to the vaccine. Preliminary results suggest that heterologous prime and boost vaccination results in increased cross-reactive antibody titers and seroprotection rates to the vaccine viruses as compared to the homologous regimen.

### Conclusion

This study indicates the potential feasibility of priming a naïve human population with a homosubtypic H5 vaccine for boosting with a matched vaccine within a relatively short antigenic distance, and the induction of cross-protective immune responses that may provide a level of protection in the event of an influenza pandemic. CI

*Keywords: avian influenza A(H5N1), Phase 1/2 clinical study, immunogenicity, heterologous prime-boost, pandemic influenza*

Topic: Clinical Sciences: Clinical Trials – Vaccines  
Abstract No: 11352

## INDUCTION OF PERIPHERAL FOLLICULAR HELPER T-CELLS FOLLOWING LIVE ATTENUATED INFLUENZA VACCINE

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### Introduction and objectives

The determinants of serum and mucosal antibody responses to live attenuated influenza vaccine (LAIV) are poorly understood. A role for peripheral follicular helper T-cells (pTfh) in seroconversion following inactivated influenza vaccine has been demonstrated. We investigated whether pTfh are induced by LAIV and associated with humoral immune responses in young children.

### Methods

Gambian children aged 24-59 months were immunised with intranasal Russian-backbone LAIV in 2017 (n=74) and 2018 (n=57). pTfh changes from baseline to day 7 and 21 post-LAIV was assessed *ex-vivo* using whole blood staining. Expression of activation-induced markers (AIM) CD25, OX40 and PDL1 were also assessed following stimulation with influenza haemagglutinin (HA)-specific overlapping peptides. Seroconversion and mucosal HA1-specific IgA responses were measured by haemagglutination inhibition and IgA protein microarray assays at baseline and day 21.

### Results

Within pTfh populations defined by CD4+CD45RO+CXCR5+ expression, CXCR3+ICOS+ and PD1+ICOS+ pTfh increased at day 7 post-LAIV. This increase was statistically significant in children seroconverting to any strain (p=0.0359 and p=0.0123) but not in non-seroconverters. An increase in CXCR3+ICOS+ pTfh at day 7 was also seen in IgA responders (p=0.0005) but not in IgA non-responders. An increase in CD25+, OX40+CD25+, CD25+PDL1+ and CD25+OX40+PDL1+ within CD4+CD45RO+CXCR5+ cells was observed primarily at 21 post-LAIV following stimulation with pH1N1 HA and H3 HA, but not influenza B HA. No clear association was seen between AIM marker upregulation and serum or mucosal antibody induction.

### Conclusion

An increase in pTfh cells at day 7 post-LAIV was observed, which was more pronounced in seroconverters and IgA responders. Using solely AIM markers to define antigen-specific cells, upregulation was observed mainly at day 21, but these cells did not appear to be associated with humoral immune responses. More in-depth characterisation of Tfh subsets that contribute to serum and mucosal antibody responses post-LAIV is warranted.



Topic: Clinical Sciences: Development of Human Disease

Abstract No: 11017

## LONG-TERM HEMAGGLUTINATION AND NEURAMINIDASE ANTIBODY TITERS AFTER INFLUENZA CHALLENGE

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**Introduction and Objectives:** Circulating anti-influenza antibody levels are inversely correlated with clinical illness and severity of influenza disease. After natural infection and vaccination, antibody titer levels can persist initially, but generally wane over time. Human influenza challenge models give us the unique ability to follow individuals from a specific, known and well-characterized exposure to measure long-term changes in antibody titers from pre-exposure baselines.

**Methods:** Healthy volunteers who completed influenza challenge studies at the National Institutes of Health Clinical Center were invited to enroll in a long-term study for 2 years after completing an influenza challenge study. Participants were seen in the clinic once every 12 weeks which included blood collection for serum antibody titers. They also completed monthly online questionnaires to monitor for symptoms of influenza-like illness in between clinic visits.

**Results:** Since July 2015, 41 participants enrolled. Thirty-three (80%) participants completed an Influenza A H1N1 challenge study, 18 (44%) completed an Influenza A H3N2 challenge study, and 7 (17%) completed both H1N1 and H3N2 challenge studies. At enrollment, participants ranged in age from 22-45 years of age with 17 (41%) female participants, 17 (41%) White, 17 (41%) Black, and 7 (17%) Hispanic participants. Participants had variable humoral responses post challenge, including quantity, quality, and timing of the responses. Titers of antibodies against the HA and NA changed over time to a variable degree in each of the participants.

**Conclusion:** There was no consistent pattern observed, demonstrating the complexity of the systemic humoral response to influenza. This study provides unique insight into how anti-influenza antibody titers change over months and years after challenge.

**Acknowledgements:** This research was supported by the Intramural Research Program of the NIH, NIAID.

**Keywords:** *influenza challenge; healthy volunteer; hemagglutinin; neuraminidase*

Topic: Clinical Sciences: Development of Human Disease

Abstract No: 11287

## Incidence and Outcome of Acute Kidney Injury in Hospitalized A(H7N9) Patients: A National Retrospective Multicenter Study

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**Objective:** To evaluate the incidence and outcome of acute kidney injury (AKI) among patients with avian influenza A(H7N9) virus infection.

**Design:** National retrospective cohort study.

**Setting and Patients:** Totally, 336 A(H7N9) patients from 137 hospitals in mainland China from October 1, 2016, to March 1, 2017.

**Interventions:** None.

**Measurements and Main Results:** We evaluated AKI according to the Improving Global Outcomes (KDIGO) criteria with screening medical records of hospitalized A(H7N9) patients. Of the 336 A(H7N9) patients, 245 (72.9%) cases were admitted to ICUs. During the entire hospital stay, 158 (47%) patients developed AKI. Of the AKI patients, 39 (86.7%) suffered from AKI stage 3, and 64 (40.5%) patients received renal-replacement therapy. The duration from illness onset to AKI was 7 days (IQR, 5-11 days). The in-hospital mortality of A(H7N9) patients was 43.8%, and it was significantly higher in AKI than non-AKI patients (71.5% versus 19.1%;  $P < 0.01$ ). We found AKI to be independently associated with death (Hazards ratio 11.0; 95% CI, 2.9-41.4), adjusting for age, gender, acute respiratory distress syndrome and requiring vasopressor support.

**Conclusions:** AKI is common in A(H7N9) patients and associated with increased mortality. Early recognition and sufficient management of AKI should be emphasized.

**Keywords:** Acute kidney injury; Avian influenza A(H7N9) virus; Critical illness; Mortality

Topic: Clinical Sciences: Diagnostics and Disease Markers  
Abstract No: 10326

## **COST-EFFECTIVENESS AND BUDGET IMPACT OF MOLECULAR POINT-OF-CARE NUCLEIC ACID AMPLIFICATION TESTING FOR THE DIAGNOSIS OF INFLUENZA IN JAPAN**

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### **Background**

In Japan it is estimated that 5 -10% of the population develops influenza annually, resulting in approximately 7,000 deaths. The current standard of care for diagnosing influenza-like illnesses (ILI) is rapid-antigen detection tests (RADTs). While RADTs are easy to perform and provide results quickly, poor test sensitivity of RADTs leads to inappropriate treatment decisions. Newer and more sensitive molecular point-of-care nucleic acid amplification tests (mPOC NAAT) are now available for use. This study evaluates the potential cost-effectiveness and budget impact (BI) to the Japanese Ministry of Health, Labour and Welfare (MHLW) of adopting mPOC NAAT to diagnose influenza.

### **Methods**

A decision-tree economic model, developed in Microsoft Excel 2016, quantified acute costs and clinical outcomes associated with the diagnosis and treatment of influenza with mPOC NAAT compared to RADT over a one year period. All model inputs were derived from the published literature, the Japanese National Institute of Infectious Diseases, or from the MHLW. Model outputs included costs, productivity loss and clinical effects measured as life years, quality-adjusted life years, mortality, hospitalizations and antibiotic utilization. One-way and probabilistic sensitivity analyses were performed to assess the impact of uncertainty on results.

### **Results**

Use of mPOC NAAT to diagnose influenza was projected to cost ¥21,350 per patient compared to ¥21,357 with RADT, with a small increase in QALYs. In a hypothetical population of 1,000,000 patients, mPOC NAAT was projected to avert 11,691 influenza-associated deaths, 2,489 hospitalizations and 53,023 antibiotic prescriptions, relative to RADTs. The BI of mPOC NAAT among this cohort was minimal, estimated at 0.2% of current budget. Findings were robust in sensitivity analyses.

### **Conclusion**

Our results suggest that adopting mPOC NAAT to diagnose influenza would be considered cost-effective with minimal financial impact. Access to mPOC NAAT would be important to optimize appropriate influenza diagnosis and treatment decisions in Japan.

*Keywords: Influenza, Point-of-Care, Diagnostics, Cost-effectiveness*

Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 10539

## CLINICAL EVALUATION OF COBAS® INFLUENZA A/B IN JAPANESE PATIENTS WITH INFLUENZA LIKE ILLNESS

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**Introduction:** In Japan, the estimated number of influenza patients in one flu season exceeds 10 million and these patients sometimes develop critical illness or death. Therefore, it is very important to diagnose these patients timely and accurately to guide the appropriate treatment. Although rapid antigen detection tests (RADT) are commonly used for the diagnosis of influenza in Japan, it has been reported that the sensitivity of these testing is not sufficient especially for the early phase of infection. We performed a clinical evaluation of cobas® Influenza A/B on cobas® Liat, a molecular point-of-care based on RT-PCR, compared against various RADTs.

**Method:** During the 2018-2019 flu season, a total of 389 patients with influenza-like illness were enrolled at three private practices and one hospital. Samples from each patient were collected and tested by both cobas® Liat and RADT, according to manufacturer's instruction and the positive rates were tabulated.

**Result:** The positive rate of cobas® Liat was significantly higher than that of RADT in overall patient population (50.4% and 43.4% respectively, Fisher's exact test,  $p < 0.05$ ). The same trend was observed in the subpopulation of 240 patients who were tested within 12 hours of onset (50.4%, 40.8%,  $p < 0.05$ ). On the other hand, there were no significant difference in subgroup of 149 patients tested after 12 hours of onset (12-24 hours; 43.6%, 42.6%,  $p = 0.5000$ , 24-48 hours; 53.9%, 46.2%,  $p = 0.3376$ ).

**Conclusion:** From the results, cobas® Influenza A/B has higher clinical sensitivity compared to RADT, especially in patients who are in early phase of infection. Hence, for earlier detection and prevention of transmission, cobas® Influenza A/B should be used for screening of influenza like illness.

*Keywords: molecular point of test; mPOC; cobas liat; cobas influenza A/B; rapid antigen detection test*

Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 10575

## EVALUATION OF GENEDIA<sup>®</sup> MULTI INFLUENZA ANTIGEN RAPID TEST KIT

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### INTRODUCTION

Accurate diagnosis of influenza is critical for clinical management, infection control and to minimize the burden of the disease. Commercially available rapid influenza diagnostic tests (RIDT) are widely used for diagnosing influenza because they are easy to use and minimal training required. More importantly, it can provide results within 15 minutes. Though RIDT is being used commonly in developing countries, little is known about its performance. The objective of this study is to find out the performance GENEDIA<sup>®</sup> Multi Influenza Antigen Rapid Test kit, compared to nucleic acid multiplex testing by Luminex NxTAG RPP.

### METHOD

845 nasopharyngeal swab samples from paediatric patients (age ≤18) suspected with respiratory tract infections were collected in 2018. Samples were tested for influenza by 2 different methods: PCR method by Luminex NxTAG RPP and GENEDIA Multi Influenza Antigen Rapid Test kit.

### RESULTS

It is shown in the study that influenza infection in Malaysia was high, with the prevalence of 20%. Test sensitivity for GENEDIA<sup>®</sup> kit was very low at 26.8%. On the other hand, test specificity was high at 99.6%. PPV and NPV were 93.8%, and 84.6% respectively. PPV for GENEDIA was 93.8% indicating 6% of positive results will be false positive. NPV of 84.6% indicating 15.4% of negative results will be false negative. Total agreement between the 2 methods were 85.1%.

### CONCLUSIONS

This evaluation revealed that GENEDIA<sup>®</sup> RIDT is a reliable test kit with PPV and NPV of 93.8% and 84.6%, respectively. High specificity of 99.6% makes it a reliable kit for influenza diagnosis. Other advantages include the ability of GENEDIA<sup>®</sup> RIDT to distinguish the pandemic (H1N1) 2009 influenza virus from seasonal influenza, quick results within 15 minutes, and it is easy to perform the test without the need to have equipment.

*Keywords: Influenza; RIDT; Genedia*

Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 10683

## TOWARDS A UNIVERSAL INFLUENZA A RT-qPCR DETECTION

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### Introduction and Objectives

The history of influenza A (IA) virus infections suggests that novel epizootic or pandemic strains can appear suddenly and from a previously unrecognized virus population. Hence, a fully validated universal RT-qPCR assay should outperform the inclusivity of the currently used assays and unify the diagnostic protocols. Here, we present a holistic approach in the development and evaluation of a universal RT-qPCR which enables to detect the IA virus without borders.

### Methods

The primers and probe were selected on the evolutionarily ultra-conserved regions identified by analysing more than 99,000 informative IA virus M-segment sequences, ie, the entire information content of the GISAID and GenBank databases from all species from 1902 to 2018.

### Results

100% of human H3N2, 100% of H1N1pdm, 98.2% of avian, 94.6% of swine and 100% of other IA virus strains exhibited absolute sequence identity to the primers and probe sequences. Overall, this means 99% detection rate at 0.5% threshold level. The SVIP-MPv2 assay exhibited reaction efficiency of 93-97% and consistent ability to detect 2 template copies/μl of nucleic acid extract or 10 IA virion equivalents per 25μl reaction in a 20+5 protocol (LOD<sub>95%</sub>). The predicted inclusivity and specificity were further confirmed on a broad panel of positive and negative clinical specimens and IA virus isolates in four human or avian national reference laboratories.

### Conclusion

Since the assay was carefully optimized and validated it should be used as a frontline screening tool to detect diverse IA virus strains in all reservoirs.

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Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 10773

## Alternate Measures of Infection Based on Serological Outcomes to Multiple Influenza Strains

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### Introduction

A four-fold rise or greater in homologous antibody titre is the long established gold standard for serologically confirmed influenza infection. This assay informs our fundamental understanding of infection attack rates during pandemic and non-pandemic periods. However, the related concepts of original antigenic sin, antigenic seniority and back-boosting raise the possibility that infection in older individuals could increase titres to historical strains more consistently than to currently circulating strains.

### Methods

We obtained paired samples from 983 individuals living in and near Guangzhou, China and tested paired antibody titres against the isolates circulated in 3 selected years of two influenza B lineages including Victoria (B/Victoria/2/1987, B/Malaysia/2506/2004 and B/Brisbane/60/2008, B/Shantou/267/2011) and Yamagata (B/Yamagata/16/1988, B/Shanghai/361/2002 and B/Florida/4/2006, B/Shantou/515/2011)

### Results

Traditional analyses of these data suggested that infection with either serotype of influenza B was low with only seroconverting 12.2% (10.2%, 14.3%) to B Yamagata 2011 and 5.06% (3.81%, 6.53%) to B Victoria 2011. However, rates of seroconversion to older strains were higher in older individuals for B Yamagata. We thus defined seroconversion to be based on different strains for different ages and found much higher rates of seroconversion for B Yamagata of %, 12.9%, (10.9%, 15.2%) but similar rates for B Victoria, 5.78% (4.42%, 7.33%).

### Discussion

The related concepts of original antigenic sin, antigenic seniority and back-boosting may all contribute to higher average rises in titre to ancestral strains of influenza B compared with currently circulating strains. Therefore, a more accurate picture of infection with influenza B can be obtained using a multi-strain definition of seroconversion. In this study, we found substantially higher rates of seroconversion in older individuals. This refined definition of seroconversion may lead to a strikingly different picture of influenza patterns than has hitherto been observed with the traditional definition of infection based on homologous seroconversion.

**Keywords:** four-fold rise antibody titre, influenza B

Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 10924

## CLINICAL RISK SCORE TO DIAGNOSE INFLUENZA INFECTION IN KAMPHAENG PHET, THAILAND

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**Introduction.** Influenza is usually characterized by an abrupt onset of high fever, cough and/or sore throat lasting several days. Typically, the clinical presentation of influenza disease is indistinguishable from other respiratory viruses. In Kamphaeng Phet province, Influenza types A and B are commonly associated with outbreaks at the end of the rainy (June-August) and winter seasons each year (October-February). This study aimed to develop a clinical risk score to help in diagnosing influenza at the first hospital visit.

**Methods.** A case-control data analysis was conducted in patients aged from 6 months old with influenza-like illness (ILI; fever  $\geq 38^{\circ}\text{C}$  and cough or sore throat) in Kamphaeng Phet, Thailand during April 2012 to February 2019 (WRAIR#1795A). Influenza infection was confirmed by RT-PCR of nasal or throat swab. The best predictors were selected by multivariable logistic regression and transformed into clinical risk scores.

**Results.** 1,880 patients experiencing ILI were enrolled, with 43.5% confirmed as influenza by RT-PCR. The best combination of clinical predictors included close contacts with ill persons, headache, malaise, runny nose and generalized muscle ache. The clinical scores can help to diagnose influenza infection correctly with an AuROC of 68.9% (95% CI; 66.4, 71.2). The likelihood ratio of positive for influenza was 0.4 in the low risk category and 2.0 (95% CI; 1.6, 2.3) in the high risk.

**Conclusions.** A simple clinical risk score can help to diagnose influenza more accurately with limitations of Influenza Rapid testing and to begin an appropriate course of treatment in time to be of the most benefit to the patient.

*Keywords: clinical risk, diagnosis, influenza*



Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 11012

## INTER-NOSTRIL VARIATION IN DETECTION OF INFLUENZA BY RAPID INFLUENZA DETECTION TESTS

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**Introduction and Objectives:** Rapid influenza diagnostic tests (RIDT) are commonly used in emergency rooms, urgent care centers (UCCs) and medical clinics for point-of-care influenza diagnosis. Testing is usually performed on a nasal swab specimen obtained from one nostril. We use data from a test-of-concept study, designed to assess patients' ability to self-swab, to assess inter-nostril differences in influenza detection.

**Methods:** The "Self- and Parent/Guardian-Collection of Specimens for Rapid Influenza Diagnostics" study enrolls patients of all ages presenting at two UCCs with acute respiratory infections. Patients, or their parents for minor children, are asked to open a provided Nasal Swab Home Collection Kit, read the instructions, and collect a nasal swab specimen (PtC) without observation. Following this, a trained research assistant obtains a nasal swab (RAC) from the other nostril. Both swabs are processed and evaluated using the Quidel Sofia Influenza A+B FIA. Residual specimens from both Sofia assays are then tested for influenza and human RNase P at the Wisconsin State Laboratory of Hygiene using rRT-PCR (IVD CDC Human Influenza Virus RT-PCR Diagnostic Panel).

**Results:** This is an interim analysis of the first 303 patients. Patients were aged from 9 months to 93 years (mean±SD: 32.6±18.2) and 57.3% were female. The majority (88.9%) had never collected a nasal self-swab specimen. Overall, 92.7% of RIDT results were concordant (20.5% influenza[+]; 72.3% influenza[-]). Nine (3.0%) specimens were RAC influenza[+] and PtC influenza[-] and 13 (4.3%) specimens were RAC influenza[-] and PtC influenza[+]. The proportions of negatively discordant specimens did not differ between RAC and PtC ( $\chi^2=0.753$ ;  $P=0.385$ ). Moreover, specimen adequacy (mean Ct for RNase P) was not different for either RAC[+]/PtC[-] ( $P=0.085$ ) or RAC[-]/PtC[+] ( $p=0.206$ ).

**Conclusion:** Whereas sampling techniques may have contributed to these findings, the most parsimonious explanation is that influenza virus antigens are present in differential amounts between nostrils.

*Keywords: influenza; diagnostics; RIDT; self-swab; variation*

Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 11024

## Use of Saliva in the Detection of Influenza Viruses at the Accident and Emergency Department During an Influenza Epidemic

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### Introduction and Objectives

Nasopharyngeal specimen has been the preferred specimen type for respiratory virus detection. However, the collection of nasopharyngeal specimen is an invasive procedure and can only be performed by healthcare workers. In contrast, saliva can be collected from patients easily. Recent studies have demonstrated that respiratory virus test results of saliva are comparable to those of nasopharyngeal specimens. Since January 2019, we have started to use saliva for the detection of respiratory viruses at the Accident and Emergency Department (AED). Here, our objectives are i) to analyze the saliva testing results during the 2019 winter season, and ii) to compare the results from nasopharyngeal specimen testing performed at the AED during the 2017/2018 winter and the 2017 summer epidemic.

### Methods

Saliva specimens were collected from patients at the AED of Queen Mary Hospital, a University-affiliated tertiary hospital in Hong Kong. Saliva was tested using Xpert® Xpress Flu/RSV assay. Saliva test results were retrieved from the Laboratory Information System.

### Results

From January 3 to February 28, 2019, a total of 487 saliva specimens were collected. The median age was 52 years, with an interquartile range of 30 to 70 years. Influenza A virus, influenza B virus and respiratory syncytial virus (RSV) were detected in 46% (222/487), 0.2% (1/487) and 1.6% (8/487) of patients, respectively. One patient had co-infection with influenza A virus and RSV. The percentage of specimens positive for influenza viruses in 2019 winter was similar to those of 2017/2018 winter influenza season (51%; 79/154) and of 2017 summer influenza season (43%; 23/53).

### Conclusion

The detection rate of influenza viruses in saliva during 2019 winter is similar to that in previous influenza seasons when nasopharyngeal specimens were used. Saliva is a convenient specimen type that can be used for respiratory virus testing at AED.

*Keywords: Influenza, Diagnosis, Accident and Emergency Department, Saliva, Specimen type*

Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 11042

## Detection of Influenza A Viruses Associated with Reduced Susceptibility to Cap-Dependent Endonuclease Inhibitor by Using the CycleavePCR™ Method

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### Introduction and Objectives

Cap-dependent endonuclease inhibitor, baloxavir marboxil (BXM), became available for influenza treatment in Japan in 2018. Several studies reported that amino acid mutations at position 38 in PA protein confer reduced susceptibility to BXM, and I38T is the most frequent mutation. We have developed real-time PCR methods for influenza A virus to detect I38T mutation in PA protein by using CycleavePCR™ (Takara Bio Inc, Shiga, Japan).

### Methods

During 2018/19 season, nasopharyngeal swabs were collected from influenza like illness patients after informed consent in pre- and post-treatment of BXM when they visited clinics and hospitals in Japan. Viral RNA was extracted from supernatants of clinical samples and transcribed into complementary DNA. CycleavePCR™ was conducted by using chimera probes that differentiate wild genotype (I38) and the mutated genotype (I38T) for influenza A/H1N1pdm09. Genetic sequencing by Sanger methods was carried out to confirm the results.

### Results

In total, 30 pairs of pre- and post-BXM treatment samples for A/H1N1pdm09 were examined by CycleavePCR™. No I38T virus was detected in pre-treatment samples (0%), but 3 I38T viruses were detected in post-treatment samples (10%). In turn, the detection rate was 3 out of 5 when the denominator was post-treatment sample positives (60%). Genetic sequencing verified 2 I38T and 1 I38S in the post-treatment samples. This method is specific to I38T and can identify the other mutant at position 38.

### Conclusion

We have developed a specific and high-throughput method to detect the I38T mutation in 3 hours after RNA extraction and cDNA synthesis. This method is useful in monitoring the most common mutant that confers reduced susceptibility to BMX. Currently CycleavePCR™ for A/H3N2 is underway. The results for 74 A/H3N2 pairs of pre- and post-BXM treatment samples will be presented in the congress.

*Keywords: Diagnostics ; Reduced Susceptibility ; mutation*

Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 11099

## Effect of a new point-of-care test (POCT) for influenza on clinical outcomes in hospitalised patients with severe influenza disease.

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### Introduction:

Hospitalisation due to influenza disease causes a significant burden to the Norwegian health care system. Clinical diagnosis of influenza is challenging. Treatment with neuraminidase inhibitors (NIs), should preferentially be started early and has shown to reduce mortality in the hospital setting. New rapid influenza POCTs could aid clinical decision making, influence antiviral and antimicrobial treatment choices, ameliorate isolation priorities, and possibly reduce length of hospital stay in severely ill patients.

### Objectives:

Our study aimed to assess the effect of the introduction of a new influenza POCT in a tertiary public university hospital, and to decipher the T-cellular immunological responses in severely ill hospitalised influenza infected patients.

### Methods:

We conducted a prospective, controlled cohort study in two university hospitals. Patients consented and were enrolled when admitted to the two largest hospitals in Bergen, Norway, during the 2018/2019 influenza season. Adult patients with suspected influenza or community acquired pneumonia (CAP), whom had a diagnostic test for influenza performed, were eligible for inclusion. One hospital had introduced an influenza POCT in the ER, while, the other relied on RT-PCR lab-based diagnostics, and was used as control. In addition, influenza positive patients from the tertiary hospital donated nasopharyngeal swabs and blood samples for immunological studies which are ongoing.

### Results:

A total of 613 patients with suspected influenza were recruited. 156/434 patients (36%) showed a positive POCT in the ER at the tertiary hospital and 69/179 patients were positive by RT-PCR at the neighbour hospital (39%) In addition, sera and PBMCs were collected from 131 influenza positive patients for immunological analyses. Positive POCT ensured early NI treatment.

### Conclusion:

Early virological confirmation in the ER influenced decision making regarding isolation use and NI treatment. Influenza was the dominating viral respiratory pathogen in both hospitals. The data-analysis of the results and immunological assays are ongoing.

*Keywords: point-of-care, diagnostics, influenza, infection*

Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 11109

## DEVELOPMENT AND VALIDATION OF ALGORITHM'S PROGNOSTIC FOR INFLUENZA CONTAGIOUSNESS

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### Introduction and Objectives

In an influenza pandemic, identifying individuals whom will subsequently become contagious before having significant contact with others would enable implementation of public health measures that could limit spread of disease. The aim of this work was to develop and validate an algorithm prognostic for contagiousness using biomarkers identified at early time points post exposure to virus, before subjects would be exhibiting substantial clinical disease symptoms.

### Methods

We utilised the human viral challenge model as it is an effective means of understanding a subject's pre-exposure biomarker levels and allows precise monitoring of clinical disease and biomarkers throughout the infection time-course.

The contagious status (phenotype) of 131 subjects was monitored by measuring clinical symptoms and taking nasopharyngeal swabs for virus quantitation. Blood samples were taken at frequent timepoints pre- and post-inoculation for microarray transcriptomics and to evaluate a panel of Luminex biomarkers. Training and test sets were created, and cubic spline analysis used to identify differentially expressed biomarkers in the training set. A parsimonious approach was taken to develop prognostic algorithms using each of the assay platforms, as well as pan-algorithms that combined the microarray and Luminex platforms. Each algorithm's performance was evaluated in the training set and the independent test set.

### Results

We identified biomarkers that had differential time-course trajectories in subjects whom later became contagious from subjects whom did not. Several prognostic algorithms showed good performance within the training set. Three of the algorithms continued to perform well when tested in the independent test set (Table) and, therefore, were successfully validated within the challenge model.

### Conclusion

Using the human viral challenge model, we have developed and validated prognostic algorithms for contagious prediction, ready for field testing. This represents an important step forward in providing additional public health tools for responding to an influenza pandemic.

*Keywords: contagiousness, human viral challenge model, prognostic, algorithm*

Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 11215

## BALOXAVIR GENOTYPIC AND PHENOTYPIC SUSCEPTIBILITY SURVEILLANCE

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### Introduction and Objectives

Baloxavir marboxil was approved in Japan and the USA for treatment of acute uncomplicated influenza A and B virus infection in 2018. Since oseltamivir resistant influenza A(H1N1) virus emerged and circulated in 2007/8 at high prevalence in countries that did not use significant amounts of the drug, capacity for surveillance of baloxavir susceptibility of circulating influenza viruses is important outside of the US and Japan.

### Methods

We validated a microneutralisation assay measuring baloxavir IC<sub>50</sub> for influenza A and B viruses. This assay detects baloxavir resistant virus even when present as a mixed population. We developed pyrosequencing assays for the most frequently detected mutations affecting baloxavir susceptibility, positions 23 and 38 in the PA protein. We also interrogated PA sequences generated by the NIC London over the last 5 years for the presence of mutations in the PA gene that may affect baloxavir susceptibility.

### Results

We tested influenza A(H1N1)pdm09, A(H3N2) and influenza B viruses isolated between 2017 and 2019 in the microneutralisation assay to determine a baseline IC<sub>50</sub> values. We established a low frequency of known mutations affecting baloxavir susceptibility by analysing >3000 sequences from 2014-19. These data contribute to the global dataset. Our pyrosequencing assays can be used to rapidly screen for mutations at position 23 and 38 in the PA gene, should baloxavir be used in the UK.

### Conclusion

Surveillance of circulating influenza virus novel antiviral phenotypic and genotypic susceptibility is important, and many laboratories have the existing capacity. Rapid global spread of drug resistant virus has occurred. The approval of new, non-neuraminidase inhibitor antivirals is an important step forward for the treatment of influenza infection and pandemic preparedness. Knowledge of the rate of emergence of virus with reduced susceptibility, and fitness to transmit is critical to maintaining the public health utility of these drugs.

*Keywords: Baloxavir; Influenza; Diagnostic; Surveillance; Susceptibility*

Topic: Clinical Sciences: Diagnostics and Disease Markers  
 Abstract No: 11315

## DEVELOPMENT AND ESTIMATION OF DIAGNOSTIC ACCURACY OF A REAL-TIME MULTIPLEX RT-PCR ASSAY FOR THE DETECTION OF INFLUENZA AND PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUSES IN SWINE

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The porcine respiratory disease complex (PRDC) is a multifactorial disease characterized by respiratory syndrome and poor growth in fattening swine, which impact the economy in the swine industry. It has been reported that infections or coinfections with Influenza A virus (IAV) and the Porcine Reproductive and Respiratory Syndrome virus (PRRSV) play an important role in the development of PRDC. Despite the relevance of IAV and PRRSV in the disease, there are no commercial kits that allow the simultaneous detection of both pathogens. We developed a Multiplex real-time RT-PCR assay by designing primers and hydrolysis probes for the simultaneous detection of IAV and PRRSV in swine samples. The Multiplex assay demonstrated a high analytical specificity and sensitivity in the diagnosis of these viruses, detecting 100 plaque forming units/ml (PFU/ml) of IAV and 1 PFU/ml of PRRSV. To validate the assay with clinical samples, the assay was performed on 803 swine samples where 93 samples were positive for IAV, 14 samples were positive for PRRSV and 3 samples were positive for both viruses. Of the clinical samples, 680 were used to compare the results with the CDC gold standard test used to detect IAV and estimated its diagnostic accuracy. The diagnostic sensitivity was 71.79% (CI 63.21-80.38%); whereas the diagnostic specificity was 99.47% (CI 98.79-100%), which validated the discriminative property of the test. The positive and negative predicted values of 96.55% (CI 92.14-100%) and 94.44% (CI 92.51-96.36%), respectively, indicated a robust performance of the assay. This Multiplex assay detects simultaneously both pathogens in clinical samples, and the validation process established its high capacity to make a confirmatory IAV diagnosis. This study confirms the clinical value of having an assay that provides a differential and timely diagnosis of two relevant pathogens for the porcine industry in Chile.

*Keywords: PRDC, IAV, PRRSV, Multiplex diagnostic, RT-qPCR*

Topic: Clinical Sciences: Diagnostics and Disease Markers  
Abstract No: 11344

## OBESITY, DIABETES, CHRONIC CARDIOVASCULAR DISEASE AND EARLY INCREASED EXPRESSION OF IL-6, IL-8 AND IL-10 ARE ASSOCIATED WITH INCREASED SEVERITY DURING INFLUENZA A VIRUS INFECTION

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**Introduction and Objectives:** Influenza virus infections cause seasonal epidemics and occasional pandemics and remain a major cause of morbidity and mortality worldwide. Disease severity depends on virological and host factors, such as extreme age, pregnancy, immunosuppression and a number of identified comorbidities. However, it is still unclear what specific molecular factors are at the basis of disease outcome in the general population. Here we evaluated whether preexisting clinical conditions differentially modulate host responses leading to severe disease.

**Methods:** We conducted a comprehensive analysis to understand the host factors that affect clinical outcome using clinical metadata from a cohort of 222 individuals (129 severe and 93 non-severe patients), and evaluated the status of their innate and adaptive immune responses by measuring pro- and anti-inflammatory cytokines during the acute phase of infection (0-7 days post symptom onset) using a Multiplex ELISA, and determined seroconversion by the hemagglutination inhibition assay.

**Results:** Bivariate analyses showed that severe individuals, including those that due to influenza infection required Intensive Care Unit (ICU) admission, mechanic ventilation and O<sub>2</sub> support, were associated with comorbidities such as, obesity (BMI>30), diabetes, and chronic cardiovascular disease (including arterial hypertension); and with an early increased expression of IL-6, IL-8. Those more severe individuals requiring ICU also associated with increased levels of IL-10, IL-15, IP-10 and MIP-1a. The presence of antibodies against HA at disease onset was not predictive of a better outcome. Additionally, multiparameter analyses identified that variables such as obesity, diabetes, IL-6, and 10 were associated with a worse clinical outcome (e.g. ICU and respiratory support). In contrast, immunosuppression, and increased IL-1b and IL-5 levels were not associated with severity.

**Conclusion:** Our results suggest that innate and adaptive immune responses play a crucial role in disease outcome, providing potential new targets for therapeutic and prophylactic interventions for those at higher risk.

*Keywords: Severe disease, Innate immune responses, ICU, Disease marker*



Topic: Clinical Sciences: Diagnostics and Disease Markers

Abstract No: 11348

## CLINICAL EVALUATION OF RAPID MOLECULAR TEST FOR INFLUENZA, ID NOW INFLUENZA A&B 2 IN COMPARISON WITH CURRENTLY AVAILABLE TESTS

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**Introduction and Objectives:** We evaluated the performance of ID NOW Influenza A&B 2 (ID NOW 2), rapid molecular test for influenza, in comparison with 3 currently available tests.

**Methods:** A total of 254 nasopharyngeal swabs (NPS) and 271 nasopharyngeal aspirates (NPA) collected from 373 children and 152 adults with influenza-like illness were tested using ID NOW 2, viral culture, rapid antigen detection test, and loop-mediated isothermal amplification test to evaluate the sensitivity and specificity compared with real-time reverse transcription polymerase chain reaction as the reference method.

**Results:** The sensitivities of ID NOW 2 for influenza A were 95.9% and 95.7% in NPS and NPA, respectively, and for influenza B were 100% and 98.7% in NPS and NPA, respectively. The specificity was 100% for both influenza A and influenza B in NPS and NPA. These sensitivities reflected the difference of analytical sensitivity among tests, and were not affected by time after illness onset and patient age.

**Conclusion:** ID NOW 2 demonstrated a high sensitivity and specificity that is useful for diagnosis of influenza in the clinical setting and infection control.

Topic: Clinical Sciences: Diagnostics and Disease Markers  
Abstract No: 11763

## PERFORMANCE OF QUICKVUE INFLUENZA A+B RAPID TEST TO DETECT INFLUENZA INFECTION DURING YEAR 2012-2019 IN KAMPHAENG PHET, THAILAND

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**Introduction.** Influenza is usually characterized by an abrupt onset of high fever, cough and/or sore throat lasting several days. Rapid diagnostic detection has been used to detect influenza A (seasonal and pandemic) and B in order to provide prompt clinical care. In Kamphaeng Phet province, influenza A and B are commonly associated with outbreaks at the end of the rainy (June-August) and winter seasons each year (October-February). This study aimed to analyze the performance of QuickVue Influenza A+B rapid test at the time for clinical care.

**Methods.** A surveillance study was conducted in patients aged at least 6 months old with influenza-like illness (ILI; fever and cough and/or sore throat) from Kamphaeng Phet provincial hospital from April 2012 to May 2019 (WRAIR#1795A and 1957). One nasal swab from each participant was collected and tested by QuickVue while confirmation by RT-PCR was done using a nasal or throat swab. QuickVue performance was characterized with respect to sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for Influenza A and B, as compared to RT-PCR.

**Results.** 5,958 patients experiencing ILI were analyzed, where 2,180 (36.6%) were confirmed positive for influenza by RT-PCR (51.2 influenza A and 48.8% influenza B) and 1,793 (30.1%) were positive by QuickVue (55% influenza A, 47.8% influenza B and 1.2% influenza A and B coinfection). Thus the sensitivity of QuickVue for influenza overall was 74.6% (95% Confidence Interval, CI: 72.7-76.4%), specificity 95.5% (95% Confidence Interval, CI: 94.8- 96.1%), PPV 90.5% (95%CI: 89.0-91.8%) and NPV 86.7% (95%CI: 85.6-87.7%). The concurrence rate of QuickVue Rapid test compared to RT-PCR result was 87.6%. (p=0.002).

**Conclusions.** QuickVue Influenza Rapid test demonstrated a sensitivity of 74.6%, specificity of 95.5% and 87.6% concurrence result for seasonal and pandemic influenza detection. This can be useful for clinician to provide antiviral drug to control the disease during the influenza outbreaks.

**Keywords:** QUICKVUE Influenza Rapid test

Topic: Clinical Sciences: Special Populations

Abstract No: 10334

## INFLUENZA AS A CAUSE OF SUDDEN DEATH - THE EXPERIENCE FROM THE NATIONAL REFERENCE LABORATORY FOR INFLUENZA, CZECH REPUBLIC

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### Introduction and Objectives

The influenza-associated sudden death is investigated only rarely. Recently, we have recorded increasing number of influenza positive autopsy samples related to sudden death. The objective of this work is to analyze pathological decision in context with the laboratory confirmation of influenza infection. As an outstanding example we present sudden death investigation of a 6-year-old girl with normal health status and without severe symptoms before death and without any macroscopic pathological signs of influenza infection during dissection.

### Methods

Clinical, respective epidemiological anamnesis and inflammatory changes in the airways, especially haemorrhagic tracheitis are the indication for virological examination.

The influenza virus (A - H1N1/H3N2, B - Yamagata/Victoria) was detected in autopsy samples from trachea, lungs and myocardium by reverse transcription real time polymerase chain reaction (RT-qPCR) and electron microscopy.

### Results

Between 2009 and 2018, 216 autopsy cases were investigated for influenza. Of the examined samples, 114 were positive, only 7 were attributed to influenza-associated sudden death. During the epidemic season 2018-19, we examined 27 autoptic sudden death material, and 14 were positive for the influenza virus (A - 4, A/H1N1 - 5, A/H3N2 - 1, B - 1, *Orthomyxoviridae* - 3). In the suddenly deceased child's case, all of the obtained autoptic material was positive for influenza A/H1N1 (trachea, lung, myocardium, blood, cerebrospinal fluid). Histological summary will be added.

### Conclusion

Our data suggest that sudden death directly connected with influenza infections occur sporadically even in young people and children after short viraemia and even without clinical signs of influenza. During the influenza epidemic sudden death cases should be investigated for the presence of influenza viruses even in the absence of clear macroscopic clinical/pathological picture afterward. This approach may broaden our knowledge regarding the complexity of influenza pathophysiology.

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Topic: Clinical Sciences: Special Populations  
Abstract No: 10394

## Hospitalization of high functioning adults for respiratory illness is associated with changes in mental status

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**Background:** Influenza is a serious respiratory illness with potentially life-threatening consequences. While the disease is self-limiting, individuals with chronic health conditions would be expected to take longer to recover. This study used the SF-12 to evaluate patients hospitalized with an acute respiratory illness (ARI) to assess physical and mental functioning prior to admission and 3-8 weeks post enrollment.

**Methods:** Adults  $\geq 18$  years of age enrolled in the Hospitalized Adult Influenza Vaccine Effectiveness Network study – Pittsburgh site with ARI with cough of  $\leq 10$  days duration were eligible. Those who consented to an enrollment and follow-up survey were included regardless of respiratory pathogen identified by respiratory viral panel testing from nasopharyngeal specimens. SF-12 respondents are asked to consider the previous 4 weeks when responding. Thus, both the enrollment and follow-up surveys covered some part of their illness and/or recovery. Respondents were grouped using cluster analysis based on SF-12 scale scores and age. Comparisons were made using Chi-square and F-tests.

**Results:** Of 50 adults who completed both surveys, 24 were grouped as the highest functioning cluster (HFC), 11 were in the lowest functioning cluster (LFC) and 15 were in the medium functioning cluster (MFC). At enrollment, the LFC more frequently reported body aches and confusion, lower pre-illness physical activity levels and other measures of physical function than the HFC ( $P < 0.016$ ). At approximately one-month post enrollment, the HFC reported significant decrements in most SF-12 scale scores; overall physical and mental component scores were significantly lower than at baseline ( $-4.26 \pm 8.1$ ;  $P = 0.017$  and  $-5.98 \pm 10.5$ ;  $P = 0.011$ , respectively). Changes in mental but not physical component scores from enrollment to follow-up differed significantly ( $P = 0.016$ ) between HFC and LFC.

**Conclusions:** Although their enrollment and follow-up SF-12 scores were higher, HFC reported larger losses in mental function during an ARI hospitalization than groups with lower scores.

*Keywords: influenza; hospitalization; physical function; mental function; acute respiratory infection*

Topic: Clinical Sciences: Special Populations  
Abstract No: 10511

## FRAILTY AND IMMUNE RESPONSE TO INACTIVATED INFLUENZA VACCINE IN OLDER ADULTS

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### Introduction and objectives:

Frailty accelerates immunosenescence; however, the impact of frailty upon immune response to influenza vaccine in older adults is mixed. This study assessed the association of frailty with immune response to influenza vaccine in adults in community to assisted living sites.

### Methods:

An observational, prospective study of 168 adults  $\geq 54$  years of age across a range of frailty levels was conducted in the Fall of 2017. Eligibility criteria included receipt of a previous season's influenza vaccine and willingness to receive the 2017-2018 seasonal influenza vaccine. Blood was drawn pre- and 28 days post vaccination for determining hemagglutination inhibition titers (HAI). The 5-item frailty phenotype measure was used. Logistic regression model outcomes were 28-day seroprotection (HAI titer  $\geq 40$ ), seroconversion (4-fold rise in titer, given baseline titer  $\geq 10$ ) and  $\log_2$  Day-28 titers. Multivariable models were run separately for each vaccine strain (influenza A/H1N1, A/H3N2-egg-based and A/H3N2-cell-based) including known frailty-associated covariates (sex, race, obesity, smoking status, baseline health status) and baseline HAI titers.

### Results:

The cohort was 68% female, 76% white, 35% frail, and 41% obese. Seventy-four percent of the cohort received high dose (HD) vaccine. Frailty was not significantly associated with seroprotection or seroconversion at Day 28 in multivariable models. Post-hoc analyses examined  $\log_2$  Day 28 titers for each vaccine strain, adjusting for covariates significant in univariable models ( $P \geq 0.20$ ) using a stepwise forward regression generalized linear model. Models were run separately for recipients of HD and standard dose (SD) vaccines. The only independent variable significantly associated with  $\log_2$  Day 28 titers across vaccine strain outcomes was  $\log_2$  baseline titer.

### Conclusions:

Vaccination resulted in similar seroprotection and seroconversion rates across the spectrum of phenotypic physical frailty. Thus, frail persons may benefit from vaccination, which in this study consisted mostly of HD inactivated vaccine.

Topic: Clinical Sciences: Special Populations  
Abstract No: 10864

## Epidemiology of Hospitalised Adults with Seasonal Influenza Infection and Risk Factors for Severe Outcome in Glasgow, Scotland

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**Introduction and Objectives.** The epidemiology of influenza-related hospitalisations in the UK are poorly characterised. We described the clinical presentation and management of adults hospitalised with influenza infection in Greater Glasgow, and determined risk factors for severe outcome (intensive care unit (ICU) admission or inpatient death).

**Methods.** We conducted a retrospective cohort study of adults ( $\geq 18$  years) hospitalised with laboratory-confirmed influenza infection at 8 Greater Glasgow acute hospitals between October 2015 and April 2016.

**Results.** Influenza was identified in 548 hospitalised adults. Median age was 54 years (range 18-94); 69.5% had  $\geq 1$  comorbid conditions. 11.1% had severe outcome (ICU admission in 7.7% and 6.6% died). Median length of stay (LOS) was 6 days (IQR 3-10), encompassing 5228 bed days. Antiviral treatment were given to 54.0% patients; 13.6% of whom received it within 24 hours after admission. Early antiviral treatment was associated with shorter LOS (median 5 vs. 8 days,  $p < 0.001$ ).

Independent predictors of severe outcome included older age (age 41-65: adjusted odds ratio (aOR) 4.09, 95%CI 1.38-12.10;  $> 65$  aOR 5.85, 95%CI 1.98-17.30; vs. 18-40), immunosuppression (aOR 2.33, 95%CI 1.16-4.65), and influenza A virus infection (aOR 6.00, 95%CI 2.21-17.18). Additionally, we identified an 'influenza severity score' comprising five clinical parameters: systolic blood pressure  $< 90$ mmHg, oxygen saturation  $< 90\%$ , respiratory rate  $> 30$  breaths/minute, Glasgow Coma Scale  $< 15$ , and radiologically-confirmed pneumonia. A score of 0, 1, and  $\geq 2$  were associated with 0.9%, 4.6%, and 33.8% risk of severe outcome, respectively.

**Conclusions.** Adults with hospitalised influenza had significant morbidity and mortality, particularly among those aged  $\geq 65$  years and with underlying immunosuppression. During winter months, clinicians should have a low threshold for influenza testing and promptly initiate antiviral treatment. The proposed influenza severity score, if validated, could be used along with point-of-care tests in a clinical pathway for the prompt diagnosis and treatment of hospitalised influenza.

*Keywords: Influenza; hospital; adult; epidemiology; risk factors*

Topic: Clinical Sciences: Special Populations  
Abstract No: 10873

## RESPIRATORY VIRUS SURVEILLANCE IN LONG-TERM CARE FACILITIES

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**Introduction.** Long-term care facilities (LTCFs) are ideal environments for acquisition and spread of infection: susceptible residents live in crowded institutional settings. Outbreaks of influenza and other acute respiratory infections (ARIs) in LTCFs often occur without identification of pathogens. Our novel surveillance approach is part of study evaluating the use of rapid influenza diagnostic testing (RIDT) in ten LTCFs.

**Method.** Nursing staff collected specimens from residents of participating LTCFs with new onset ARI symptoms using a nasal swab for RIDT. Following processing for Quidel Sofia Influenza A+B FIA, the residual swab was placed into viral transport medium and forwarded to the Wisconsin State Laboratory of Hygiene and tested for influenza using RT-PCR (IVD CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel) and for 17 other viruses (Luminex NxTAG Respiratory Pathogen Panel [RPP]). The numbers of viruses were compared using chi-square for the first two years of data.

**Results.** Totals of 155 and 189 specimens were submitted during 2016-2017 and 2017-2018, respectively; 93 specimens have been submitted and tested as of 30-March-2019 for the 2018-19 season but have not been included in analyses at this time. No differences were found in overall virus detection rates between years (56.6% vs. 57.8%). Two-year average detection rates for influenza A (22.7%), influenza B (16.0%), RSV (19.1%) and hMPV (17.0%) accounted for 74.8% of all detections, while coronaviruses (17.0%), rhino/enteroviruses (7.7%) and parainfluenza (2.6%) were less common. Specific distribution of viruses varied significantly across the first two years (Table:  $\chi^2=44.9$ ,  $df=6$ ;  $p<0.001$ ).

**Conclusion.** Surveillance in LTCFs using nasal swabs collected for RIDT is feasible. Influenza viruses are common and accounted for 35.2-43.1% of detections. Significant differences in virus distribution occurred across the two study years; third year data is forthcoming. Simple approaches to surveillance may provide a more comprehensive assessment of respiratory viruses in LTCF settings.

**Keywords:** Long-term care facilities; acute respiratory infections; influenza virus; rapid influenza diagnostic testing; nasal swab; surveillance

Topic: Clinical Sciences: Special Populations  
Abstract No: 10975

## OSELTAMIVIR TREATMENT PATTERNS AMONG IMMUNOCOMPROMISED PATIENTS IN U.S. INSURANCE CLAIMS DATA DURING THE 2017-18 INFLUENZA SEASON

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**Introduction:** Immunocompromised patients who develop influenza are significantly more likely to develop severe or complicated influenza, as compared to immunocompetent patients. Moreover, recent evidence suggests immunocompromised patients clear the influenza virus less rapidly than their immunocompetent counterparts. Immunocompromised patients are therefore a priority population for prevention and treatment of influenza. Further understanding of real-world clinical management with influenza antivirals among immunocompromised patients is needed.

**Methods:** We estimated prevalence of oseltamivir use among immunocompromised patients, overall and specifically for therapeutic and prophylactic use, using US insurance claims from MarketScan Commercial and Medicare Supplemental Databases from 1 October 2017 to 31 March 2018. Patients were required to have continuous enrollment and be 13 years or older to be eligible for study inclusion. Immunocompromised status was defined as at least 2 fills of an “immunomodulating medication” during the study period, as defined by the World Health Organization (L01-L04 ATC codes). We estimated prevalence ratios (PR) and 95% confidence intervals (CIs) for oseltamivir use among immunocompromised patients compared to a general population cohort.

**Results:** We identified 285,504 patients that met criteria for immunocompromised status. In this immunocompromised cohort, prevalence of at least one pharmacy fill for oseltamivir was 6.4%. When stratifying by type of use, prevalence of therapeutic fills was 5.1%, and prevalence of prophylactic fills was 1.2%. When compared to a general population cohort aged 13 and older for the same study period, immunocompromised patients were more likely to receive an oseltamivir fill (PR: 1.39, 95% CI: 1.37, 1.41). This finding held true for therapeutic oseltamivir fills (PR: 1.37, 95% CI: 1.35, 1.40) and prophylactic fills (PR: 1.61, 95% CI: 1.56, 1.67).

**Conclusions:** In an observational analysis of U.S. claims data, we observed that oseltamivir use was more prevalent among immunocompromised patients as compared to the general population during the 2017-18 influenza season.

*Keywords: immunocompromised; oseltamivir; antivirals; epidemiology*



Topic: Clinical Sciences: Special Populations  
Abstract No: 10977

## OSELTAMIVIR TREATMENT PATTERNS DURING PREGNANCY IN U.S. INSURANCE CLAIMS DATA (2015-2018)

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**Introduction:** Pregnant women with influenza are at higher risk of mortality and morbidity, as compared to non-pregnant influenza patients. Moreover, the potential effects of influenza treatment and influenza infection itself on the developing fetus add serious complexity to developing clinical guidance. The Centers for Disease Control and Prevention (CDC) recommend prompt treatment with antivirals for pregnant women with suspected or diagnosed influenza. With the exclusion of pregnant women from most randomized controlled trials of investigational agents, real-world data in this high-risk population is especially crucial. We sought to shed light on real-world treatment patterns by estimating the prevalence of oseltamivir use at any point during pregnancy using recent data from U.S. insurance claims.

**Methods:** Data were ascertained from the MarketScan Commercial Insurance Claims Database from 1 January 2015 to 31 March 2018. Women with a diagnosis or procedure code for any type of delivery were included. Look-back windows of 280 days (40 weeks) prior to delivery date were used to estimate pregnancy intervals. Patients were required to be continuously enrolled for the entirety of their pregnancy interval. Treatment was ascertained from pharmacy fill data, indicating that patients actually filled the prescription for oseltamivir.

**Results:** We identified 459,361 pregnant patients that met inclusion criteria. In this cohort, 6,217 patients had at least one pharmacy fill for oseltamivir (1.35%). When stratifying by type of use, 4,224 patients had at least one therapeutic oseltamivir fill (0.92%), and 1,772 patients had at least one prophylactic fill (0.39%) (see Table 1).

**Conclusions:** In an observational analysis of U.S. insurance claims data, oseltamivir use during pregnancy was observed for a nontrivial proportion of patients. Therapeutic use of oseltamivir was more common than prophylactic use in this population. Next steps include understanding how treated and untreated pregnant populations differ and investigating predictors of antiviral use during pregnancy.

*Keywords: Pregnancy; prenatal; oseltamivir; antivirals; epidemiology*

Topic: Clinical Sciences: Special Populations  
Abstract No: 11003

## Prematurity associated with maternal influenza hospitalization

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### Introduction:

The risks to fetuses born to mothers hospitalized with influenza are poorly described. This study is a matched case-control study evaluating the effects of influenza-associated hospitalizations on infants born to mothers who were hospitalized with influenza.

### Methods

Women who were hospitalized with laboratory-confirmed influenza were collected prospectively through the Emerging Infections Program (EIP) influenza surveillance. Any patient pregnant at the time of the influenza hospitalization was considered a case-mother. Infants were linked to the mothers using state birth certificate record data. Using the birth certificate records, infants with similarly estimated dates of confinement were identified as possible controls. To qualify as a control, the mother could not have a hospitalization for any respiratory illness during her pregnancy as identified by the state hospital discharge database. Only singleton pregnancies were included. Matching was done based on mother's admission year, maternal race, mothers age within 3 years, and estimated confinement date within 3 weeks. Descriptive analyses were performed. An ordinal mixed effects model was conducted for the ordinal outcome of prematurity, defined as <37 weeks of gestation. The main exposure variable was influenza hospitalization. Covariates included mothers age, education level, insurance, high risk medical condition(s), and tobacco.

### Results

99 pregnant women were identified by the EIP database. 94 cases were singleton pregnancies. One case had no matched controls and hence was dropped, leaving 93 cases and 279 controls. The table outlines the demographics of both mother and babies for cases and controls. The average week of gestation for controls was 39.1 ±1.5 weeks compared to cases which was 38.9±3.3 weeks. Cases were more likely to have a premature birth < 37 weeks (OR: 3.3; 95% CI: 1.2, 8.7).

### Conclusion

Infants born to mothers hospitalized with an influenza infection are more likely to be born premature.

*Keywords: pregnancy; infants; prematurity*

Topic: Clinical Sciences: Special Populations

Abstract No: 11085

## Influenza A(H3) hospital outbreak in Portugal during 2017/2018 season. NGS - a tool for investigation.

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### Introduction

During December/2017 an influenzaA(H3) outbreak has occurred among patients and health care workers at an Hospital Center in Lisbon. In outbreak analysis, next generation sequencing (NGS)was key not only for whole genome characterisation but also for monitoring of reassortment events and intra-host single nucleotide variants(iSNV) in order to understand the chain of infection. Present work aims to reconstitute this chain of infection by genetic characterisation using deep sequencing.

### Methods

*Ten nasopharyngeal swabs related to an hospital influenza A(H3) outbreak were received at Portuguese-NIC. After PCR multiplex amplification, whole genome sequences were obtained by deep sequencing on a MiSeq platform. Bioinformatics analysis was performed on INSaFLU (<https://insaflu.insa.pt/>). Beyond whole genome consensus sequences INSaFLU allowed to obtain iSNVs and reassortment patterns of viral genomic segments constellation based on the highest nucleotide similarity against reference strains.*

### Results

B/Yamagata influenza viruses have predominated (57%) during 2017/2018 season. Influenza A(H3) were detected in 14% of flu cases, mostly represented by 3C.2a2 subgroup, to which viruses from this study belonged (with the characteristic HA substitutions T131K,R142K and R261Q). Studied viruses presented 10 amino acid substitutions distributed by PB2, HA, NP and NA genes. Were found 57 iSNVs distributed by different viral genomic segments. However, only 1 iSNV(HA: A197G) with a 20% frequency in one patient has become fixed in another individual. Although outbreak viruses belonged to 3C.2a2 genetic subgroup, INSaFLU revealed a more complex reassortment profile. Based on nucleotide similarity: PB1, PA, HA and NS were more closely related to 3C.2a2 subgroup, while PB2, NP, NA and M were closest to 3C.2a3 subgroup.

### Conclusions

Substitution and reassortment patterns found exclusively in outbreak viruses among 2016/17 and 2017/18 A(H3) viruses characterised in our lab, confirm the closest relatedness of viruses detected in this outbreak. Analysis of minority variants disclosed an hypothetical transmission between 2 outbreak patients.

*Keywords: A(H3) outbreak; Whole genome; NGS; iSNV*

Topic: Clinical Sciences: Special Populations  
 Abstract No: 11114

## OBESITY IS ASSOCIATED WITH A DECREASE IN ANTI-INFLUENZA IgG AND AN INCREASE IN ANTI-INFLUENZA IgA ANTIBODY RESPONSES

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Influenza vaccines fail to provide optimal protection in obese individuals, while obesity is a risk factor for developing severe influenza infection, making vaccination of utmost importance for this high-risk population.

To study the effect of obesity on anti-influenza IgG and IgA antibody repertoires in healthy weight (HW, Body Mass Index:  $17 \leq \text{BMI} < 25$ ) and obese ( $\text{BMI} > 30 \text{ kg/m}^2$ ) patients, we used two cohorts: (1) Serum samples collected pre- and 30 days post-vaccination by the 2010-2011 seasonal influenza trivalent inactivated vaccine ( $\text{BMI}: 19.5\text{-}46.9$ ,  $n=222$ ); (2) and serum samples from patients that underwent bariatric surgeries at the surgery day ( $\text{BMI}: 17\text{-}40.6$ ,  $n=46$ ).

Antigen microarrays spotted with whole inactivated viruses and recombinant hemagglutinin (HA) proteins from >43 influenza strains were used to profile the anti-influenza antibody repertoires in these two cohorts.

In the vaccinated cohort, pre- and post-vaccination anti-influenza IgG levels to the whole viruses were significantly lower in obese patients (magnitudes of A/H1N1, A/H3N2 and B subtypes;  $p=0.000003\text{-}0.03$ ), while post-vaccination IgA antibodies to the vaccine strains were significantly higher in the obese ( $p=0.001\text{-}0.01$ ). Pre- and post-vaccination IgG levels to recombinant HA proteins of A/H1N1 and B subtypes were also significantly higher in HW as compared to obese patients ( $p=0.0001\text{-}0.01$ ), and a similar trend was observed for A/H3N2 HA proteins. Surprisingly, post-vaccination anti-HA IgA levels in the obese group were not higher than HW patients. Similar results were observed in the bariatric surgery cohort, with lower anti-influenza IgG levels and higher IgA levels in the obese group compared with the HW.

While a previous study reported that obese individuals have higher levels of overall IgG and IgA, our findings suggest that healthy weight and obese individuals generate distinctly different anti-influenza IgG and IgA antibody responses. The vaccine-induced Ab repertoire is also different in HW and obese subjects, with opposite effects on the IgG and IgA levels.

*Keywords: obesity, influenza, antigen microarrays, antibodies*

Topic: Clinical Sciences: Special Populations

Abstract No: 11182

## VIRAL RESPIRATORY INFECTIONS IN ADULTS FOLLOWING HEMATOPOETIC STEM CELL TRANSPLANT (HSCT) AT A TERTIARY CARE HOSPITAL IN INDIA

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**Introduction and objective:** Immunocompromised population like HSCT recipients are at a significant health risk of respiratory viral infections with morbidity and mortality, especially during their initial immunosuppressive regimen. Only limited data are available from India and this study was intended to fill this lacuna.

**Method:** HSCT patients enrolled from January 2017 to September 2018 at AIIMS, Delhi, India, were followed up for 3 to 18 months for episodes of respiratory illness until December 2018. Nasal and throat swabs collected from suspected acute respiratory infection were tested for influenza virus, respiratory syncytial virus (RSV-A/B), adenovirus, rhinovirus, human metapneumovirus (HMPV) and parainfluenza virus (PIV1-4) by real time polymerase chain reaction (RT-PCR).

**Results:** A total of 191 episodes of suspected acute respiratory infection were recorded during the follow-up of 76 enrolled HSCT patients (mean follow-up, 310 days). Samples could be collected and tested from 70 of these episodes (30 involving the upper, and 40, the lower respiratory tract) in 55 patients, with 1 to 4 episodes occurring per patient. Of the 70 episodes, 33(47.1%) tested positive for one or more respiratory virus. Of these virus-positive episodes, 15 occurred within the first month post-HSCT. Overall, rhinoviruses, which are not differentiated from enteroviruses by the RT-PCR, were the commonest viruses detected in 18(51.4%) of the 35 isolates (from the 33 virus-positive episodes). HMPV(10%) and RSV-B(5%) were detected exclusively in acute lower respiratory tract infection (ALRTI), and were not detected in any episodes of upper respiratory tract infection. Influenza viruses (A/H3N2 and B) and parainfluenzaviruses (PIV-3 and PIV-4) were found in both upper and lower respiratory tract infections.

**Conclusion:** This study highlights the role of respiratory viral infections in HSCT patients, particularly ALRTI associated with RSV and HMPV, and the high rate of infection in these patient, particularly in the first month after transplant.

*Keywords: Respiratory viral tract infection; real time RT-PCR; Stem cell transplant; AURI; ALRI*

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 10139

## Yeasts Ribonucleic Acid efficiency in a treatment of patients with Acute Respiratory Infectious Yeasts Ribonucleic Acid efficiency in a treatment of patients with Acute Respiratory Infectious

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*Background.* Influenza and other acute respiratory infections (ARI) are the most common human diseases. The idea of fast elimination of an agent from the cells of infected body is still a topical issue. It is established that medications based on yeast ribonucleic acid (Nuclex) possess have a broad spectrum of antiviral effect. It contains highly purified RNA.

*Material and methods.* 164 patients were observed. To determine the aetiology in patients with ARI the swabs were evaluated by PCR.

*Result.* A complex Nuclex treatment of the study group of patients had a positive effect on the course of the disease, it accelerated patients' recovery. This drug contributed to significant decrease in duration of febrile period. So, it lasted in average 1.8 days in the study group and 2.6 days in the control one,  $p < 0.001$ . Cough lasted for 2-3 days in 61.2% of patients of the study group, while the number of persons with cough was much lower in the control group 32.6%,  $p < 0.01$ . Duration of cough for six days or longer in 31.2% patients of the study group was significantly lower,  $p < 0.01$ , if compared to the control one 52.2%. In average, in the group treated with Nuclex cough lasted for 3.8 and 5.9 days in the control group of patients,  $p < 0.01$ . By PCR in early convalescence only in 8.2% of study group patients and in 60.2% of the control the pathogens were found again,  $p < 0.01$ .

*Conclusion.* Comprehensive treatment with antiviral drug Nuclex improved the clinical findings significantly; decreased the duration of fever, coughing and hospital stay duration of patients. Treatment with Nuclex caused eradication of viruses in patients with influenza and other ARI that was evidenced by PCR in dynamics. Pathogens were found in 8.2% of patients from the study group and in 60.2% of patients of the control one,  $p < 0.01$ .

*Keywords:* Treatment, Influenza, ARI, Nuclex

Topic: Clinical Sciences: Therapeutics – Antivirals  
 Abstract No: 10510

## CLINICAL AND VIROLOGICAL EFFICACY OF BALOXAVIR MARBOXIL IN CHILDREN WITH INFLUENZA A

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### Introduction and objectives

This study aimed to investigate the clinical and virological efficacy of baloxavir marboxil, a novel Cap-dependent endonuclease inhibitor, in children.

### Methods

During the 2018/19 influenza season, we enrolled 15 and 17 outpatients who were diagnosed with influenza A using a rapid antigen detection kit and were treated with baloxavir and oseltamivir, respectively. We collected three nasal swab samples from each patient before the antiviral therapy (day 0), on days 2 or 3, and between day 4 and 6. These samples were provided for the influenza A subtyping and the measuring the viral RNA load by a real-time RT-PCR. Using a questionnaire, we also obtained patients' background and clinical symptom scores over time. This study was approved by each IRB and informed consent was obtained from patients' parents.

### Results

There was statistical differences in age (due to differences in the dosage form, including only tablets for baloxavir and dry syrup for oseltamivir) and in influenza A subtype, but no differences were observed in other patient-specific background factors between patients treated with baloxavir and oseltamivir (Table 1). In all patients, antiviral therapy was started within 24 h after onset. The median residual viral RNA load in baloxavir- and oseltamivir-treated patients was 0.3% and 4.2% (Mann–Whitney *U* test, *P* = 0.013) on day 2 or 3 and 0.09% and 0.14% (Mann–Whitney *U* test, *P* = 0.34) on day 4 to 6, compared to day 0 levels, respectively. We observed no significant difference in fever duration (Table 1) and clinical symptom scores over time after antiviral therapy between baloxavir- and oseltamivir-treated patients.

### Conclusion

The clinical effects of baloxavir are comparable to those of oseltamivir. Baloxavir can decrease the viral load more rapidly than oseltamivir. Of note, the drug susceptibility assay and the *PA* gene analysis are currently under investigation.

*Keywords: Influenza; children; antiviral therapy; baloxavir marboxil; oseltamivir*

Topic: Clinical Sciences: Therapeutics – Antivirals

Abstract No: 10613

## ANTIVIRAL ACTIVITIES OF MULBERRY (*MORUS ALBA*) JUICE AND ITS GALLIC ACID AGAINST INFLUENZA VIRUSES

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**Introduction:** Influenza viruses cause acute respiratory infection responsible for seasonal epidemics and pandemics, imposing a huge toll on both human health and the economy worldwide. The emergence of drug-resistant viral strains requires new approaches for the treatment of influenza. *Morus alba* juice (MAJ) and seeds (MAS) are rich in polyphenols with biological activities that may impact positively on human health. In this study, we examined the antiviral activities of the MAJ and MAS against influenza viruses.

**Methods:** Inhibitory effects of MAJ and MAS were evaluated using time-of-addition plaque assays against influenza strains, A/Brisbane/59/2007(H1N1) (BR59), pandemic A/Korea/01/2009(H1N1) (KR01), A/Brisbane/10/2007(H3N2) (BR10), and B/Florida/04/2006 (FL04). To evaluate the antioxidant effects of MAJ on the virus-infected cells, DPPH radical scavenging and ferric ion-reducing activities of MAJ were analyzed. GSH levels in the virus-infected cells were also examined. Polyphenols of MAJ were quantitatively analyzed using an LCMS-8040<sup>TM</sup> liquid chromatograph mass spectrometer.

**Results:** MAJ showed much higher antiviral activities than MAS against BR59, KR01, BR10 and FL04 in a dose-dependent manner in the pre- or co-treatment of virus. MAJ at 4% concentration exhibited 1.3 log inhibition against FL04, a type B virus. MAJ exhibited significant GSH restoration, DPPH radical scavenging and ferric ion-reducing activities in a dose-dependent manner. Cyanidin-3-rutinoside, the most abundant polyphenol compound of MAJ identified by LC-MS, showed weak inhibitory effects against FL04, whereas gallic acid, a minor compound of MAJ, revealed significant antiviral effect.

**Conclusion:** MAJ and gallic acid showed antiviral effects at the early stage of viral infection, and the significant enhancement of GSH levels in influenza virus-infected cells. MAJ can be developed as a novel plant-derived antiviral against influenza viruses.



Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 10794

## A SYSTEMATIC REVIEW OF THE UPTAKE AND EFFECTIVENESS OF OSELTAMIVIR IN TREATMENT OF INFLUENZA ILLNESS IN PATIENTS WITH CHRONIC CARDIO-PULMONARY DISEASE

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### Introduction and Objectives

Patients with chronic cardiopulmonary disease are more susceptible to influenza-associated complications including death. Oseltamivir is recommended in this high-risk population for influenza treatment. We conducted a systematic review to determine the uptake and effectiveness of oseltamivir in reducing severity of influenza illness in patients with cardio-pulmonary conditions.

### Methods

Medline, EMBASE, Cochrane Controlled Register of Trials (CENTRAL) and CINAHL were searched. Quality appraisal of final studies was conducted using Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidelines. Data were extracted using a pre-developed template. Main outcomes measured included the rate of use of oseltamivir for influenza-like-illness and its effectiveness in reducing disease severity in patients with cardiopulmonary diseases. Outcomes measured for effectiveness were influenza-related complications such as respiratory infections, hospitalization rates and time to freedom from illness.

### Results

A total of 330 articles were retrieved. Final analysis was conducted on nine articles. Administration rate of oseltamivir ranged from 31-100% in patients with cardio-pulmonary disease depending on country and hospital setting. Incidence rates of influenza-related respiratory tract infections were reportedly between 11-19.8% in oseltamivir group compared to 18-69% in comparator group. Hospitalization rates due to influenza-related symptoms were between 0.6-7.4% in oseltamivir group compared to 1.3-18.5% in comparator group. Median time to alleviation of illness for patients receiving oseltamivir was lower (37.9-123.9hrs) than those who did not receive oseltamivir (53.8-134.3hrs).

### Conclusion

Data on use and effectiveness of oseltamivir in this high-risk population remains limited. Published studies suggest that oseltamivir is beneficial in reducing disease severity however the use of oseltamivir in this cohort is suboptimal. Further research is needed to determine the real-world effectiveness of oseltamivir in this high-risk population.

*Keywords: oseltamivir; cardiopulmonary conditions; influenza*

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 10830

## Defective interfering particles (DIPs): Development of production systems and identification of determinants of antiviral activity

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**Introduction and objectives:** Influenza epidemics and pandemics threaten public health. This threat is not adequately met by currently available antivirals and vaccines and novel options to fight influenza are required. Defective interfering (DI) viral RNAs, usually harboring a deletion, can arise during viral replication and DI RNA containing particles, termed DI particles (DIPs), can suppress the spread of wt virus. We are interested in developing DIPs for influenza therapy and prevention and seek to understand how DIPs exert antiviral activity.

**Methods:** We are using reverse genetics and MDCK cells stably equipped with influenza A virus (IAV) proteins to produce genetically defined DIPs. Mini-replicon systems are employed to study suppression of viral genome replication by DI RNAs.

**Results:** We report the generation of MDCK cells expressing codon-optimized PB2 that allow efficient production of the segment 1-derived prototypic DIP, DI 244. DIP production was independent of wt virus, could be traced using DI RNAs encoding fluorescent proteins and resulted in particles that exhibited potent antiviral activity. In order to understand how DIPs interfere with IAV infection, we asked whether DI RNA length is an important determinant of antiviral activity. We show that a deletion is sufficient to convert a genomic IAV segment into a DI RNA, as judged by the mini-replicon assay. Moreover, we demonstrate that the inhibitory activity of these DI RNAs is inversely correlated with DI RNA length and extends to diverse IAV genomic segments. Our ongoing studies aim to clarify whether DI RNA length is also critical for antiviral activity of DIPs produced in the systems described above and the results will be presented.

**Conclusions:** We report cell systems that allow efficient production of DIPs in the absence of infectious IAV and we provide evidence that DI RNA length is an important determinant of antiviral activity of DIPs.

*Keywords: defective interfering particle; PB2; DI 244*

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 10889

## THE FACTS OF INFLUENZA ANTIVIRALS SUSCEPTIBILITY SURVEILLANCE IN RUSSIA

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**Introduction and Objectives** The objective of the study was to assess the epidemic influenza viruses susceptibility to antivirals used in Russia.

**Methods** A total of 137 influenza viruses, collected in 2017-2019, were assessed for neuraminidase inhibitors (NAI) susceptibility: most of them were isolated in Moscow region (115) and different cities of Russia (22). The strains were received from different groups of population (pregnants – 61, SARI patients – 13, patients with lethal outcomes – 4). The 50% inhibitory concentration was determined for oseltamivir and zanamivir by neuraminidase inhibition assay. Also the cell-based ELISA assay for determining susceptibility to antivirals and genetic analysis for identifying of amino acid substitution (AAS) responsible for influenza viruses' resistance were used as well.

**Results** The majority strains of A(H1N1)pdm09 (73) and A(H3N2) (31) viruses exhibited normal inhibition (NI) by NAI. Highly reduced inhibition (HRI) by oseltamivir was exhibited in 5 A(H1N1)pdm09 strains isolated from pregnant women. Three women had received oseltamivir treatment. In all women treated with oseltamivir, fever ended on the first or second days of antiviral therapy, which may indicate the effectiveness of treatment. All these strains contained H275Y AAS, associated with HRI by oseltamivir, but not by zanamivir. All tested B viruses (28) exhibited NI by NAI. In January 2019 the epidemic strain of influenza A(H1N1)pdm09 - A/Moscow/246/2018 showed the reduced replication in MDCK cells in rimantadine presence (0,5-10,0 µg/ml). This strain had not AAS in 31 position of M2 protein. Also all tested in 2015-2016 epidemic season A(H1N1)pdm09 strains were susceptible to umifenovir (10,0 µg/ml). Moreover, the percentage of the reproduction inhibition for 25 strains isolated from autopsy materials was comparable to that for strains isolated from nasopharyngeal swabs of recovered patients.

**Conclusion** It demonstrates that continued monitoring and sharing of surveillance data on all existing antivirals susceptibility are important.

*Keywords: Antivirals susceptibility; neuraminidase inhibitors; rimantadine; umifenovir; surveillance*

Topic: Clinical Sciences: Therapeutics – Antivirals

Abstract No: 10940

## VICTORIA AND YAMAGATA: EXPLOITING LINEAGE-SPECIFICITY OF INFLUENZA B VIRUS NEURAMINIDASE INHIBITOR NATURAL SUSCEPTIBILITY AND SELECTIVE PRESSURE STRENGTH AT RESISTANCE-ASSOCIATED SITES.

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**Introduction:** B/Victoria(B/VIC) and B/Yamagata(B/YAM)-lineages have been co-circulating among humans for >30 years. Antigenically and genetically different, it is always questioned in what these two lineages may further differ. Here, we aimed to investigate the lineage-specificity of (1) virus natural susceptibility to neuraminidase inhibitors(NAIs); and (2) strength of selective pressure(SP) on the neuraminidase (NA) sites associated with (highly) reduced inhibition((H)RI) *in-vitro*.

**Methods:** Susceptibility of influenza-B viruses circulating in Portugal (2004/2005-2012/2013, N=142) to oseltamivir(OS) and zanamivir(ZA) was evaluated by MUNANA-based assay, using IC<sub>50</sub> baseline median as measure of *in-vitro* natural susceptibility. NA sequences were obtained by Sanger sequencing and aligned by Clustal-W in MEGA7. PyMOL enabled structural mapping. Datasets for SP analyses were constructed using all potentially-complete NA sequences available at GISAID-EpiFlu™/NCBI databases (November-2013). Following alignment and manual-inspection in MEGA7/Jalview, datasets included ≈1440(B/VIC) and ≈2000(B/YAM) sequences. PhyML enabled maximum-likelihood inferences and HyPhy2.2 was used to estimate site-specific SP by SLAC, FEL and iFEL methods (p-value≤0.05).

**Results:** B/VIC-lineage viruses exhibited a significantly lower overall natural susceptibility to OS (VIC<sub>IC50</sub>=21.27nM, YAM<sub>IC50</sub>=17.28nM; p=0.002) and, particularly ZA (VIC<sub>IC50</sub>=14.35nM, YAM<sub>IC50</sub>=4.82nM; p<0.0005), compared to B/YAM viruses. Individual season results supported overall data, although only for ZA. B/VIC-lineage-specific NA K219N and K373E mutations lie closely to the active site and may explain the differences observed. SP analysis revealed only slightly differences in the profile of the (H)RI-associated sites between the two lineages (>negatively selected-B/VIC; negatively selected/non-significant dN/dS<1-B/YAM). Ever, only in B/VIC RI-associated site 395 was found under positive selection. Although detected at similar frequencies in both lineages (0.05%-0.3%), type B NA (H)RI-conferring mutations occurred essentially in B/VIC-lineage viruses (n=24; B/YAM:8 mutations).

**Conclusions:** A lower natural *in-vitro* susceptibility to NAIs, particularly ZA, and a potential higher capacity to tolerate (H)RI-conferring mutations distinguished B/VIC from B/YAM-lineage viruses. Evidence of positive SP alerted for a potential higher risk of spread of a RI variant (A395E) in B/VIC-lineage.

**Keywords:** Influenza B lineage; neuraminidase inhibitors; natural *in vitro* susceptibility; resistance-conferring mutations; selective pressure

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 10950

## OPTIONS FOR THE ANALYSIS OF AN ORDINAL ENDPOINT, THE HOSPITAL RECOVERY SCALE IN SEVERE INFLUENZA

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Pimodivir is a novel influenza anti-viral in Phase 3 development including to treat patients who are hospitalized for severe influenza A. No single best endpoint for evaluating influenza treatments in this patient population has been identified, following lack of proof to show efficacy of NAIs using time to clinical stability endpoints.

Based on the use of an ordinal endpoint in retrospective and prospective data collections of severe influenza cases requiring hospitalization, and promising results in Phase 2 development of pimodivir, the Hospital Recovery Scale (HRS) has been defined to assess the efficacy of pimodivir in the Phase 3 program. The HRS consists of 6 ordered mutually exclusive categories, assessing the actual clinical status of patients: (1) Not Hospitalized; (2) Non-ICU Hospitalization, Not Requiring Supplemental Oxygen; (3) Non-ICU Hospitalization, Requiring Supplemental Oxygen; (4) Admitted to the ICU, Not Requiring Invasive Mechanical Ventilation; (5) Requiring Invasive Mechanical Ventilation; (6) Death.

The proportional odds model is a standard tool to analyze ordinal data and is more efficient than logistic regression following dichotomization of outcomes. However, also methods like Partial Proportional Odds Model, Generalized Estimation Equations, Sliding Dichotomy, MultiState Markov Modeling and Time to Clinical improvement on the HRS may be used to analyze ordinal data or derived endpoints. The different methods will be presented and compared, in the setting of severe influenza treatment.

The clinical relevance of the ordinal or derived endpoints, their analysis methods and differences in interpretation of the results will be discussed, supported by analysis of simulated data sets comparing 2 treatment groups, for different effect sizes and stratification by onset of influenza symptoms to first dose of study drug. The methods vary in identifying an overall treatment effect, in terms of statistical significance, for different data sets with the same underlying treatment effect.

*Keywords: Influenza, antiviral, ordinal endpoint, treatment effect, pimodivir, modeling*

Topic: Clinical Sciences: Therapeutics – Antivirals

Abstract No: 10959

## LIMITED USE OF ANTIVIRALS FOR TREATMENT OF INFLUENZA IN GERMANY, 2014-2018: ANALYSIS OF THE IMS-EU ELECTRONIC MEDICAL RECORDS DATABASE

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### Introduction and Objectives

Anecdotal reports suggest patients in Europe (EU) seldom visit healthcare providers for influenza treatment, and EU providers are unlikely to prescribe antivirals. We investigate this using German data from the IMS-EU electronic medical records database between 2014-2018. We assess the proportion of patients visiting practices for influenza, what proportion receive antivirals, whether patients receiving antivirals differ from those who do not, and characteristics of high- and low-prescribing practices.

### Methods

Patients with ICD-10 code for influenza (J09, J10, J11) during flu seasons 2014/2015—2017/2018 were selected. Patients with claim for antivirals (oseltamivir, zanamivir, or peramivir) within +/- 5 days of flu diagnosis were “AV-treated;” patients with flu diagnosis but no antiviral claim were “AV-untreated.” Descriptive statistics: 1) patient demographics and clinical characteristics, stratifying by AV-treated/untreated status; 2) characteristics of healthcare practices, stratifying by whether >5% (“high”) or ≤5% (“low”) of influenza patients at that practice received antivirals.

### Results

Of 121,409 influenza patients, 4,912 (4.0%) received antivirals. AV-treated patients were more likely to be older and/or privately insured. 8.7% (n=427) of 4,912 AV-treated patients received antibiotics within five days of influenza diagnosis, and 15.4% (n=17,886) of 116,497 AV-untreated patients. Of 1,314 practices treating influenza patients, 182 (13.8%) were “high-” prescribing; these were more likely to be in eastern than western Germany. Practices saw a median of 2,640 patients over two quarters, but just five influenza patients per season — of whom a median of zero received antivirals (Table 1).

### Conclusion

Use of influenza antivirals in Germany is limited. Less than 5% of influenza patients identified received treatment. Furthermore, contrasting the median number of influenza patients seen by practices against: a) the total number of patients seen over two quarters, and b) influenza prevalence in Germany (~8%) suggests that the vast majority of German influenza patients do not present to clinics.

*Keywords: Antivirals; Germany; treatment patterns; prescribing practices*

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 10978

## AQUEOUS ZANAMIVIR GLOBAL COMPASSIONATE USE PROGRAM – 2009-2019

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### Introduction and Objectives

Zanamivir is a neuraminidase inhibitor active against influenza A and B viruses. In parallel with the intravenous (IV) zanamivir development programme for the treatment of complicated influenza, a global compassionate use program (CUP) was initiated in 2009 at the onset of the influenza pandemic to provide zanamivir aqueous solution (IV or nebulised administration) on a named-patient basis.

### Methods

The CUP was administered via applicable country regulatory requirements. Serious adverse event (SAE) reporting was mandatory in accordance with local regulations. A Physician's Guidance Document was provided, including recommendation on dosing, administration methods and eligibility criteria.

### Results

As of 31Jan2019, requests for treatment were received for 3781 patients globally. Over 96% of patients received zanamivir intravenously. Patients ages ranged from <1 month to 98 years, including 3379 adults, 83 adolescents aged 13-17 years and 304 children/infants <13 years. At least 20 patients were 0-6 months (12 born prematurely with a gestation age of 23 -35 weeks); 41 were <1 year, five 1 to <2 years, 120 2-5 years, and 98 6-12 years of age.

SAEs (fatal and non-fatal) were reported for 446/3781 patients and reflected a severely ill hospitalised influenza population. Fatal SAEs were reported for 361 patients (~10% mortality rate) and were generally similar to those reported in IV zanamivir clinical studies and other reports of critically ill patients with influenza. There were 138 fatal cases considered by the treating physician as related or possibly related to zanamivir. Where sufficient information was available, most of these fatal cases were confounded by severe underlying medical illness.

### Conclusion

This CUP has provided access globally to IV zanamivir for a significant number of hospitalised patients. Compared with zanamivir's known safety profile, no new safety concern has been identified from the CUP.

*Keywords: Intravenous zanamivir, hospitalised patients, compassionate use, serious adverse event (SAE), safety*

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 11057

## Antiviral susceptibility of influenza A viruses during 2018/19 season, Portugal

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### Introduction:

Antiviral drugs are indicated to prevent and treat influenza illness, at present oseltamivir and zanamivir are the two neuraminidase inhibitors (NAIs) licensed and most widely used drug against influenza.

To assess the extent of susceptibility to the two most commonly used NAIs, during 2018/19 influenza season in Portugal, was measured the 50% inhibitory concentration (IC<sub>50</sub>) of NAIs for influenza A(H3) and A(H1)pdm09 viruses. Were assessed the *amino acid substitution linked with antiviral resistance (NAIs and baloxavir)*.

### Methods:

Influenza virus were isolated in MDCK/MDCK-siat1 cell lines from influenza positive nasopharyngeal swabs collected from influenza-like illness (ILI) patients. A neuraminidase inhibition assay using a fluorescent substrate 2`-(4-methylumbelliferyl)-a-D-N-acetylneuraminic acid (MUNANA) was performed to determine the IC<sub>50</sub> for 60 influenza A(H1N1)pdm09 and 19 influenza A(H3N2). After whole genome sequencing, 14 viruses were monitored for neuraminidase (NA) amino acid substitutions related to oseltamivir and zanamivir resistance. Ten polymerase acidic protein (PA) amino acid substitutions (associated to baloxavir resistance) were also monitored in 16 viruses.

### Results:

None of the influenza A(H3) and A(H1)pdm09 viruses showed phenotypic resistance to oseltamivir and zanamivir, all the viruses presented a normal inhibition.

The median for IC<sub>50</sub> values for oseltamivir and zanamivir, were 0.57 (range 0,19-4,09) and 0.47 (range 0,14-1,44) for A(H1)pdm09, 0.37 (range 0,15-0,63) and 0.66 (range 0,22-1,70) for A(H3N2), respectively.

NA and PA sequencing didn't revealed any substitution currently known to be associated with antiviral resistance to NAI and baloxavir.

### Conclusions:

The currently epidemic influenza A(H1N1)pdm09 and A(H3N2) viruses are susceptible for both NAIs with no evident trend toward decreased of susceptibility compared to viruses detected in previous seasons. Monitoring of mutations in PA linked with baloxavir resistance determined the susceptibility of influenza A recently circulating virus.

*Keywords: antivirals, NAI, baloxavir, influenza*



Topic: Clinical Sciences: Therapeutics – Antivirals

Abstract No: 11101

## DEFINING FUNCTIONAL RESISTANCE OF AVIAN INFLUENZA VIRUSES TO NEURAMINIDASE INHIBITOR DRUGS

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Neuraminidase inhibitors (NAIs) are important antivirals used for treatment of influenza virus infections in humans. Mapping of functional resistance to currently licensed NAIs has been limited to human strains with only sporadic reports investigating avian influenza (AIV). Past pandemics as well as the increasing number of humans infected with AIV have shown the importance of having information about avian NAIs that could cross the species barrier. In this study we introduced four NAI resistance-associated mutations most commonly found in human influenza into the NA of six prevalent AIV strains presenting zoonotic potential: H7N9, H5N6, H6N1, H5N8, H5N2 and H4N6. Using the established MUNANA assay we showed that R292K substitution significantly impaired NA activity in all strains, whereas E119V, H274Y and N294S had more variable effects. The impact of these mutations on NAI susceptibility was drug- and strain-specific and no universal signature conferring resistance to all NAIs in all virus backgrounds was found. However we did identify multidrug-resistant mutants as well as one mutation leading to oseltamivir-resistance in all viruses. Despite compromised NA activity drug-resistant H5N6 and H6N1 viruses replicated to comparable or significantly higher titres in primary chicken cells as compared to wild type. The competitive fitness of NAI-resistant H5N6 was also confirmed *in ovo*. Single amino acid substitutions found in the haemagglutinin (HA) of two H5N6 mutants reduced their receptor binding avidities, thus compensating the sialidase deficiency and enhancing viral fitness. Our results demonstrate that there are no universal NAI resistance determinants for all influenza strains and that whereas some mutations significantly reduce viral fitness, others can be rapidly compensated by concurrent changes in the viral genome. Our findings highlight the importance of dissecting molecular mechanisms of NAI-resistance in AIV and incorporating them in the routine surveillance programmes in birds.

*Keywords: antiviral resistance, neuraminidase inhibitors, avian influenza*

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 11152

**Contribution of antibody-dependent cellular cytotoxicity to the antiviral function of the vestigial esterase-targeting monoclonal antibody 9F4 against H5N6 infections *in vivo***

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Highly pathogenic avian influenza virus (HPAIV) strains have been a major health concern ever since they were found to infect and cause death in humans. Current treatment options include several small molecule antiviral drugs that function as neuraminidase inhibitors and can aid individuals resolve influenza A virus (IAV) infections when administered within 48 hours from the onset of flu-like symptoms. In order to better manage IAV infections, several other classes of antivirals such as monoclonal antibody (mAb) based therapeutics are being developed. The mAb 9F4 was described previously to neutralise several clades of H5N1 *in vitro* and confer protection in mice infected with the A/Smew/Sweden/V820/06 H5N1 virus. In this study, 9F4 was found to bind and prevent entry of pseudotyped particles containing the HA from the recently reassorted H5N6 (A/Guangzhou/39715/2014) in host cells. An antibody-dependent cellular cytotoxicity (ADCC) deficient 9F4 variant was also generated by the introduction of a L234A, L235A (9F4-LALA) mutation; wild type 9F4 but not 9F4-LALA was found to induce mouse Fc gamma receptor 4 (mFcγRIV) activation *in vitro*. To evaluate the *in vivo* efficacy of 9F4 and 9F4-LALA against H5N6 viruses, mice were intranasally infected with a 100 PFU dose of recombinant influenza virus rgPR8/H5N6 containing seven segments from A/Puerto Rico/1934 (PR8) and the HA segment of H5N6. A single 10mg/kg dose of 9F4 or 9F4-LALA given intraperitoneally 24 hours after rgPR8/H5N6 infection was able to confer protection in infected mice. Virus titration of lung homogenates from infected mice revealed that while both 9F4 and 9F4-LALA both reduced virus titres compared to control antibody, 9F4-LALA had reduced antiviral activity compared to wild type 9F4. Collectively, these findings demonstrate the utility of 9F4 as a potential antiviral against newly emerging reassortant H5 HPAIV strains and that ADCC augments but is dispensable for 9F4's antiviral activity *in vivo*.

*Keywords: monoclonal antibody, antiviral, H5N6, vestigial esterase targeting mab, ADCC*

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 11175

## STRUCTURAL STUDIES ON THE INTERACTION BETWEEN A H5NX NEUTRALIZING MONOCLONAL ANTIBODY AND H5N6 HEMAGGLUTININ

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Monoclonal antibody (MAb) 9F4 is a mouse IgG2b antibody that binds hemagglutinin (HA) of several H5Nx including H5N1, H5N2, H5N6 and H5N8. MAb 9F4 conferred both prophylactic and therapeutic protection against a lethal viral challenge of a clade 2.2.2 H5N1 in mice. Furthermore, mAb 9F4 also protected mice against H5N6 infection when given therapeutically, suggesting its potential for use in passive immunotherapy in the event of a H5Nx pandemic. In order to obtain structural information on the interface of antibody-antigen interaction, Fab of 9F4 was obtained and used for crystallization. Crystals were subjected to X-ray diffraction and the data was used to solve the three-dimensional (3D) structure of Fab 9F4. Next, the ectodomain of H5N6-HA was expressed as trimers in mammalian cells, purified and subjected to deglycosylation. The mixture of Fab 9F4 and H5N6-HA was subjected to gel filtration analysis and antibody-antigen complex was obtained for cryo-electron microscopy (EM). The complex is homogeneously distributed as evident from EM micrographs and its 3D structure is solved. Based on escape mutation and binding studies, mAb 9F4 is known to bind to a novel epitope present in the vestigial esterase sub-domain of HA. Taken together with the complex structure from cryo-EM and crystal structure of Fab 9F4, detailed information on the interface of antibody-antigen interaction is presented here.

*Keywords: mAb 9F4, H5N6-HA*

Topic: Clinical Sciences: Therapeutics – Antivirals  
Abstract No: 11760

## EFFICACY OF CB-012, A NOVEL ANTIVIRAL Fc-CONJUGATE, AGAINST INFLUENZA A (H1N1) IN A LETHAL MOUSE MODEL OF SEVERE COMBINED IMMUNODEFICIENCY (SCID)

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### Introduction

Cidara Therapeutics is developing a new generation of antivirals that couple a small molecule antiviral agent to the effector domain (Fc) of a human IgG1 antibody. These long-acting, antiviral Fc-conjugates (AVCs) directly inhibit viral replication while simultaneously engaging the immune system. The long half-lives and potent, intrinsic antiviral activities of AVCs make them well suited for potential use as preventative agents in patients with significant immunodeficiencies. CB-012 is a lead AVC candidate against influenza A/B currently undergoing evaluation for use in both immune-competent and -deficient populations.

### Methods

Efficacy was evaluated in male BALB/c *scid* mice (~7 wks) (Jackson Labs, #001803) challenged intranasally with 3x the LD<sub>95</sub> of influenza A/Puerto Rico/8/1934. CB-012 was administered intravenously as a single dose at 0.3, 1, or 3 mg/kg two hours after viral challenge. Body weights (BW) were monitored daily for 5 weeks, with 20% BW loss recorded as a mortality.

### Results

Over the first 3 weeks, all CB-012 concentrations were fully protective while vehicle-treated animals succumbed to viral infection by Day 9. Importantly, no significant BW loss was detected in CB-012 treated groups. By Day 35, the group treated with the lowest dose of CB-012 (0.3 mg/kg), a dose that is protective in immune-competent BALB/c mice, reached 80% mortality, indicating suppression of infection but incomplete viral eradication.

In contrast, groups treated with a single dose of CB-012 at 1 or 3 mg/kg were fully protected for the 5-week duration of the study. Mice in these groups had transient, insignificant BW loss and demonstrated net weight gain on Day 35 (2% and 8%, respectively).

### Conclusion

CB-012 demonstrated robust efficacy in a mouse model of severe immunodeficiency. This result supports further development of the AVC CB-012 as a novel antiviral for prevention against influenza in high-risk patients, including those with immunodeficiencies.

Topic: Clinical Sciences: Therapeutics – Immunomodulators, Supportive Care, Others  
 Abstract No: 10938

## Antiviral potential of IFN $\alpha$ subtypes against influenza infection in a human lung explant model

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**Introduction:** Respiratory infections due to influenza virus (IVs) are currently treated and prevented by using antiviral drugs that target viral proteins, for which resistance can emerge. IFN $\alpha$  is induced after IV infection, participates in host antiviral response and represents 12 different human subtypes with underexplored anti-influenza properties. Here, we make use of human lung explants as a pre-clinical model to evaluate the antiviral potential and immunomodulatory profile of IFN $\alpha$  subtypes.

**Methods:** Human lung explants were freshly obtained from patients undergoing lung surgery. The tissue infected with seasonal influenza strain A(H3N2) was evaluated for cytokines and ISGs induction by multiplex and real time analysis. For IFN $\alpha$  subtypes treatment, tissue was pre-incubated and further infected with A(H3N2). Also, IC<sub>50</sub> for selected IFN $\alpha$  subtypes was determined in A549 cells. Additionally, antiviral ISGs induction was evaluated in treated lungs and A549 cells. Finally, IFN $\alpha$  antiviral effect dependency on the restriction factor MxA was assessed in MxA Crispr/Cas knock-out cells.

**Results:** Initially, we observed that influenza A(H3N2) infection of human lung explants led to upregulation of antiviral ISGs, such as MxA, RIGI, OAS and ISG15, and cytokines, including IFN $\alpha$ 2, IFN $\beta$ , IFN $\gamma$ , IL1 $\beta$ , IL6 and MCP1, among others. IFN $\alpha$  subtypes treatment displayed distinct antiviral activities against influenza infection. IFN $\alpha$ 1,  $\alpha$ 8 and  $\alpha$ 21 did not present antiviral activity, whereas the other subtypes significantly suppressed viral replication, with  $\alpha$ 4,  $\alpha$ 5, and  $\alpha$ 16 presenting the highest activities, in comparison to  $\alpha$ 2. Transcriptional upregulation of antiviral ISGs was subtype-specific and positively correlated with their antiviral activity. Surprisingly, subtype antiviral activity was differentially dependent on the restriction factor MxA, indicating the involvement of other virus-specific ISG.

**Conclusions:** Our study reveals that eight IFN $\alpha$  subtypes presented anti-influenza potential compared to the clinically licensed IFN $\alpha$ 2 and should be further investigated for therapeutic treatment of severe IVs infections.

**Keywords:** influenza, treatment, IFN, subtypes, MxA

Topic: Clinical Sciences: Therapeutics – Immunomodulators, Supportive Care, Others  
Abstract No: 11141

## CHARACTERIZATION OF BROADLY CROSS-REACTIVE NEUTRALIZING ANTIBODIES AGAINST THE INFLUENZA B VIRUS HEMAGGLUTININ AND THEIR MECHANISM OF PROTECTION IN MICE

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**Introduction and Objectives:** Influenza B viruses cause significant morbidity and mortality in humans worldwide, especially in children. While highly conserved epitopes on the hemagglutinin (HA) of influenza A virus have been studied to great lengths using monoclonal antibodies (mAbs), far less is known about those on the antigenic surface of the influenza B virus HA.

**Methods:** Using hybridoma technology, we isolated a panel of mouse mAbs against the influenza B virus hemagglutinin (B HA) and tested their reactivity (via ELISA) to an array of purified influenza B viruses.

**Results:** Several of these mAbs exhibited broad binding activity against divergent influenza B virus strains (ranging from the ancestral B/Lee/1940 strain to the more recent B/Phuket/3073/2013 strain). Specifically, four of these mAbs were found to have broad neutralization activity as tested by microneutralization and plaque reduction neutralization assays. The target epitopes for these mAbs were mapped by the generation and characterization of viral escape mutants. Two mAbs appear to bind to the head domain of the HA while two target the broadly conserved stalk domain. The mechanism of neutralization of these mAbs were characterized to determine the stage at which these mAbs target the influenza virus life cycle (entry, uncoating, or egress). The mAbs were also evaluated in a therapeutic and prophylactic setting *in vivo* in mice against lethal influenza virus challenge.

**Conclusion:** Overall, the knowledge gained regarding the location of the specific epitopes that these mAbs target, mechanism of neutralization, and *in vivo* protective efficacy against lethal challenge, can aid in the design of novel vaccines intended to elicit broadly protective immunity against influenza B virus infection.

**Keywords:** Monoclonal Antibodies; Influenza B; Neutralization; Cross-reactive; Hemagglutinin

Topic: Co-Infection: Bacterial Co-infections with Influenza  
 Abstract No: 10336

## EXPERIMENTAL MUCOSAL VACCINE AGAINST INFLUENZA AND PNEUMOCOCCAL INFECTIONS

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**Introduction and objectives.** Influenza and its bacterial complications are a significant cause of morbidity and mortality. Development of mucosal vaccines based on live influenza vaccine (LAIV) and surface pathogenicity factors of pneumococci is a novel approach to prevent influenza-related bacterial complications. The objectives of the study included estimating the components of innate and acquired immunity, correlating with resistance against influenza-related *S. pneumonia* infection.

**Methods.** For intranasal immunization of mice, we used A/17/California/09/38(H1N1)pdm09, A/17/Mallard Netherlands/00/95(H7N3) or trivalent LAIV of 2017-2018 influenza season formulation. The bacterial vaccine components administered simultaneously with LAIV were 1) chimeric protein (PSPF) composed of three pneumococcal surface protein fragments associated with fragments of flagellin; 2) group B streptococcus recombinant peptide ScaAB. We evaluated the protective effect of mono- or combined vaccine preparations against challenge with influenza viruses and *S. pneumonia* serotype 3 when using different schemes of viral or bacterial infections.

**Results.** The ScaAB potentiated LAIV immunogenicity, while PSPF did not provide the same effect. Nevertheless, associated immunization using LAIV and PSPF more effectively prevented post-influenza pneumococcal pneumonia than either vaccine alone. When immune mice were infected with *S. pneumonia* on 24 hours before the onset of influenza infection, LAIV demonstrated a protective effect on both lethality and pneumococcal lung infection. Immunization with LAIV protected mice against lethal influenza infection on day 5 after immunization, and this was associated with increased type 1 interferons expression in the lungs.

**Conclusion.** A mouse model for the preclinical study of viral-bacterial vaccines has been developed. Immunization of mice with an experimental vaccine based on LAIV and recombinant peptides was effective in preventing post-influenza pneumococcal pneumonia. LAIV provided early protection against homologous and heterologous viral infection and has a positive effect on the course of virus-bacterial infections.

**Keywords:** Influenza, *S. pneumonia*, associated vaccination, chimeric recombinant protein

Topic: Co-Infection: Bacterial Co-infections with Influenza  
Abstract No: 10901

## Establishment of avian influenza virus/*Acinetobacter baumannii* co-infection model in mice

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**Introduction:** Patients with avian influenza virus infection manifest a wide range of disease severity. In most of the patients with severe situation, complication caused by secondary bacterial infection was frequently found and was believed as a major factor of the adverse clinical outcome. In particular, subsequent infection of gram-negative bacteria *Acinetobacter baumannii* was frequently identified in these patients upon the infection. However, there is so far no model for studying the pathogenesis of the avian influenza/bacteria co-infection and understand the interplay between the two pathogens.

**Objective:** To establish a co-infection model of avian influenza (H9N2) virus and *Acinetobacter baumannii* in mice.

**Results:** The pathogenesis was enhanced in the condition of co-infection using the H9N2 virus with or without mammalian adaptation at PB2-E627K. Interestingly, there was no difference on the viral and bacterial loads in the lung as well as the influx of various immune cells between those mice infected by co-infection and H9N2 alone.

**Conclusion:** This model mimics the clinically relevant condition of patients with avian influenza virus infection and suggests that subsequent bacteria co-infection can increase the severity. However, the exact mechanism on the pathogenicity will need to be further explored.

*Keywords: Avian influenza, co-infection, bacteria*



Topic: Co-Infection: Bacterial Co-infections with Influenza  
Abstract No: 10957

## INCREASED BURDEN OF CARE AMONG PATIENTS HOSPITALIZED WITH INFLUENZA AND CO-INFECTED WITH PNEUMONIA IN THE UNITED STATES, 2009-2018: ANALYSIS OF THE PREMIER HEALTHCARE DATABASE

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### Introduction and objectives

Bacterial pneumonias contribute to influenza-associated morbidity and mortality; their prevention and treatment deserves better understanding. We analyzed influenza patients with pneumonia in a large US hospital database between 2009-2018 to establish demographic and clinical characteristics, in-hospital mortality, duration of hospitalization/ICU stay/ventilation, and performance on an ordinal endpoint used in hospitalized influenza trials.

### Methods

Retrospective analysis of patients hospitalized for influenza in the Premier healthcare database from October 2009 through May 2018, in hospitals with continuous enrollment (<sup>≥</sup>1 patient per year) over this period. We compare three influenza cohorts: bacterial pneumonias, non-bacterial pneumonias and all flu patients. Secondary bacterial pneumonia was defined as either: 1) specific ICD-9/10 code for bacterial pneumonia, or 2) non-specific code and positive result on lab culture. "Non-bacterial" pneumonia was defined as either: 1) specific code for viral pneumonia, or 2) non-specific code and no result, or negative result, on lab culture. Descriptive statistics on baseline characteristics and outcomes were generated for all seasons from 2009-2018; we present 2009-2018 and—given its severity in the US—2017-2018 flu season data.

### Results

Bacterial pneumonia was not more prevalent in 2017-2018 versus 2009-2018. At baseline, a numerically higher prevalence of COPD and congestive heart failure was observed in influenza patients with bacterial pneumonia, versus those with other pneumonias, and the overall flu cohort. Bacterial pneumonia patients spent longer: a) in hospital; b) in the ICU; and c) on a ventilator, and had poorer performance on an ordinal endpoint scale than the overall flu cohort or influenza patients with non-bacterial pneumonias. [Table 1].

### Conclusion

Influenza patients co-infected with bacterial pneumonias had greater healthcare resource use and poorer outcomes. Future work will investigate antibiotic, antiviral, and procedural treatment patterns and outcomes (including costs and long-term outcomes and complications) for patients co-infected with influenza, stratifying by key bacterial pathogens.

*Keywords: Co-infection; real-world data; pneumonia*

Topic: Co-Infection: Bacterial Co-infections with Influenza  
Abstract No: 11086

## OCCURRENCE OF INFLUENZA AND BACTERIAL CO-INFECTIONS IN UPPER-TORSO CANCER PATIENTS UNDERGOING RADIOTHERAPY IN GHANA

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**Introduction:** Influenza is a common febrile illness with significant impact on children, the elderly and immunocompromised. Bacterial co-infections associated with cases of influenza are a leading cause of acute respiratory infections (ARI) and mortality especially among high-risk groups. Cancer patients undergoing therapy are prone to ARI associated with influenza viruses and bacteria such as *S. pneumoniae* and *S. aureus*. However, ARI-like symptoms developed by upper-torso cancer (UTC) patients undergoing radiotherapy are perceived to be the side effects of the radiation, hence diagnosis of infectious pathogens such as viruses and bacteria implicated in ARI among this is not investigated. This pilot study therefore aimed to determine the incidence of influenza virus and bacteria associated with ARI in UTC patients undergoing radiotherapy in Ghana.

**Methods:** Patients with cancers of the breast, head and neck were randomly selected from the Radiotherapy department of the Korle Bu Teaching Hospital (KBTH) in August 2018 and interviewed using a structured ARI study questionnaire. Oropharyngeal and nasopharyngeal swabs were collected from consented patients. Molecular detection of respiratory pathogens was by polymerase chain reaction (PCR) assay.

**Results:** 10 patients were enrolled into the pilot study with mean age 54.2 years. 80% of participants were females. Out of the study participants, 90% exhibited clinical symptoms of cough, sore throat and difficulty in breathing and 70% tested positive for at least one respiratory pathogen. Influenza virus was detected in 20% of patients as single infections and in 50% of patients as co-infections with bacteria. Further analysis of samples identified H1N1pdm09, B/Victoria lineage, *S. pneumoniae*, *H. influenzae* and *S. aureus* as the main respiratory pathogens isolated.

**Conclusion:** ARI experienced by UTC patients undergoing radiotherapy at the KBTH are not solely side effects of radiation but could also be due to influenza and other bacterial infections.

Topic: Co-Infection: Bacterial Co-infections with Influenza  
Abstract No: 11136

## BACTERIAL CO-INFECTION WITH INFLUENZA AMONG THE HOSPITALIZED PATIENTS WITH SEVERE ACUTE RESPIRATORY INFECTION

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**Introduction:** Bacterial co-infection with influenza in Severe Acute Respiratory Infection (SARI) cases is an important cause of morbidity and mortality. It is often under reported in Nepal. The objective of this study was to describe the etiology of bacterial co-infection with influenza among the hospitalized SARI patients during the winter season in Nepal.

**Materials and Methods:** A descriptive cross sectional study was conducted at National Influenza Center (NIC), Nepal during the winter of 2018/019. A total of 240 throat swab and bronchoalveolar lavage (BAL) were collected from the patients with SARI following WHO case definition. Total nucleic acid was extracted using Pure Link viral RNA/DNA mini kit (Invitrogen) and multiplex real-time RT-PCR assays were performed.

**Results:** Of the total 240 samples; respiratory pathogens were found in 194 (80.8%) samples. Infection with influenza virus was predominantly higher (50.0%) than other respiratory pathogens (30.8%). Influenza A/H1N1 pdm09 94 (78.3%) was the most frequent, co-infection of bacterial 76 (63.3%), viral 40 (33.3%) and mixed infection 16 (13.2%) were comparatively higher in influenza negative cases than patients with influenza positive SARI cases. Dual infection of *Chlamydia pneumoniae* and *Streptococcus pneumoniae* 18 (15.0%), rhinovirus and *S. pneumoniae* 12 (10.0%) followed by mono infection of *C. pneumoniae* 10 (8.3%) and *S. pneumoniae* 10 (8.3%) were detected in influenza negative cases. The incidence of dual bacterial infection with influenza in the months of January, 2019 and December, 2018 were 69 (57.5%) and 52 (43.3%), respectively.

**Conclusions:** This study highlights the bacterial co-infection with influenza in clinically well defined hospitalized patients. It could have consequence, both in pandemic and seasonal episodes. Further, year-round study is required for better understanding of influenza and bacteria associated co-infection and seasonality in Nepal.

**Keywords:** Bacterial co-infection, Influenza, Nepal, SARI

Topic: Co-Infection: Bacterial Co-infections with Influenza  
 Abstract No: 11264

## THE PREVALENCE OF INFLUENZA-ASSOCIATED CO-INFECTIONS IN CHILDREN UNDER 5 YEARS WITH ACUTE RESPIRATORY INFECTIONS IN MADAGASCAR, 2018.

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### Introduction and Objectives

Young children present a higher risk of serious complication associated to influenza especially in low income countries. We aimed to determine the frequency of influenza and co-infections among children suffering from respiratory tract infections.

### Methods

Nasopharyngeal swabs and clinical/demographic data were collected from children under 5 years presenting influenza-like illnesses (ILI) or severe acute respiratory infections (SARI) from sentinel sites during 2018. Specimens were analyzed using the FTIyo Respiratory pathogens kits (Fast Track Diagnostics) that simultaneously detect 21 respiratory pathogens comprising viruses and bacteria.

### Results

A total of 475 specimens associated with ILI (n=364) and SARI (n=111) were tested. Age ranged from 1 day to 59 months (median age=15 months). Overall, influenza viruses were detected in 32% (152/475) of specimens tested and other respiratory pathogens were detected in 81% (383/475) of specimens regardless of co-infection. The detection rates for influenza were similar among ILI (31.9%, 116/364) and SARI (32.4%, 36/111) patients. Amongst influenza positive specimens, 81% (123/152) were co-infected with other pathogens. Age group 7 to 30 months presented the highest rate of influenza co-infection (52%, 64/123). Enteroviruses, RSV and *Mycobacterium pneumoniae* were the most common pathogens found in association with influenza (68, 41 and 32 cases, respectively), with a positive correlation observed between influenza and RSV co-infections ( $r=0.7$ ). By examining clinical spectrum of SARI patients, 58% (64/111) had serious complication during hospitalization of which influenza (n=24) and RSV (n=24) were the most prevalent. Most of influenza-associated severe cases (88%; 21/24) were found to be co-infected.

### Conclusion

Our results suggested that influenza-associated co-infection played a role in seriousness of the disease in infants. These data strongly support the need to prioritize influenza vaccination among these at-risk group in low income countries. More efforts are needed to decipher the drivers of severity and the exact role of co-infections.

*Keywords: Influenza; co-infection; ILI; SARI; Madagascar*

Topic: Co-Infection: Viral Coinfections with Influenza  
Abstract No: 10695

## COINFECTIONS OF INFLUENZA AND OTHER RESPIRATORY VIRUSES IN FLORIDA SURVEILLANCE SPECIMENS JANUARY 2014 – FEBRUARY 2019

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### Introduction

Patients coinfecting with influenza and one or more additional viruses are at an increased risk of greater disease duration and severity. Physicians regularly perform rapid influenza testing on suspect influenza like-illness (ILI) patients, without considering the possibility of additional viral infections. With the introduction of multiplex PCR assays, identification of coinfections is more common.

### Methods

The Florida Department of Health, Bureau of Public Health Laboratories (BPHL), Laboratory Information Management System was queried for all samples tested by BPHL for influenza A, influenza B, and an extended panel which includes respiratory syncytial virus (RSV), parainfluenzas 1-3, adenovirus, human metapneumovirus, rhinovirus, enterovirus, and seasonal coronaviruses HKU1, NL63, 229E, and OC43 by real time (RT)-PCR from January 2014-February 2019. Samples identified as having a coinfection of influenza and at least one other virus were analyzed.

### Results

From January 2014-February 2019, 1,872 patients positive for influenza virus were tested for at least one additional virus, of which 162 (9%) patients were identified to have a viral coinfection. Of these, 27 (17%) were coinfecting with influenza and two or more additional viruses. Influenza A comprised 69% of coinfections, followed by 30% influenza B, and 1% both influenza A and B. Of these 162 patients with coinfections, over three quarters (77%) were identified in patients age 0-18 years. The most common viruses present in these coinfections were rhinovirus, adenovirus, RSV, enterovirus, and coronavirus OC43.

### Conclusion

High rates of coinfections among patients infected with influenza shows the importance of considering the presence of additional viruses in patients with ILI. By increased respiratory virus surveillance and expanded use of extended testing, antiviral medication and vaccine development efforts can be modified to guide treatment and decrease the disease burden of ILI.

*Keywords: Coinfections; Florida; PCR; Surveillance*

Topic: Co-Infection: Viral Coinfections with Influenza  
Abstract No: 11076

## Influenza and other respiratory viruses, 2015-2018, Portugal

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### Introduction

Viruses are among the major causes of acute respiratory tract illness. There have been limited number of studies focused on the impact of the wide range of other respiratory viruses (RV) besides influenza. The aim of this study was to describe the seasonality and prevalence of respiratory virus weekly reported by the Portuguese Laboratory Network for the Diagnosis of Influenza Infection (PLNDII) during 2015-2018.

### Method

During 2015-2018, fifteen hospitals from the PLNDII performed the laboratory diagnosis of RV by PCR in samples from patients with respiratory tract infections. The seasonality of NIRV and influenza was evaluated. Distribution by age groups, co-infections and hospitalization were analysed.

### Result

were reported 4597 positive for NIRV: 1509(2015/16), 1283( 2016/17) and 1805(2017/18). Co-infections between NIRV and influenza ranged between 2.5-5.8%. RSV was the most commonly detected NIRV ranging from 607(40%) positive cases in 2015/16 to 1057(59%), in 2017/18. RSV preceded influenza peak (the week with higher number of influenza cases) in 2015/16, was more frequent after influenza peak in 2016/17 and in 2017/18 higher number of RSV were observed during the influenza peak. Picornavirus (hRV/hEV/hPeV) represented 12% to 18% of the NIRV, were the first detected viruses in the beginning of the winter seasons. PIV, hCoV, hMPV, AdV and hBoV were confirmed in less than 7% of the NIRV positive cases, being more frequent after the influenza peak. PIV, hCoV, hMPV, AdV, hBoV viruses were frequently detected in co-infections, mainly in children 0-4 years followed by the 65+. The RSV were most frequently detected NIRV hospitalized patients.

### Conclusion

The implementation of respiratory virus's surveillance systems will add value to the greater knowledge of the aetiology of respiratory infections. Besides influenza, NIRV might also be associated with burden and mortality. Further studies, both hospital-based and population-based, are required to fully understand the aetiology of respiratory infections.

*Keywords: co-infections, respiratory virus, hospitalizations, surveillance*

Topic: Co-Infection: Viral Coinfections with Influenza

Abstract No: 11153

## Investigation of pneumonia outbreak among under five years children in a tribal area of Maharashtra, India

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**Background:** - The increased number of pneumonia associated deaths among under 5 children during month of August from tribal area, Amravati was reported. With the state health help National institute of virology Pune investigated root cause.

**Objective:-** identify the viral causative organism causing sever acute respiratory illness among the children of tribal area, Maharashtra.

**Methods:-** Investigation was carried out during 25<sup>th</sup> to 30<sup>th</sup> September 2018 ; children(<5 yrs) with Acute/ Sever respiratory illness (ARI/SARI) admitted/attending at tertiary care IPD's, sub-district hospitals IPD, Primary health centers IPD and OPD, siblings of pneumonia death cases and children around the death case, unattended children with ARI in community, Anganwadi and schools and children coming for health Camp were also screened for ARI. Total 94 nasal/throat swabs were collected and tested for respiratory viruses by Real time RT PCR.

**Result:** - Nearly 350 children were screened and 94 specimens were collected, of which 34 (36.1%) were Respiratory Syncytial Virus (RSV), 14 (14.8%) were Influenza, 05(5.3%) Para-Influenza virus, 03(3.1%) Adenovirus, 01(1.06%) rhinovirus and 03/94 (3.9%) had mixed infection (Influenza A & RSV). RSVB 33(97%) was predominant subtype and among Influenza A(H3N2 ) was detected in 12 cases (85.7%). RSV was detected mostly (88.2%) in children less than 2 years and presented with ARI symptoms without fever.

**Conclusion:-** Investigation reveals that the RSV B was predominant causative agent and co-circulation of different respiratory viruses were observed. Study also recommends need of well-ventilated wards with sufficient distance between two patients during hospitalization to minimize nosocomial spread of RSV. School child with ARI was also found positive for RSV; hence school health services can be utilized to screen the children with ARI.

**Keywords:** Children; co-circulation; co-infection; Influenza; Respiratory Syncytial Virus;

Topic: Co-Infection: Viral Coinfections with Influenza

Abstract No: 11166

## VIRAL CO-INFECTIONS WITH INFLUENZA AMONG SEVERE ACUTE RESPIRATORY INFECTION (SARI) CASES IN THE PHILIPPINES, 2016–2017

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### Introduction and Objectives:

Severe Acute Respiratory Infection (SARI) causes significant mortality and morbidity worldwide. In the Philippines, a SARI sentinel surveillance is being conducted to describe influenza seasonality. Also, other viral pathogens are being tested other than influenza to detect etiology of SARI cases in the country. This study aims to describe the epidemiology of viral co-infections with influenza in the SARI surveillance in the country.

### Methods

One nasopharyngeal and one Oropharyngeal swab were collected from patients presenting acute respiratory infection with history of fever of  $\geq 38\text{ C}^\circ$  and cough with onset within the last 10 days and requires hospitalization. Patients were admitted in six (6) regional hospitals located in the country. The samples were tested for real-time RT-PCR test for Influenza A (InfA) and B (InfB), and other respiratory viral pathogens such as Human Respiratory Syncytial Virus (HRSV), Parainfluenza virus types 1 and 3 (PIV-1,3), Herpes Simplex Virus types 1 and 2 (HSV-1,2) Enterovirus (EV), Human Metapneumovirus (HMPV) and Adenovirus (ADV).

### Results:

From years 2016 to 2017, a total of 1,998 samples were collected for SARI Surveillance. Among the samples, 2.65% (53/1998) with influenza viral co-infection. Of these, 58% came from male patients and only 42% came from female patients. Majority of the cases were from agegroup less than 2 years of age (37.73%) followed by 65 years old and above (20.75%). Among the 53 co-infections, the most common combinations were InfA- HMPV (20.75%), InfA-HSV-1 (18.87%) and InfA-ADV (16.98%).

### Conclusion:

Detection of other respiratory viruses other than influenza is very important since this will provide an important contribution to identifying the true rate of co-infections and their correlation with the clinical symptoms and severity of disease.

*Keywords: viral co-infections*



Topic: Co-Infection: Viral Coinfections with Influenza  
Abstract No: 11188

## Viral Co-Circulation and Co-Infection with Influenza during an Epidemic of Severe Acute Respiratory Illness among Children in Southern Province, Sri Lanka

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### Introduction and Objectives

During the typical influenza peak in southern Sri Lanka in 2018, the number of severe and fatal cases of acute respiratory illness among previously healthy children was unexpectedly high. Consequently, an outbreak response effort was organized at Teaching Hospital Karapitiya (THK), the regional tertiary referral hospital which operates one of only four pediatric ICUs in the country.

### Methods

Nasopharyngeal swabs were obtained from patients who were either 1) referred to the infection control team of THK (passive surveillance), or 2) met the active surveillance case definition, defined as recent fever plus  $\geq 1$  symptom of respiratory illness (cough, difficulty breathing, shortness of breath, hypoxia, or tachypnea). In children <5 years, additional age-appropriate symptoms of respiratory illness were accepted as per WHO guidelines. Real-time PCR was used to identify the following viral targets: influenza A (INF-A), influenza B (INF-B), human adenovirus (AdV), and respiratory syncytial virus (RSV).

### Results

Of the patients tested for  $\geq 1$  viral target, 64 (17.8%) were INF-A/B positive, 69 (24.3%) were AdV positive, and 66 (23.8%) were RSV positive. An additional 34 (34.7%) INF-A/B, 49 (17.3%) AdV, and 46 (16.6%) RSV positive cases showed evidence of co-infection with another virus.

For cases with a known illness outcome, 17 (4.6%) were fatal, including 3 (4.7%) INF-A/B positive, 4 (5.8%) AdV positive, 2 (3.0%) RSV positive, and 2 (1.6%) co-infection cases. Respiratory support (mechanical ventilation or supplemental oxygen) was required in 64 (33.3%) cases, including 4 (6.3%) INF-A/B positive, 21 (30.4%) AdV positive, 23 (34.8%) RSV positive, and 11 (8.5%) co-infection cases.

### Conclusion

INF-A/B, AdV, and RSV were co-circulating during a peak influenza period and in some cases resulted in confirmed co-infection. This outbreak of unexpectedly severe acute respiratory illness in Sri Lankan children underscores the importance of testing for co-circulating viruses during peak influenza activity.

*Keywords: Viral co-infection; Viral co-circulation; epidemic; Sri Lanka*

Topic: Co-Infection: Viral Coinfections with Influenza  
Abstract No: 11762

## MOLECULAR CHARACTERISTICS AND SEASONALITY OF RESPIRATORY VIRUSES CO-INFECTION AMONG INFLUENZA POSITIVE CASES IN NEPAL

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**Introduction and Objectives:** Acute respiratory infections are one of the major public health problems. Data on epidemiology and seasonality of respiratory viruses are scarce, poorly characterized and hence empirical antimicrobial agents are used in Nepal. The objective of this study was to characterize the co-infection of respiratory viruses with influenza by real time PCR assay.

**Materials and Methods:** A descriptive cross sectional study was conducted at National Influenza Center, Nepal during the year 2016. A total of 882 throat & nasopharyngeal swab were collected from the children with influenza like illness according to WHO case definition. Total nucleic acid was extracted using Pure Link viral RNA/DNA mini kit (Invitrogen) and multiplex RT-PCR assays were performed.

**Results:** Of the total 882 specimens; respiratory viruses were found in 556 (63.0%) specimens. Respiratory syncytial virus 130 (14.7%) was most frequently detected in children followed by rhinovirus 121 (13.7%), metapneumo virus 37 (4.2%) and enterovirus 36 (4.0%) respectively. The rate of respiratory syncytial virus 60 (15.7%) co-infection was high than rhinovirus 51 (13.4%), metapneumo virus 13 (3.4%) and enterovirus 12 (3.1%) infection in influenza positive cases. Children (<2 year) were more commonly co-infected with respiratory syncytial virus than influenza, rhinovirus and enterovirus. Respiratory viruses were detected year-round in Nepal. Incidence of respiratory syncytial virus infection was throughout the year with two peaks in summer (July-Aug) and winter (Jan-Feb) season.

**Conclusion:** Respiratory viruses are significantly higher in infections and thus these findings suggest for clinical management and minimize the use of antimicrobial agents for respiratory infections in Nepal.

*Keywords: Acute respiratory infection; Etiology; Influenza like illness; Multiplex PCR; Nepal*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10396

## EXPLORATION OF M SPLICING VARIANTS IN INFLUENZA A VIRUSES

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### Introduction and objectives:

The influenza A virus M segment generates four mRNA transcripts (M1, M2, mRNA3, and mRNA4) through alternative splicing. Another splicing isoform, M42, was previously identified as a novel splice variant form and suggested to functionally replace M2 in several M2-deficient influenza strains. It is postulated that other novel M splicing forms may exist to facilitate viral functions for host adaptation of the other influenza A viruses. The avian H9N2 virus is considered to be the principal gene pool for the H5 and H7 subtypes of influenza A viruses which have caused human infections in the last two decades. It is therefore important to understand the role of M splicing in H9N2 virus replication and host adaptation.

### Methods:

RT-qPCR was used to measure the relative abundance of RNA. Co-IP and Immunofluorescence assay was used to test protein-protein interactions. Growth kinetics in cells was studied to characterize virus replication ability.

### Results:

We identified a novel mRNA (M6) expressed by the H9N2 virus. Subsequent investigation revealed that the M6 mRNA splicing donor site and accept site. An affinity purified anti-M6 specific antibody was raised against a peptide corresponding to the predicted junction region of the putative M6 protein and used to identify M6 protein in H9N2 and H7N9 virus-infected cells by western blot analysis, confirming that a viral protein is expressed from M6 mRNA. This novel viral protein also appears likely to bind to M1. Further characterization of M6 protein and mRNA functions in H9N2 influenza virus infection and replication, using M6-deficient viruses and cells stably expressing M6, is currently underway.

### Conclusion:

This study revealed a novel viral protein, M6, expressed from a spliced isoform of M segment derived mRNA. The biological significance of the M6 protein and regulation of M mRNA splicing for H9N2 virus-host adaptation will be explored.

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10418

## Three-dimensional ultrastructural analysis of cells from lung autopsy case of A/H1N1pdm09 Influenza Virus Infection

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**Background:** We previously reported pathological and molecular biological characteristics of autopsied lung tissues with A/H1N1pdm09 infection. Majority of A/H1N1pdm09 in the lung had an aspartic acid-to-glycine substitution at position 222 (D222G) in hemagglutinin protein and infected Type II alveolar epithelial cells (AEC-II). In this study we analyzed the lung specimens from the same patient using transmission electron microscopy (TEM) and novel scanning electron microscopy (SEM) methods to observe virus particles and virus-related structures in an AEC-II, a monocyte/macrophage (M/MØ), and a neutrophil (Neu), respectively.

**Method:** Autopsied lung tissue were fixed in glutaraldehyde and embedded in epoxy resin. The novel SEM technique involves mounting single or serial ultrathin sections on a glass slide and staining them with heavy metals, thereby enabling imaging at a resolution comparable with that of TEM by recording back-scattered electrons. By digitally 'stitching' together contiguous SEM images, a large-scale two-dimensional image with high resolution can be obtained. Serial section array (SSA)-SEM analyzes each cross-sectional image throughout a single whole cell to reveal the distribution of specific structures. After manual tracing of specific structures such as virus particles, cytoplasm, and nucleus in each serial section, the structures are visualized in a three-dimensional model.

**Results:** In AEC-II, a lot of intranuclear dense tubules, which are associated with matrix 1 protein of influenza virus, were observed in the nucleus. In Ms/MØs, many various formed of intravesicular virus particles were observed although neither budding of virus particles nor intranuclear dense tubules were observed. Whole cell analysis found the presence of a M/MØ harboring numerous virus particles.

**Conclusion:** The differences among AEC-II, Ms/MØs and Neus with respect to the distribution of virus particles and virus-related structures suggest that each cell type plays a different role in A/H1N1pdm09 -D222G pneumonia.

*Keywords: Three-dimensional, A/H1N1pdm09*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10422

## PREVENTING AN ANTIGENICALLY DISRUPTIVE MUTATION IN EGG-BASED H3N2 SEASONAL INFLUENZA VACCINES BY MUTATIONAL INCOMPATIBILITY

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**Introduction and Objectives:** Egg-based seasonal influenza vaccines are the major preventive countermeasure against influenza virus. However, their effectiveness can be compromised from antigenic changes arising from egg-adaptive mutations on influenza hemagglutinin (HA). The L194P mutation is commonly observed in egg-based H3N2 vaccine seed strains and significantly alters HA antigenicity. An approach to prevent occurrence of L194P would therefore be beneficial. **Methods:** We show that emergence of L194P during egg passaging can be impeded by pre-existence of a G186V mutation, revealing strong incompatibility between these mutations. X-ray structures illustrate that, when both G186V and L194P are present, the HA RBS is severely disrupted. Importantly, wild-type HA antigenicity is maintained in G186V, but not in L194P.

**Results:** Our results demonstrate that epistatic interactions can be taken advantage of to prevent emergence of mutations that adversely alter antigenicity during egg adaptation.

**Conclusions:** Overall, this study provides important insight into the optimum selection of egg-based influenza vaccine seed strains.

*Keywords: H3N2 vaccine; Antigenic; Mutational incompatibility*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10431

## FluChip-8G Characterization of Seasonal and Non-Seasonal Influenza

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**Introduction and Objectives:** Rapid influenza tests provide low information content and exhibit poor sensitivity, whereas sequencing can be time-consuming and data intensive. FluChip-8G is a microarray-based assay platform developed for pandemic preparedness and is capable of same day characterization of seasonal and non-seasonal influenza A and influenza B in a single, multiplexed assay with automated, neural-network based data analysis. A clinical assay is pending 510(k) clearance for the detection of A/H1N1pdm09, A/H3N2, “non-seasonal” A, and lineage differentiation for influenza B. A research use only version of the assay (FluChip-8G Insight) provides HA subtyping of H1, H3, H5, H7, and H9, and NA subtyping of N1, N2, N7, N8 and N9 viruses utilizing the same assay with alternative software. We present results for the clinical and analytical validation of the clinical assay and non-seasonal influenza A subtyping performance data using FluChip-8G Insight.

**Methods:** Clinical validation was conducted in a 3-site study of 1565 specimens, with analytical validation of key performance metrics. Subtyping of non-seasonal influenza A viruses was evaluated using k-fold cross validation of 280 results obtained from samples contrived in nasopharyngeal swab specimens using 193 unique non-seasonal A strains representing 49 unique subtypes.

**Results:** Clinical sensitivity and specificity exceeded 91% and 98%, respectively, compared to the CDC panel of real-time RT-PCR assays, and similar limits of detection to other multiplexed molecular assays observed. Non-seasonal subtyping exceeded 93% PPA for H1, H3, H5, H7, N1, N2, N7, N8, and N9 (H9 was ~89%) and 95% NPA for all targeted subtypes.

**Conclusion:** FluChip-8G is a powerful tool for detection, characterization, and surveillance that provides same day identification of potentially pandemic viruses while also providing detection and differentiation of seasonally circulating influenza viruses. Continued analysis of additional non-seasonal subtypes as they emerge will allow rapid software updates for continuous improvements to performance.

*Keywords: molecular diagnostic; multiplex; surveillance; pandemic preparedness; microarray*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10432

## Correlation Between VaxArray HA and NA Multiplexed Immunoassays and Immunogenicity in Mice

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**Introduction and Objectives:** Influenza vaccine potency via single radial immunodiffusion assay (SRID) is labor and reagent intensive and often unsuitable with emerging manufacturing technologies. Alternative assays predictive of immunogenicity are needed as a replacement for SRID. VaxArray HA and NA multiplexed microarray-based immunoassays utilizing subtype specific, broadly reactive monoclonal antibodies are available, and allow the user to quantify HA and NA concentration from all components in a multivalent vaccine using 2 hour, multiplexed assays. This study probed the relationship between VaxArray-measured potency and immunogenicity.

**Methods:** Five H3N2 vaccine formulations (5 to 80 µg/mL of HA) and an 80 µg/mL HA sample heat-treated at 56 °C for 20 h were assessed for HA concentration using SRID and VaxArray, and for NA using a MUNANA-like activity assay and VaxArray. Antibody response was then measured by hemagglutination inhibition (HAI) and neuraminidase inhibition (NAI) assays after subsequent injection into mice. A second cohort of mice received a second dose of the five formulations prior to assessment of antibody titers.

**Results:** For HA, VaxArray vaccine potency was equivalent to potency measured by SRID, and these amounts were predictive of immunogenicity as evidenced by a strong correlation between VaxArray measured potency and HAI geometric mean titers (GMT). Likewise, VaxArray measured NA content was predictive of the NAI GMT. The HA potency measured by VaxArray in the heat-treated sample was very low, with the VaxArray NA assay reporting non-detectable levels of intact NA. These data demonstrate that the VaxArray NA assay measures the native, active form of NA.

**Conclusion:** These data support the conclusion that VaxArray HA and NA assays measure the immunogenic forms of the A/H3N2 antigens investigated, with this initial study indicating that VaxArray assays can be used to assess the potency of HA and NA components in influenza vaccines as a proxy for immunogenicity.

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10464

## REFERENCE ANTIGEN-FREE AND ANTIBODY-FREE LTD-IDMS ASSAY FOR INFLUENZA PANDEMIC VACCINE *IN VITRO* POTENCY DETERMINATION

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Influenza vaccines are the most effective intervention to prevent the substantial public health burden of seasonal and pandemic influenza. Hemagglutinin (HA), as the main antigen in inactivated influenza vaccines (IIVs), elicits functional neutralizing antibodies and largely determines IIV effectiveness. HA potency has been evaluated by single-radial immunodiffusion (SRID), the standard *in vitro* potency assay for IIVs, to predict vaccine immunogenicity with a correlation to protective efficacy. SRID relies on strain-specific reference antigens and antisera which require a time-consuming process for generation and calibration, and can delay vaccine release especially during pandemic.

We previously reported that limited trypsin digestion (LTD) selectively degraded stressed HA, so that an otherwise conformationally insensitive biophysical quantification technique could specifically quantify trypsin-resistant, immunologically active HA. Here, we demonstrate that isotope dilution mass spectrometry (IDMS), a method capable of quantifying the absolute HA concentration without reference antigen use, can be further expanded by adding LTD followed with precipitation to selectively quantify the active HA. We test the LTD-IDMS assay on pandemic vaccines and showed that this method, unlike SRID, has no requirement for strain-specific reference antigens or antibodies and can generate potency values that correlate with SRID. Thus, LTD-IDMS is a promising alternative *in vitro* potency assay for influenza vaccines to complement and potentially replace SRID in a pandemic when strain specific reagents may not be readily available.

**Keywords:** LTD, IDMS, Potency, Pandemic, Vaccine



Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10467

## Expression of type I and II interferon in the lungs of immune mice after viral - bacterial infection

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Bacterial co-infections are common in patients infected with influenza, which lead to complications. In this study, we evaluated whether associated nasal vaccine based on live attenuated influenza vaccine and Group B streptococcus (GBS) recombinant polypeptides can protect against H7N9 influenza infection. We also made an attempt to reveal the role of cytokines expression in such protection.

**Methods** A reassortant influenza /17/Mallard/(H7N3) virus LAIV was provided from the Virology department of IEM. The Group B Streptococcus Vaccine (GBSV) contained recombinant GBS polypeptides from Microbiology Department of IEM. Groups of mice were vaccinated intranasally with of LAIV, GBSV or mixed vaccine LAIV + GBSV. On 3 weeks after mice were double infected with (H1N1)influenza virus or (H7N9) and GBS 24 hours apart. Using PCR with Sybr Green we compared the increase in cytokines expression, IFN- $\gamma$ , 1 $\beta$  TNF- $\alpha$  and IL-6. For this purpose we analyzed the mRNA expression in the lungs of mice on 48 and 72 hours after the initial virus infection.

**Results** We determined a significant IFN- $\gamma$  increase among the LAIV+GBSV immunized animals compared to other groups on 72 hours after primary viral challenge with influenza virus. In this group of mice was protection against virus-bacterial infection, determined by the reduction of viruses and bacteria levels in the lungs. At the same time, the expression of IFN-1 $\beta$  was lower in LAIV+GBSV immunized mice compared to other vaccine groups.

**Conclusion** LAIV and GBS combined vaccination provided effective protection against heterologous influenza virus infections with improved viral and bacterial clearance from the lungs of mice. The highest level of protection correlated with elevated IFN- $\gamma$  m-RNA expression which may indicate T-cell immunity induction. The decrease in the expression of IFN-1 $\beta$  correlated with decrease in the reproduction of viruses and bacteria in the lungs when combined vaccination was the most effective against GBS secondary infection

**Keywords:** LAIV GBSV IFN- $\gamma$  IFN-1 $\beta$  TNF- $\alpha$  IL-6

Topic: Virology and Pathogenesis: Cellular & Molecular Virology

Abstract No: 10504

## IDENTIFICATION OF A NOVEL HOST FACTOR: TMPRSS9 IS REQUIRED FOR REPLICATION OF INFLUENZA A VIRUS

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Influenza virus causes annual epidemics and occasional pandemics, and thus represents a threat to human health world-widely. Its prevention is compromised by the rapid evolution of the virus and lack of a universal vaccine. The effectiveness of influenza treatment is also limited by the scarcity of good antiviral agents and emergence of resistant viral strains. Host factors required for influenza virus replication may provide alternative targets for antiviral or anti-influenza agents. For this reason, we identify host factor essential for the replication of influenza A virus that could serve as potential antiviral targets in this study.

Utilizing RNA interference technique, 2,732 human genes from On-TARGETplus were screened in cells infected with influenza A virus. Experiments were performed using recombinant A/PR/8/1934 H1N1 influenza virus that expresses a GFP-conjugated NS1A protein. A small interfering RNA designed to silence gene expression was used to assess the function of host proteins under influenza infection. Image-based and biochemical assays were carried out to investigate the mode of action during the replication of influenza A virus.

Our genome-wide siRNA screening assay identified six cellular factors regulating influenza A virus. Among those hits, we focused on the TMPRSS9 in this study. Knockdown of TMPRSS9 in cells infected with influenza A virus decreased the expression of the viral NP, M2 and NS1A proteins. We confirmed that silencing TMPRSS9 also decreased the levels of viral RNA. Additionally, we observed that the knockdown of TMPRSS9 inhibits the infection of influenza A virus from early time point.

In this study, we identified TMPRSS9 as a novel host factor, which suppresses the replication of influenza A virus. From now on, we need to study the mechanism of action on TMPRSS9 against influenza A virus. To the best of our knowledge, TMPRSS9 is expected to represent a potential target for anti-influenza drug development.

*Keywords: Influenza A virus; siRNA; TMPRSS9*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
 Abstract No: 10517

## Regulation of Cellular Ceramide in Response to Influenza A Virus Infection

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**Introduction:** Annual influenza outbreaks are associated with significant morbidity and mortality worldwide despite the availability of seasonal vaccines. Influenza pathogenesis depends on the manipulation of host cell signaling to promote virus replication. Ceramide is a sphingosine-derived lipid that regulates diverse cellular mechanisms. Studies highlighted the differential role of ceramide *de novo* biosynthesis on the virulence and lifecycles of some viruses. Whether ceramide plays a role in influenza virus replication is not known. In this study, we assessed the potential interplay between influenza virus and ceramide biosynthesis pathways.

**Methods:** Accumulation of ceramide in human lung adenocarcinoma epithelial cells (A549) infected with influenza A/H1N1 virus (A/PR8/34) was evaluated using thin layer chromatography and confocal microscopy. Virus replication and cell death were assessed upon inhibition of *de novo* and salvage ceramide synthesis and upon treatment of cells with exogenous ceramide.

**Results:** Significant accumulation of ceramide was observed starting at 24 hour-post-infection (hpi) and peaked at 48 hpi. The increase in ceramide synthesis was also dependent on the dose of the virus inoculum. Inoculating the cells with UV-inactivated IAV or treating them with sialidase prior to inoculation with IAV did not result in ceramide accumulation in the cells. The treatment of A549 cells with inhibitors of *de novo* ceramide biosynthesis significantly decreased ceramide accumulation compared to untreated, infected cells. This inhibition was accompanied by an increase in cell death and enhanced virus replication. The addition of exogenous C6-ceramide prior to infection mediated an increase in cellular ceramide levels and significantly attenuated IAV replication.

**Conclusion:** IAV triggers the accumulation of ceramide in epithelial cells via activation of the *de novo* biosynthesis pathway in a dose- and time-dependent manner. Inhibiting *de novo* ceramide biosynthesis and salvage pathway enhanced IAV replication suggesting an antiviral role for ceramide.

**Keywords:** influenza A, sphingolipid, ceramide, *de novo* pathway, anti-viral

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10658

## Stabilization of Foreign Gene Insertion into the Influenza Virus Genome

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We previously attempted to establish a reporter influenza virus by inserting the gene of the Venus fluorescent protein into the NS segment of influenza A/Puerto Rico/8/34 (PR8, H1N1) virus to yield WT-Venus-PR8. However, the inserted Venus gene was deleted immediately during serial passages of WT-Venus-PR8. Here, we found that the PB2-E712D mutation stabilizes the Venus gene. The mechanisms by which Venus gene deletion occurs and how the polymerase mutation stabilizes the Venus gene remain unknown.

We hypothesized that the stability of the Venus gene was determined by the fidelity of the virus polymerase. To analyze the change in fidelity associated with the polymerase mutation, we compared the mutation rates between WT-PR8 and PR8-PB2-E712D by deep sequencing analysis. Next, we co-infected mutant influenza viruses to examine whether the Venus gene deletion was the result of gene recombination between NS-Venus segments. We also passaged WT-Venus-PR8 in MDCK cells to identify additional stabilizing mutations.

There was no significant difference in the mutation rates between WT-PR8 and PR8-PB2-E712D, suggesting that the stability of the Venus gene is not influenced by the change in fidelity of the virus polymerase. In the co-infection analysis, we saw no evidence of gene recombination. After serial passages of WT-Venus-PR8 in MDCK cells, we identified additional mutations that stabilized the Venus gene.

Our results suggest that the deletion of the Venus gene from the influenza virus genome was not caused by low fidelity of the virus polymerase or gene recombination. The Venus gene deletion may be caused by internal deletion following polymerase jumping. The additional mutations we found in the virus polymerase that stabilized Venus gene insertion may shed further light on the stabilization of foreign gene inserts into the influenza virus genome.

*Keywords: genetic stability; viral polymerase; foreign gene insertion;*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
 Abstract No: 10687

## Effect of proline mutagenesis on fusion proteins of enveloped RNA viruses

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**Background:** The influenza virus hemagglutinin (HA), the S proteins from human coronavirus (HCoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), and the respiratory syncytial virus (RSV) and Nipah virus (NiV) F proteins belong to the class I fusion proteins. The prefusion structures of these viral fusion proteins reveal an overall mushroom-like architecture, with very similar membrane fusion mechanisms for host entry. Recent work on the MERS-CoV S protein showed that proline substitutions in the loop between the helices in the central coiled-coil region increased expression yields of prefusion forms.

**Methods:** The recombinant viral fusion proteins derived from A/Thailand/CU44/2006 (CU44), HCoV-229E, MERS-CoV KOR\_KNIH\_016\_CuI, HRSV-A/IC688/12, and Ind-Nipah-07-FG were produced in insect cells using recombinant baculovirus expression vectors. With the cloned construct with a foldon domain and 6xHis-tag, mutant genes were prepared by site-directed mutagenesis. The recombinant bacmids were generated according to the Bac-to-Bac expression system protocol and the recombinant mutant proteins were expressed in insect cells, purified using chromatographic methods, and characterized by gel electrophoresis and size-exclusion chromatography-multi-angle laser light scattering (SEC-MALS).

**Results:** We have designed proline mutations in the loop near the central helix in the fusion proteins from five different enveloped RNA viruses, influenza virus, HCoV and MERS-CoV, and RSV and NiV. The proline substitution induced a 2-fold increase in the expression of HA, MERS-CoV S and RSV F proteins. Furthermore, the wild type fusion proteins of HCoV and NiV were little expressed, whereas their proline substitutions increased expression yields similar to those of HA, MERS-CoV S or RSV F.

**Conclusion:** As the loop near the central helix in the fusion proteins is critical in the loop-to-helix transition during membrane fusion, this proline substitution enhanced expression of the fusion proteins, which will improve fusion protein production for highly pathogenic enveloped RNA viruses.

*Keywords: influenza surface proteins, proline mutagenesis, MERS, human CoV, RSV,*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10708

**Title: Method for determining percentage split virion by Nanosight**

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**Background:** The commercial manufacture of the split virion vaccines requires an assessment of the level of virion disruption achieved to confirm that the process conditions utilized have produced a predominantly split vaccine formulation. Accurate quantitation of the percentage split virion in drug substance is complex due the variation in size/shape of the species being assessed (whole and split virion material). This report details the development of a novel method for quantitation of the percentage split virion in vaccine.

**Methods:** The method utilizes the Nanosight instrument to determine the level of virion disruption in vaccine. The assay considers both particle concentration and size in the drug substance to enable quantitation of the level of virion disruption.

**Results:** Data generated through development has demonstrated the test method is capable of assessing the splitting of influenza A and Influenza B strains with quantitation achieved through the use of a strain matched standard curve. Robustness of the method was assessed across multiple assays and through the use of spiking studies to calculate spike recoveries.

**Conclusion:** The Nanosight may prove to be suitable for the quantitative assessment of the percentage split virion in influenza vaccine.

*Keywords: Vaccine; Assay; Nanosight;*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10727

## Intra-host and intra-population Sequence Diversity of pH1N1 and Associated Clinical Outcomes

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Emergence of influenza virus antigenic variants is widely reported, and it typically results in influenza vaccine virus mismatch. Here we report on within- and across-host pH1N1 virus population diversity and dynamics at consensus and sub-consensus levels over a three-year period. We also investigated the association between emergence of new variants and their clinical relevance.

Viral RNA was extracted from nasal samples (n=100, 2015-2017) and used to amplify all eight genes. DNA libraries were constructed using Nextera XT DNA library preparation kit and sequenced using Illumina Miseq Machine. Data were extracted and analyzed using various softwares.

Analysis of virus diversity at sub-consensus level showed that majority of low-frequency variants were reported in individual patients in all genes except for HA, NA and NS genes. In HA, 45% and 30% of low-frequency variants arose independently within multiple patients and across years. Likewise, 38% of variants in NA were found repeatedly in multiple patients and 24% were found across years. Investigating the emergence of low-frequency variants at consensus level demonstrated that intra-host pH1N1 evolution recapitulates many evolutionary dynamics observed at the global scale. Analysis of intra- and inter- host genetic diversity at haplotype level revealed the clustering of low-frequency haplotypes from early 2015 with dominant strains of 2016, indicating rapid haplotype evolution. In all years, clustering pattern was not always patient specific, instead, haplotypes from different patients were showing closer nucleotide similarities than when compared to corresponding consensus sequences of the same sample, strongly suggesting the transmission of haplotypes among patients. Importantly, all patients with respiratory and/or cardiac complications were exhibiting higher number of haplotypes, and on the other hand, not all patients with higher diversity were suffering from severe outcomes.

Sub-consensus virus diversity analysis is essential to explain variabilities in clinical outcomes that couldn't be explained by either analysis alone.

*Keywords: Influenza, NGS, Diversity*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10730

## REPLICATION COMPETENCE AND TROPISM OF INFLUENZA B VIRUSES IN HUMAN AIRWAY ORGANIDS

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### Introduction and Objectives

Although influenza B virus (IBV) can infect millions of people worldwide resulting in substantial morbidity and mortality during seasonal epidemics, research on IBVs is still lacking. In this study, we investigate the tropism of IBVs in the physiologically relevant human airway organoids, which are three-dimensional cultures with similar tissue-specific histological properties, cellular diversity and sialic acid profiles to the human bronchus.

### Methods

Human airway organoids derived from human lung progenitor cells were used to investigate the cellular tropism of Yamagata-like and Victoria-like IBVs in comparison to human seasonal H1N1 and H3N2 viruses using immunohistochemical co-staining with specific cellular markers. Replication kinetics of IBVs and seasonal IAVs in human airway organoids were studied and compared to those in ex-vivo explants of human bronchus and differentiated primary human bronchial epithelial cells (dHBECs). In addition, the inhibitory effect of mucus on the replication of IBVs and seasonal IAVs was investigated using dHBECs.

### Results

Both IBVs and seasonal IAVs showed highly productive replication in human airway organoids with mean peak titres being  $>10^5$  TCID<sub>50</sub>/ml at 24 or 48hpi which are comparable to the results observed in ex-vivo explants of human bronchus and dHBECs. In human airway organoids, IBVs and seasonal IAVs infected various cell types, including ciliated cells, club cells, goblet cells, and basal cells. IBVs and seasonal IAVs were effectively inhibited by dHBEC-secreted mucus with 2.5 to 4.5 log TCID<sub>50</sub>/ml reduction in viral titres on average in the presence of mucus.

### Conclusion

Our results demonstrated the non-negligible virulence of IBVs which demand more attention and further support the application of human airway organoids as a physiologically relevant experimental model for future pandemic risk assessments.

*Keywords: Influenza B; seasonal influenza; airway organoids; tropism; replication*



Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10733

## Influenza like illness and severe acute respiratory infection surveillances in Mongolia in 2018/19 influenza season

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**Background:** An influenza epidemic occurs annually between pandemics by seasonal viruses continually evolving to escape human immunity. Mongolian National Influenza Center is conducting the national influenza surveillance laboratory network, which includes 5 laboratories and 160 sentinel sites. We are reporting influenza surveillance results conducting in influenza season in 2018/19 years, Mongolia.

**Method:** The nasopharyngeal specimens were tested for influenza by rt-RT-PCR, for other respiratory pathogens detection by multiplex rt-PCR. DNA sequences for HA and NA gene were performed using NIID, Tokyo primers, analyzed by an ABI 3130xl Genetic Analyzer and antiviral susceptibility testing by chemiluminescence-based NAI assay.

**Results:** During the study period from 01 October 2018 to 01 August 2019 have been registered 263.699 influenza and ILI per 10,000 which were 6.1% (874.4) of all the registered outpatient visits 4,388.497. Totally, 31.859 patients hospitalized due to pneumonia and SARI in the hospital-based surveillance sites. 51 patients died and 28 (54.9%) of them were under 4 years of age. Influenza activity has been reached the peak in the 3<sup>rd</sup> week, 2019. We tested 2552 specimens and influenza positivity rate was 536(21%) including 412(76.9%) A(H1N1)pdm09, 124(23.1%) A(H3N2) and 0(0%) B.

Genetically, A(H1N1)pdm09 and A(H3N2) viruses were belonged to the A/Michigan/45/2015(H1N1)pdm09 group (6B.1) and to the A/Singapore/INFIMH-16-0019/2016(H3N2) group (3C.2a1), respectively and sensitive to Oseltamivir.

There were tested 441 randomized samples for detection of other respiratory viruses and it has been detected 206(48.8%) positive samples among them 46(22.3%) Rhinovirus, 37(18%) RSV, 37(18%) PIV, 13(6.3%) Adenovirus, 9(4.3%) Coronavirus, 8(3.8%) HBoV, 6(3.%) Mpneu and 5(2.4%) Enterovirus, respectively.

**Conclusion:** In Mongolia, A(H1N1)pdm09 and A(H3N2) viruses are mostly created infection in the cold season of 2018/19. The viruses that were active in the Mongolian territories were similar to the vaccines which were recommended by WHO and were sensitive to Oseltamivir.

*Keywords: Surveillance, influenza, respiratory viruses, Mongolia*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10755

## Development of an enzyme linked immunoassay for the quantitation of Influenza hemagglutinin

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Influenza vaccine is updated annually to provide optimum coverage for circulating strains. Each vaccine requires potency determination to ensure sufficient antigen dose to induce immunity. While the current compendia potency assay has served the world for many decades, the development of an alternative assay with higher throughputs and more robust reacting reagents is gaining momentum. We have previously reported the development of a capture detection enzyme linked immunoassay (ELISA/EIA) that utilises subtype specific monoclonal antibodies (mAbs). The unique sandwich-like arrangement of the EIA utilises single mAbs for both capture and detection of antigen allowing for select binding of trimeric and oligomeric forms of HA only. The adoption of parallel line regression analysis has allowed the incorporation of strict intra-assay controls which has led to improved accuracy and precision of the EIA. Significantly, the introduction of a zwitterionic detergent pre-treatment step has broadened the applicability of the EIA to be compatible with all Influenza vaccine antigen presentations. Our EIA approach has demonstrated resistance in the presence of well-known adjuvants, excipients and preservatives. We have demonstrated instances where these additives have caused significant inhibitory effects on single radial immune-diffusion (SRID) while having no effect on the EIA. The additional development of an accelerated stability model which correlates with long term stability has been utilised for rapid screening and selection of stability indicating mAbs utilised in our EIA. This extension of previous findings and the many advantages described indicate this assay is well placed to be a candidate as an alternate for the current method.

*Keywords: Potency Assay, Influenza, Vaccination, Assay Development, Hemagglutinin,*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10763

## Establishment of a yeast-based system to study influenza proteins

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**Introduction and Objectives:** Every year, the influenza virus causes up to 500,000 deaths globally. Efficient therapy does not exist because of: i) Our understanding of the virus and its proteins is far from complete. ii) The virus constantly evolves resulting in highly virulent strains that readily acquire drug resistance. Current experimental systems for studying the viral proteins are limited. We propose a systematic approach in which several viral proteins will be studied in yeast. In this insulated and experimentally accessible system, we will reveal the proteins' structure-function relationships, identify cellular targets through which the viral proteins affect the cell and eventually use the model for drug repurposing to find new drugs. We have chosen to begin with non-structural protein 1 (NS1), nucleoprotein (NP) and the three components of the viral RNA polymerase (Polymerase Acidic protein (PA), Polymerase Basic protein 1 (PB1) and Polymerase Basic protein 2 (PB2)) to be expressed in yeast as these are highly conserved across various influenza A strains.

**Method:** Viral proteins are expressed via several types of vectors, in a variety of strains/mutants and a growth phenotype was observed.

**Results:** By transforming NS1, NP and PB1 into *Saccharomyces cerevisiae* laboratory strain BY4741, we have found that the inducible expression of NS1 and NP confers a slow growing phenotype. By optimizing the growth conditions of the model, we have found that the slow-growing phenotype conferred by NS1 is reproducible in different yeast strains. We have also found that the slow-growing phenotype may not due to autophagy processes, but the viral protein does affect the cell's metabolism.

**Conclusion:** We propose further study into characterizing the slow-growing phenotype imparted by NS1 and NP in yeast to help us further understand the function and host protein interaction to discover new therapeutics against influenza.

**Keywords:** Influenza A virus; *Saccharomyces cerevisiae*; NS1; NP

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10766

## Effects of Codon Usage and CpG Frequency in Influenza Virus Expressing GFP

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**Introduction and objectives:** Seasonal influenza epidemics and occasional pandemics threaten public health worldwide. Influenza viruses carrying exogenous genes, such as reporters, are of great use for basic and translational researches. However, the effect of codon bias-related modification on exogenous gene carried by influenza virus is still unclear. In this study, we generated recombinant influenza viruses to express NS1-GFP-(2A)-NS2 polyproteins in infected cells. The effects of codon usage pattern and/or CpG frequency in the GFP ORF on virus replication and GFP protein expression green fluorescent protein (GFP) reporter were studied.

**Methods:** The codon usage bias and/or CpG frequency observed from the GFP sequence were compared to that observed from the NS segment of avian influenza viruses (AIV) or human influenza viruses. A total of 6 mutated influenza viruses with different combinations of codon usage and CpG frequency in the GFP ORF were rescued. These growth kinetics and GFP expressions of these viruses were investigated.

**Results:** All virus mutants had similar replication rates in mammalian cells and embryonic chicken eggs. The mRNA expressions of these mutated segments were also similar. However, high-CpG-modification and AIV-rare-codon-modification improved the GFP expression compared with the corresponding counterparts. The expression of NS1-GFP fusion protein in each mutant positively correlated with the GFP signal intensity, while negatively correlated with the NEP protein expression.

**Conclusion:** Our current study suggested that both high-CpG-pattern and AIV-rare-codon-usage can improve the GFP expression while keep the replication nature of original virus. These findings put forward construction a traceable influenza virus and a promising recombinant virus carried functional gene to control infection.

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10785

## A humanized MDCK cell line for the efficient isolation and propagation of human influenza viruses

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**Introduction:** Recent human influenza A/H3N2 viruses do not replicate well in traditional Madin-Darby canine kidney (MDCK) cells and therefore derivatives engineered to overexpress human virus receptors (e.g.,  $\alpha$ 2,6-sialoglycans), such as AX4 cells, are used to propagate human influenza viruses. Here, we developed an MDCK cell line (hCK) that expresses high levels of  $\alpha$ 2,6-sialoglycans and very low levels of  $\alpha$ 2,3-sialoglycans (avian virus receptors).

**Methods:** We compared the efficiency of isolation from clinical samples and growth of human A/H1N1pdm, A/H3N2, and B influenza viruses in hCK cells with that in parental MDCK and AX4 cells.

**Results:** We found that hCK cells were markedly better than MDCK or AX4 cells in supporting efficient A/H3N2 virus isolation and growth. Moreover, A/H3N2 viruses propagated in hCK cells maintained higher genetic stability than those propagated in MDCK or AX4 cells.

**Conclusion:** Our findings demonstrate the value of hCK cells for influenza research, particularly human A/H3N2 virus studies, and potentially for vaccine production.

*Keywords: H3N2; Receptor; Virus replicaton; Genetic stability*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10818

## IMPROVEMENT OF LIVE ATTENUATED INFLUENZA VACCINE STRAIN SELECTION BY EMPLOYMENT OF SITE-DIRECTED MUTAGENESIS

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Live attenuated influenza vaccine (LAIV) is the only commercially available influenza vaccine by which candidate vaccine virus (CVV) strains are generated by reverse genetics (RG). Unlike inactivated influenza vaccines, which rely on classical reassortment and serial egg-passage, RG vaccines have the potential to be improved using site-directed mutagenesis (SDM). Improvements by mutagenesis including increased CVV yield, enhanced human cell replication, better antigenic cross-match to cell/circulating viruses, have been performed to produce effective A/H3N2 and A/H1N1pdm09 LAIV CVVs, during 2016-17 and 2017-18 influenza seasons, respectively.

HA and NA genes from egg-propagated wild-type influenza viruses are cloned and reassorted with internal genes of A/AnnArbor/6/1960 to generate live-attenuated 6:2 reassortants. LAIV 6:2 viruses are characterised to assess egg-yield, genetic and HA stability, antigenic properties, and replicative fitness in human nasal epithelial cells (huNEC). SDM was performed on strains with suboptimal CVV properties between 2014 and 2019.

Introduction of amino acid substitutions H183L+L194P into the HA protein of A/H3N2 CVV A/NewCaledonia/71/2014 improved antigenic crossmatch to cell/circulating wild-type strains and gave moderate vaccine effectiveness during 2016-17. Introducing the same substitutions to A/Alaska/252/2016 and A/Singapore/INFIMH-16-0019/2016 strains generated CVVs that appeared non-immunogenic in ferrets as measured by HAI-assay. Introduction of N125D+D127E+D222G+R223Q substitutions into the HA protein of A/H1N1pdm09 CVV A/Slovenia/2903/2015 improved egg-yield and huNEC replicative fitness. Introducing these substitutions into CVV A/Switzerland/3330/2017 HA resulted in reduced huNEC replication and antigenic cross-match.

SDM can be a valuable tool for improving LAIV CVVs. For A/NewCaledonia/71/2014, antigenic cross-match to cell/circulating wild-type strains was improved, and for A/Slovenia/2903/2015, egg-yield and replicative fitness were enhanced. Introducing the same residues into recent, genetically-distinct strains, did not provide improvements. This demonstrates the contextual impact that sequence and structure have on key HA functional residues. A comprehensive structure-functional understanding of HA and NA proteins is important for development of optimised influenza CVVs by SDM.

*Keywords: live attenuated influenza vaccine; LAIV; mutagenesis;*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10841

## Development of an adjuvant nanosphere for delivery of influenza virus proteins

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**Introduction and Objectives:** Influenza is an infectious respiratory illness caused by influenza viruses. Despite yearly updates, the efficacy of influenza vaccines is significantly limited by antigenic drift and/or antigenic shift. These constant changes to the influenza virus make-up also challenge the development of a universal flu vaccine, which requires conserved regions in specific proteins that are also shared by influenza viruses of different subtypes. We propose that it is possible to bypass this challenge by the development of an influenza vaccine based on soluble proteins encapsulated in an adjuvanted nanoparticle system.

**Methods:** In this study, we aim to generate an intranasal influenza vaccine using *N*-trimethylated chitosan nanoparticles (TMC-nPs) as the carriers of a recombinant influenza nucleoprotein (NP). The purified NP recombinant protein was confirmed by SDS-PAGE and western blotting. The toxicity of the NPs was evaluated using primary human intranasal epithelium cells (HNEpCs). The properties of the NP TMC-nPs platform were characterized using Zetasizer. We also assessed the vaccine toxicity and viability as an intranasal vaccine.

**Results:** The size of NP protein was approximately 62 kDa. TMC-nPs showed no toxicity in HNEpCs at 100 µg. Purified NP was encapsulated into TMC-nPs to form NP TMC-nPs. After optimization, the NP TMC-nPs had an average diameter of  $489.8 \pm 11.72$  nm with a PDI of  $0.424 \pm 0.012$  and positive surface charge ( $11.6 \pm 0.153$  mV). NP was efficiently entrapped in TMC-nPs, as it showed a loading efficiency of 98 %. The result showed that TMC-NPs delivered NP more efficiently into HNEpCs than soluble NP alone. For cellular uptake of NP TMC-NPs, the percentage of positive cells was  $97.3 \pm 0.8$  for 48 h of treatment. Cellular uptake was also dose-dependent.

**Conclusion:** The results indicated that TMC-nPs can be used as delivery carriers of NP protein to HNEpCs.

**Keywords:** nanoparticles, influenza virus, nucleoprotein

Topic: Virology and Pathogenesis: Cellular & Molecular Virology

Abstract No: 10844

## COMPREHENSIVE ANALYSIS OF PROTEASE RECOGNITION AND FUSION CHARACTERISTICS OF THE FOUR SEASONAL INFLUENZA VIRUS HEMAGGLUTININS

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### Introduction and objectives

Membrane fusion by influenza A (IAV) or B (IBV) virus hemagglutinin (HA) requires proteolytic cleavage of their HA0 precursor. Which host proteases are involved is only partially solved for IAV and hardly addressed for IBV. In this study, we analyzed the protease recognition and fusion characteristics of the four seasonal IAV and IBV viruses, plus the monobasic HAs of 1918 A/H1N1 and avian A/H7N9. We covered 18 type II transmembrane serine proteases and 16 kallikreins.

### Methods

The effect of siRNA-mediated protease knockdown on virus replication was monitored by immunofluorescence. HA-protease coexpression served to assess HA0 cleavage by Western blot and HA-mediated fusion by polykaryon assay. Protease expression in cells and human lung tissue was analyzed by RT-qPCR.

### Results

Among 34 siRNAs evaluated in Calu-3 cells, only TMPRSS2-siRNA gave significant reduction of A/H1N1 and A/H3N2 IAVs. Its effect on B/Yamagata and B/Victoria was negligible. All four HA0s were 100% cleaved when coexpressed with TMPRSS2 in HEK293T cells. Cleavage was also prominent for TMPRSS-4, -6, -11F and -13 and hepsin, and less pronounced for TMPRSS-5, 11A, -11D and matriptase. Among the kallikreins, only KLK14 cleaved HA0. Most HA-activating proteases were highly expressed in human lung tissue. TMPRSS4 was found to be abundant in MDCK cells, rationalizing their trypsin-independent propagation of IBV and 1918 H1N1 IAV. All HA-cleaving proteases, except KLK14, generated fusogenic HA, as evidenced by polykaryon assay in which also the fusion pH was measured. For B/Victoria HA, polykaryons were formed at 33 °C but not 37 °C, consistent with lower HA protein expression at 37°C. This temperature effect was seen for IBV but not IAV.

### Conclusion

Several proteases related to TMPRSS2 activate the HAs of seasonal IAV and IBV. Besides broad protease recognition, IBV HA displays an intriguing temperature dependence which is possibly related to human adaptation.

*Keywords: Airway proteases; hemagglutinin; cleavage; fusion; temperature*



Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
 Abstract No: 10897

## MOLECULAR CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF INFLUENZA VIRUSES CIRCULATING IN THAILAND BETWEEN 2009 and 2018.

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**Introduction.** Seasonal influenza viruses are responsible for annual influenza outbreaks, worldwide. Timely monitoring to assess the potential effectiveness of seasonal influenza vaccines, composed of the most current epidemic influenza viruses, plays an important role in the prevention of influenza. Our objective was to identify the antigenic and molecular characteristics of circulating influenza viruses in Thailand as compared to the vaccine strains.

**Methods.** 11,930 respiratory samples collected from patients with influenza-like illness in central, northern and southern Thailand from 2009-2018, were tested by influenza real-time RT-PCR. Influenza positive samples with Ct value <27 were randomly selected for genome sequencing by Sanger sequencing and Next Generation Sequencing (NGS).

**Results.** 4,284 of 11,930 (36%) samples were found positive for influenza viruses. Influenza A viruses accounted for 2,731 of 4,284 (64%) and influenza B viruses for 1,553 of 4,284 (36%). Influenza A subtype viruses were as follows: 1,462/2,731 (53%) A/H3N2 and 1,269/2,731 (47%) A/pdmH1N1. Phylogenetic analysis revealed that A/H3N2 belonged to clades 7 (2009 virus collection), 1 (2009-2010), 5 (2010), 6 (2010), 3A (2011), 3B (2011-2012), 3C.1 (2011-2013), 3C.2 (2013-2014), 3C.3 (2012 and 2014), 3C.2a (2014-2017) and 3C.2a1 (2014-2018). A/pdmH1N1 viruses belonged to clades 1 (2009 virus collection), 3 (2009-2010), 4 (2009-2010), 5 (2009-2011), 7 (2010-2011), 6A (2012), 6B (2013-2016) and 6B.1 (2016-2018). B Victoria-lineage viruses belonged to clades 5 (2011 virus collection), 1B (2010-2012) and 1A (2010-2017). B Yamagata-lineage viruses belonged to clades 2 (2011-2015 virus collections) and 3 (2012-2018). All influenza viruses sequenced in this study fall in the same clades as the vaccine strains of each year.

**Conclusions.** This study revealed the HA gene sequences of circulating influenza viruses in Thailand over a 10 year period (2009-2018). Sustained epidemic influenza surveillance coupled with timely antigenic and genetic evolution analysis is critical to health authorities to guide policy decision making.

*Keywords: Influenza viruses, Next Generation Sequencing, Molecular epidemiology*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology

Abstract No: 10898

## MONITORING OSELTAMIVIR RESISTANCE IN INFLUENZA VIRUSES IN NORTHERN VIETNAM, 2013-2017

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**Introduction:** Oseltamivir resistance in influenza viruses has been monitored since 2009 in Vietnam. In 2012, National Influenza Centre - National Institute of Hygiene and Epidemiology - implemented the oseltamivir resistance monitoring in influenza viruses by fluorescence neuraminidase inhibition assay with support from WHO and Sanger nucleotide sequencing.

**Objective:** To continue the data published from 2009 to 2012, we summarize the data of oseltamivir influenza resistance from 2013-2017 on both genotype and phenotype.

**Methods:** Total 454 influenza isolates (172 A/H1N1pdm09, 165 A/H3N2 and 117 influenza B) collected from throat swabs, nasopharyngeal swabs in National influenza surveillance, Severe acute respiratory infection surveillance and Severe viral pneumonia surveillance during 2013-2017. The NA-Fluorescence substrate MUNANA kit from ABI was used followed WHO instruction, IC<sub>50</sub> data was interpreted by JASPR-US CDC and R softwares.

**Results:** The NAI assay results showed that the IC<sub>50</sub> value for A/H1N1pdm09 was 0.371 ± 0.28 nM, A/H3N2 was 0.247 ± 0.2 nM, influenza B viruses was 10,243 ± 4,307nM. Two isolates showed IC<sub>50</sub> values as 125,02nM (A/Vietnam/IS0213167/2013) and 124,27nM (A/Vietnam/HS1814041/2014), 337 and 335 times higher than the normal IC<sub>50</sub> value, were at the highly reduce oseltamivir susceptibility level. The remain influenza isolates maintain the normal susceptibility to oseltamivir. The oseltamivir resistance rate is 1,16% (2/172) in the north of Vietnam. The mutation related to oseltamivir resistance was identified as H275Y on NA proteins. For other mutation related to oseltamivir resistant in A/H1N1pdm09, although they are not found yet but still be screened in the NIC laboratory annually. Both of two resistant viruses belonged to subgroup 6B.1 in HA and NA phylogenetic trees, similar to those circulated in Vietnam at the same period.

**Conclusion:** Two A/H1N1pdm09 viruses were resistant to oseltamivir (2013 and 2014) in northern Vietnam, the remain influenza isolates showed the normal inhibition of oseltamivir from 2013 to 2017.

**Keywords:** influenza resistance, oseltamivir, northern Vietnam, 2013-2017

Topic: Virology and Pathogenesis: Cellular & Molecular Virology

Abstract No: 10976

## GENOTYPIC CHARACTERIZATION OF INFLUENZA A VIRUSES COLLECTED BY THE CANADIAN SENTINEL PRACTITIONER SURVEILLANCE NETWORK DURING THE 2018-19 BIMODAL A(H1N1)PDM09 AND A(H3N2) EPIDEMIC

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### INTRODUCTION/OBJECTIVES

The 2018-19 influenza season in Canada has been characterized by an initial wave of dominant A(H1N1)pdm09 activity peaking in January followed by a second wave of A(H3N2) peaking in March. The community-based Canadian Sentinel Practitioner Surveillance Network (SPSN) reports the genetic profile of contributing viruses.

### METHODS

Influenza A viruses were characterized by Sanger sequencing of the hemagglutinin (HA) gene. Vaccine-virus relatedness was based on amino acid substitutions in established antigenic sites for A(H1N1)pdm09 (Sa/Sb/Ca1/Ca2/Cb) and A(H3N2) (A-E) and in proximity to the receptor-binding site (RBS). Phylogenetic analysis established clade distribution.

### RESULTS

Sequence information was available for 369/946(39%) A(H1N1)pdm09 and 118/201(59%) A(H3N2) viruses. All A(H1N1)pdm09 viruses belonged to clade 6B.1A, differing from the clade 6B.1 vaccine strain (A/Michigan/45/2015) by at least five HA substitutions: S74R(Cb), S164T(Sa), and I295V, in addition to M209K and R223(RBS) associated with egg-passaging of the vaccine. Additional heterogeneity was observed in 6B.1A viruses compared to previous seasons. Several parallel substitutions are present in multiple 6B.1A subgroups including S183P (introducing a large aromatic ring in proximity to site Sb) in 313/369(85%) viruses, and T185I (site Sb) in 185/369(50%) viruses. Most A(H3N2) viruses (77/118;65%) belong to clade 3C.2a1b, a descendant branch of clade 3C.2a1 to which the vaccine strain (A/Singapore/INFIMH-16-0019/2016) belongs, the latter bearing three egg-adaptation mutations at positions 160, 194 (both antigenic site B) and 225 (RBS). A minority of A(H3N2) viruses belong to clades 3C.2a2 (20/118;17%) and 3C.3a (19/118;16%). 3C.2a1b viruses are also diversifying, with parallel substitutions acquired by multiple A(H3N2) subgroups, particularly T135K (site A, loss of a potential glycosylation site (-CHO)), T128A (site B)(-CHO) and R142G (site A), the latter two resembling substitutions present in 3C.3a viruses since 2011-12.

### CONCLUSIONS

Rapid A(H1N1)pdm09 diversification and multiple parallel substitutions in A(H3N2) viruses require monitoring for emergence of dominant immunological escape variants, with implications for vaccine protection.

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 10997

## vRNA-vRNA interactions in influenza A virus HA vRNA packaging

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### Introduction and Objectives

The genome of the influenza A virus is composed of eight single-stranded negative-sense RNA segments (vRNAs). The eight different vRNAs are selectively packaged into progeny virions. This process likely involves specific interactions among vRNAs via segment-specific packaging signals located in the 3' and 5' terminal coding regions of vRNAs. However, it remains unclear how the eight vRNAs interact with each other for selective genome packaging. The aim of this study is to identify vRNA(s) that interact with HA vRNA during genome packaging.

### Methods

Mutant viruses, which have several silent mutations in the 3' or 5' packaging signal region of HA vRNA, were generated by reverse genetics. One mutant virus, which demonstrated reduced growth, was serially passaged in MDCK cells until the growth was restored. The efficiency of vRNA incorporation into virions was quantified by RT-qPCR, and vRNA-vRNA interactions were examined by gel shift assay using *in vitro* transcribed vRNAs.

### Results and Conclusion

Of the mutant viruses, HA5m2 virus, with five silent mutations introduced into nucleotides 1664 to 1676, showed a specific defect in HA vRNA incorporation, which reduced the viral growth efficiency. After serial passaging in cells, the virus acquired additional mutations in the 5' terminal packaging signal regions of both HA and PB2 vRNAs. These mutations contributed to the recovery of viral growth and packaging efficiency of HA vRNA. A direct RNA-RNA interaction between the 5' ends of HA and PB2 vRNAs was confirmed *in vitro*. Our results indicate that direct interactions of HA vRNA with PB2 vRNA via their packaging signal regions are important for selective genome packaging and enhance our knowledge on the emergence of pandemic influenza viruses through genetic reassortment.

*Keywords: genome packaging, HA segment, RNA-RNA interaction*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology

Abstract No: 11020

## STABLE HAIRPIN SECONDARY STRUCTURE IN NS GENE RNA ENHANCES EXPRESSION OF NS1 IN INFLUENZA INFECTED CELLS

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**Introduction and objectives:** Formation of conserved RNA secondary structures has been demonstrated in a number of viruses, including influenza. Currently, there is no doubt that identification of specific RNA structures and clarification of their roles in viral pathogenesis have the potential to expand our list of therapeutic antiviral drug targets. Earlier, two regions in influenza NS gene RNA (nucleotide positions 82-148 and 497-564) were found to form stable secondary structures that differ between various influenza strains. In this research, we have attempted to show whether the described RNA structures can influence viral properties such as NS1 protein expression.

**Methods:** Using site-directed mutagenesis, we introduced mutations into the 82-148 and 497-564 regions of the A/Puerto Rico/8/34 (H1N1) NS gene in order to obtain 4 strains featuring different mRNA secondary structures. The corresponding viruses were constructed by reverse genetics, propagated in Vero cells, and characterized for infectious activity (TCID<sub>50</sub>). After infection (moi=10) of MDCK or A549 cells, NS1 expression was measured by ELISA at different time points.

**Results:** The obtained viruses, bearing different NS mRNA secondary structures, did not show significant differences in multiple-cycle infection dynamics in cell cultures. However, yield of rescued virus after transfection (null passage) was higher in viruses featuring stable RNA hairpins in both regions. In addition, different levels of NS1 protein expression were observed during the first infection cycle. Viruses which contained a stable structure in the first NS RNA region (82-148) produced significantly higher levels of NS1 than those without it. Structures at the 497-564 region had no observable effect on NS1 expression.

**Conclusion:** Stable hairpin in the 82-148 region of NS mRNA enhanced NS1 expression level, and may influence on infectivity in particular influenza strains.

Financial disclosure: This study was supported by Russian Science Foundation grant number 18-74-00130.

**Keywords:** influenza virus; NS gene; RNA secondary structure; RNA hairpin; reverse genetics.

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 11037

## Molecular genetic analysis of influenza A viruses identified during the 2018-2019 epidemic season in the Republic of Kazakhstan

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**Background:** The first half of the epidemic season of influenza was characterized by active circulation of influenza A viruses - up to 98% of the number of positive findings on influenza, with the dominant influenza A/H1N1pdm09 virus (73%) in almost all regions of the country.

**Aims:** The study of the molecular genetic properties of influenza A viruses in Kazakhstan in the epidemic season of 2018-2019 and determination their similarity to the vaccine strains of the current epidemiological season.

**Methods:** Using the Sanger sequencing method, we studied the sequences of 22 influenza A viruses, of which 12 A/H1N1pdm09 and 10 A/H3N2. Isolates and clinical samples of influenza were delivered to the NIC from the virology laboratories of different regions of the country. Phylogenetic analysis was performed on available haemagglutinin (HA) sequences to compare them to the 2018/19 vaccine virus sequences.

### Results:

Phylogenetic analysis of influenza A/H1N1pdm09 viruses showed, that all viruses studied belonged to group 6B.1 and were similar to the Michigan/45/2015 (H1N1) vaccine strain. The obtained data, along with the analysis of the strains of the previous season 2017-2018, indicate a slow antigenic drift of viruses of this subtype with the accumulation of point mutations in the HA gene.

In the study of nucleotide sequences of influenza A/H3N2 viruses, it was found that all influenza viruses fell into the component block of the current 3C.2a1 vaccine, represented by strain A/Singapore/INFIMH-16-0019/2016. In addition, all analyzed influenza A/H3N2 viruses were grouped into subclid 3C.2a1b, represented by the reference strain A/Alsace/746/2018, of which the majority of viruses were closely related to the reference strain A/Mauritius/2287/2018.

**Conclusions:** The results of the study demonstrated that all of the studied A influenza viruses detected in Kazakhstan were similar to the vaccine strains recommended by WHO for the epidemiological season 2018-2019.

*Keywords: influenza A, molecular genetic analysis, vaccine strains*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology

Abstract No: 11065

## INCREASING THE SUSCEPTIBILITY OF HEK293FT CELL LINE TO INFLUENZA INFECTION BY CRISPR-CAS9 MEDIATED KNOCKOUT OF PARTICULAR GENES

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**Introduction and objectives:** Human origin cell line that is highly permissive to influenza virus could be an ideal substrate for viral isolation and propagation, providing original viral glycosylation profile and antigenic properties, essential for influenza surveillance and vaccine production. Earlier the development of permissive cell lines was nearly random process based on scientist perseverance and good luck. In this study we explored if CRISPR-Cas9 mediated knockout of cell genes associated with influenza host restriction factors could be a direct way to obtain human cell line highly permissive to influenza.

**Methods:** By CRISPR-Cas9 genome editing we obtained several clones of HEK293FT cells with knockout of the ANXA6 or IRF7 genes. We compared replication dynamics of several influenza viruses in original and mutated cells in growth curve experiments. TCID50 assay, flow cytometry and immunofluorescence staining were used to measure the level of virus reproduction.

**Results:** We showed that accumulation of influenza A virus in the mutant HEK293FT-ANXA6<sup>-/-</sup> cells significantly exceeded the virus titer in the original HEK293FT cells. We also observed the tendency of increased virus growth in HEK293FT-IRF7<sup>-/-</sup> cells. However, the enhancement we seen was not sufficient to regard HEK293FT mutant cells as a substitute for highly permissive MDCK cell line.

**Conclusion:** Depletion of particular genes by CRISPR-Cas9 technique can increase influenza virus yield in HEK293FT cells. Though multiple gene knockout may be required to obtain more permissive human cell line suitable for influenza virus research.

Financial disclosure. This work was supported by Russian Science Foundation grant 18-75-10069.

**Keywords:** influenza virus; CRISPR-Cas9-mediated knockout; permissive cell line; genome editing

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 11098

## INFLUENZA VIRUS REPLICATION IN A549 CELLS IN THE PRESENCE OF ANTIVIRAL AGENT AMIZON (ENISAMIUM IODIDE)

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### Introduction

Antiviral properties of enisamium iodide (trade name Amizon) which was licensed for the treatment of influenza-like illness in Ukraine and other 11 countries remained not fully elucidated.

Enisamium inhibits influenza virus replication in NHBE cells, reducing viral titers, M-gene and hemagglutinin expression but not in MDCK cells due to extremely low permeability (<0.1%).

The aim of current study was to test A549 cells regarding their ability to support antiviral effect of enisamium iodide against influenza virus.

### Methods

A549 cells were infected by A/Dnipro/158/2019 (H1N1)pdm virus (MOI 1.0). Enisamium iodide was synthesized by Farmak JSC and added to culture medium in concentrations of 50, 100, 150, 250, 500 µg/ml simultaneously with the virus inoculation. M-gene expression in A549 cells was accessed 24 h post-infection by RT-PCR. Cellular uptake of enisamium iodide was evaluated by LC-MS/MS. Cytotoxicity was measured by MTT assay.

### Results

Uptake of enisamium iodide from culture medium containing 1000 µM (or approx. 250 µg/ml) of this compound by A549 cells was 4000 ng/10<sup>6</sup> cells which was similar to NHBE cells – 3300 ng/10<sup>6</sup> cells. These results provided opportunity to assume that enisamium at the mentioned above concentration could be effective against influenza virus inoculated in A549 cells, suppressing its replication.

Enisamium iodide provided significant reduction of viral titers and M-gene expression in A549 cells; EC50 was found to be less than 250 µg/ml and IC50 – between 24,5 to 31,6 mg/ml. Selectivity index, – approx. 100, – suggested this molecule is not toxic and its antiviral efficacy is not linked to cytotoxicity.

### Conclusions

Antiviral effect of enisamium iodide has been demonstrated using A549 cells to be essentially similar to NHBE cells. Uptake of the test article by A549 cells was comparable to that of NHBE cells. The antiviral effect of enisamium was not linked to cytotoxicity.

*Keywords: Enisamium iodide; Antiviral; A549 cells*



Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 11245

## DEVELOPMENT OF AN ALTERNATIVE POTENCY ASSAY TO MEASURE THE HA CONTENT OF TWO INFLUENZA B VIRUS IN QUADRIVALENT INFLUENZA VACCINE IN JAPAN.

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### Introduction

Single radial immuno-diffusion (SRID) assay is currently used to measure HA content of each vaccine component. It has been reported that cross-reactivity of antisera between two lineages of influenza B virus (IFVB) hampered accurate quantification of HA content in quadrivalent influenza vaccine (QIV) by SRID assay. Here, we produced new monoclonal antibodies (mAbs) specific for B/Yamagata-lineage (BYam) and B/Victoria-lineage (BVic) viruses, and developed an antigen-capture ELISA as an alternative potency assay to quantitate the HA protein of each IFVB in QIV.

### Materials and Methods

Two BYam-specific and 6 BVic-specific mAbs were tested for an antigen-capture sandwich ELISA. Three monovalent split vaccines of BYam (B/Wisconsin/01/2010(BX-41A) (BX41A), B/Massachusetts/02/2012(BX-51B) (BX51B), B/Phuket/3073/2015 (PHK)) and 3 vaccines of BVic (B/Brisbane/60/2008 (BR60), B/Texas/2/2013 (TX2), B/Maryland/15/2016(BX-69A) (BX69A)) were tested. SRID antigen (SRIDAg) or purified HA protein (pHA) of each strain was used as an ELISA standard.

### Results

One BYam-specific-mAb reacted with all tested BYam strains, and two BVic-specific-mAbs reacted with all BVic strains. Some mAbs detected HA1 in Western blot and had neutralization activity. We established the antigen-capture sandwich ELISA each lineage-specific using the same mAb for capture and detection. This ELISA could measure robustly the HA content of BX41A in QIV, which was impossible to measure by SRID due to interfering the formation of precipitin rings. The decreased potencies of heat inactivated vaccines measured by ELISA were well correlated with that by SRID, indicating that the ELISA measures biological active structures. Some vaccines showed consistency in HA content between SRID and ELISA using SRIDAg as a standard, but some did not. The discrepancy could be improved using pHA as a ELISA standard, which had more similar dispersion state of the vaccine than SRIDAg analyzed by TEM and SEC-HPLC.

### Conclusion

This ELISA using each lineage-specific-mAbs could be useful as an alternative potency assay for QIV.

*Keywords: Potency assay, SRID test, lineage-specific-mAbs, antigen-capture ELISA*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology

Abstract No: 11251

## DETECTION OF VICTORIA LINEAGE INFLUENZA B VIRUSES WITH $\Delta$ 162-163 DELETIONS IN THE HEMAGGLUTININ (HA), SOUTH AFRICA, 2018

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### Introduction and objectives

A group of Victoria lineage influenza B viruses with a two amino acid deletion in the hemagglutinin (HA) at residues K162 and N163, designated as V1A.1, was detected during the 2016–17 Northern Hemisphere influenza season and continues to spread geographically. This double deletion was sufficiently different from influenza B viruses in the 2017/2018 vaccines to potentially reduce vaccine effectiveness. We describe the first identification of viruses with these deletions from South Africa in 2018.

### Methods

Nasopharyngeal samples were obtained from the syndromic surveillance programs. Real-time reverse transcription polymerase chain reaction was used for virus detection and lineage determination. Hemagglutination inhibition (HAI) assays assessed antigenic characterization of influenza viruses. Influenza genetic characterization was done using next-generation sequencing on the MiSeq platform. The duration of virus circulation was determined using thresholds calculated using the Moving Epidemic Method; duration was used as an indicator of disease transmission and impact.

### Results

In 2018, 41% (460/1119) of influenza-positive specimens were influenza B viruses – ranging between 33–44% in the different ILI and SRI surveillance programs. Of 460 influenza B-positive samples, 408 (87%) had the lineage determined of which 77% (313/408) were Victoria lineage. The South African influenza season peaked as moderate during influenza B circulation, indicating low impact and moderate transmission. All influenza B/Victoria V1A.1 strains isolated (n=6) were poorly inhibited (4-6 fold lower HAI titre) by antisera raised against B/Brisbane/60/2008-like virus. We sequenced 10% (30/313) B/Victoria positive samples and all had  $\Delta$ 162-163 deletions, belonging to the V1A.1 group.

### Conclusion

We report the first detection of the  $\Delta$ 162-163 deletion variant of influenza B/Victoria viruses in South Africa in 2018. These deletions putatively effect the antigenic properties of the viruses because they border an immune-dominant region at the tip of the HA. Therefore, close monitoring of these newly emerging viruses is essential.

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 11308

## Overview of seasonal flu Inf (A/H3 )infection in Nepal

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### Background

Influenza is a highly contagious viral respiratory infection caused by influenza viruses whose epidemic and pandemic has resulted in significant mortality and morbidity. It has been reported that annual epidemic of influenza result in an estimated 3 – 5 million cases of severe illness and about 290000 – 650000 deaths globally. This study was aimed to investigate the types of influenza viruses prevailing in Nepal.

### Methods

A descriptive cross sectional study was carried out at National Public Health Laboratory, Kathmandu, Nepal for the period of one year (Jan – Dec 2016). A total of 1683 throat swab specimen was collected from patients of different age group referred to NPHL. The specimen was primarily stored at 4°C and processed using RT-PCR method for the identification of influenza viruses.

### Results

Of the total 1683 patients suspected of having influenza infection, influenza viruses were isolated from 614 (36.5%) patients with male predominance. Among the total 614 infection, the highest number of infection was caused by influenza A/H3 strain (51.0%) followed by influenza B (40.4%) and influenza A (H1N1) pdm09 (8.6%). There were two peaks of infection, one in the month of February and the other in the month of August in year 2016.

### Conclusion

We found that influenza A/H3 serotype is the major cause of influenza infection despite of some pandemic caused by influenza A (H1N1) pdm09 in Nepal.

*Keywords: Influenza, Throat swab, RT-PCR, Prodominant,*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 11310

## DEFECTIVE INTERFERING vRNA ARE NOT A MAJORITY POPULATION IN LIVE ATTENUATED INFLUENZA VACCINE AND APPEAR TO NOT DRIVE VACCINE EFFECTIVENESS

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### Introduction.

In the 2013/14 and 2015/2016 influenza seasons, the pandemic H1N1 (A/H1N1pdm09) component of the quadrivalent live attenuated influenza vaccine (Q/LAIV) was shown to have reduced clinical vaccine effectiveness (VE). More recently, using RT-PCR techniques, it was published that substantial quantities of defective interfering (DI) vRNA were present in commercial 2014/15 Q/LAIV nasal sprayers. These DI vRNA are truncated genomic RNA segments which are known to play a role in viral replication and innate immunity. It was therefore postulated that the presence of large amounts of DI vRNA in LAIV could impact clinical VE.

### Methods.

RT-PCR based methods were used to perform qualitative assessments of DI vRNA content in formulated Q/LAIV. Nanopore sequencing and a novel digital polymerase chain reaction (dPCR) assay was used to provide a quantitative assessment.

### Results.

The RT-PCR based methods confirmed published observations that DI vRNA do exist in specific Q/LAIV formulations. Then, using nanopore sequencing, we were able to show that the abundance and genetic structure of DI vRNA in RT-PCR products was dependent on LAIV subtype. The subsequently developed novel dPCR assay provided absolute quantification of PA DI vRNA relative to its full-length gene segment, in the absence of RT-PCR amplification bias.

### Conclusion.

This demonstrated that, in contradiction to the RT-PCR based observations, DI vRNA did not make up a substantial proportion of the PA gene segment population (5-35%) in any of the LAIV strains tested. Furthermore, our data suggested that there was no correlation between LAIV DI vRNA content and clinical effectiveness. Additional experiments evaluating the precise role of DI vRNA in LAIV replication and innate immunity are ongoing.

*Keywords: Vaccine Effectiveness; Defective Interfering; Live Attenuated Influenza Vaccine*

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 11737

## NGS INVESTIGATION OF A(H1N1)pdm09 HA-D222G/N POLYMORPHISM ASSOCIATED WITH INFLUENZA CASES IN RUSSIA IN 2017-2019

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**Introduction and Objectives:** High genetic variability of influenza A virus may lead to occurrence and selection of pathogenicity related mutations. It is known that the presence of HA-D222G/N mutations in A(H1N1)pdm09 influenza virus provides increased tropism to the lower parts of the human respiratory tract, which can lead to pneumonia, and often correlates with increased disease severity and mortality. The objective of the study was to analyze the presence of the HA-D222G/N polymorphism in A(H1N1)pdm09 viruses from fatal cases in Russia in 2017-2019.

**Methods:** Clinical influenza samples were collected at the local Sanitary-and-Epidemiological Centers of Rospotrebnadzor in accordance with the regulations of the Russian Federation. Viral strains were isolated in MDCK according to the WHO manuals. Whole genome sequencing was performed on an Illumina MiSeq.

**Results:** NGS analysis of A(H1N1)pdm09 viruses from 29 influenza cases (19 fatal) in the epidemic season of 2017-2018 revealed the presence of HA D222G/N mutations in major virus variant in four fatal cases. The presence of D222G/N mutations only in minor virus variants was detected in two fatal cases. Optimized NGS analysis of 67 A(H1N1)pdm09 cases (41 fatal) in the epidemic season of 2018-2019 revealed the presence of D222G/N mutations in major virus variant in 16 fatal cases. The presence of D222G/N mutations only in minor virus variants was detected in eight fatal cases. Simultaneous presence of viruses with D222G and D222N mutations was detected in 27% of the analyzed fatal cases. Presence of D222G/N mutations in major and minor viral variants as well as simultaneous presence of viral variants with different mutations may contribute to a more severe disease.

**Conclusion:** NGS revealed A(H1N1)pdm09 HA-D222G/N polymorphism, which may lead to increased disease morbidity and mortality, in 32-59% of studied fatal cases. Detection of the D222G/N polymorphism is of particular importance for epidemiological analysis and prognosis.

**Keywords:** A(H1N1)pdm09; fatal; D222G/N; polymorphism; NGS

Topic: Virology and Pathogenesis: Cellular & Molecular Virology  
Abstract No: 11773

## Heterologous Prime-Boost Regimens with Ad5 and NDV Vectors Elicit Stronger Immune Responses to Ebola Virus Than Homologous Regimens in Mice

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**Introduction and Objectives:** The 2013 Ebola outbreak in West Africa resulted in more than 11,000 deaths, highlighted the need for a vaccine. Recently, a Phase I clinical trial of adenovirus type 5 (Ad5) vector-based EBOV vaccine has shown that a homologous prime-boost regimen with Ad5 vaccine could elicit greater humoral responses, but little cellular immune response.

**Methods:** We generated a NDV recombinant, based on the LaSota strain, expressing EBOV variant Makona GP protein using reverse genetics techniques. Expression of EBOV GP protein in the recombinant virus-infected cells was detected by immunofluorescence assay. To investigate whether prime-boost with heterologous vectors could improve the immunity, the humoral and cellular immune responses to heterologous regimens combined Newcastle disease virus (NDV) and Ad based EBOV vaccines were assessed in mice.

**Results:** The ELISA and ELISPOT results showed that mice in groups primed with rLS/EB-GP and boost with Ad5-MakGP (NDV+Ad5) or reversed Ad5-MakGP prime and rLS/EB-GP boost (Ad5+NDV) elicited stronger EBOV GP-specific antibody and cellular immune responses than homologous regimens alone. In addition, the strongest EBOV GP-specific antibody and T-cell responses were detected after the Ad5-MakGP prime and rLS/EB-GP boost (Ad5+NDV) in mice.

**Conclusions:** A Ad5 prime NDV boost regimen is more effective in stimulating EBOV specific immunities than homologous regimens alone. Our results may indicate the potential boosting ability of NDV vector in human vaccine use.

*Keywords: adenovirus type 5; Newcastle disease virus; EBOV vaccine; homologous prime-boost regimen*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 10360

## FIRST CASE OF HUMAN INFECTION WITH A NOVEL TRIPLE-REASSORTANT H1N1 VIRUS IN NORTHERN CHINA

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**Introduction and Objectives** The Eurasian avian-like swine influenza A (H1N1) virus (EA-H1N1) spread into China around 2007, while the first report of EA-H1N1 in pigs in Tianjin, Northern China, was in 2016. Here we reported the first case of EA-H1N1 infection in a 9-year-old boy with upper respiratory tract infection in Tianjin in 2018.

**Methods** Throat swabs were collected from a boy with fever, cough, sore throat and headache in Tianjin, China. Viruses were isolated using MDCK cells and embryonated eggs. Full genome sequencing, phylogenetic analysis, antigenic characteristic analysis and antiviral resistance assays were performed. 4 close contacts, and 28 surrounding environment specimens where the patient lived were collected for influenza virus test.

**Results** After whole genome sequencing, the influenza virus isolate from the patient was identified as a novel triple-reassortant H1N1 virus with genes containing from Eurasian avian-like swine (HA, NA), A (H1N1) pdm09 (PB2, PB1, PA, NP, M) and classical swine (NS) lineages, termed influenza A/Tianjin-baodi/1606/2018(H1N1) virus(A/TJ/1606). Complete sequences of A/TJ/1606 virus were 94.9%–97.1% identical in all 8 gene segments with A/Fujian-cangshan/SWL624/2016 (another previously reported triple-reassortant H1N1 virus). HI assay showed that A/TJ/1606 was antigenically similar to the A (H1N1) pdm09 vaccine strain A/Michigan/45/2015(H1N1). Antiviral resistance test indicated A/TJ/1606 was sensitive to Oseltamivir. The close contacts of the patient did not develop any influenza-like symptoms during the longest incubation period. And no influenza viruses were identified from the close contacts and surrounding environment specimens.

**Conclusion** This is the first human infection with a novel triple-reassortant H1N1 virus infection identified in Northern China. No human to human transmission was observed. Though no influenza virus was detected in the surrounding environment at that time, EA-H1N1 infection in pigs in Tianjin has been reported before. Enhanced surveillance of influenza should be instituted among swine and humans in the future.

**Keywords:** Eurasian avian-like swine influenza A (H1N1) virus, phylogenetic analysis, antigenic characteristic analysis

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
 Abstract No: 10420

## Emergence of influenza A/H7N4 virus in Cambodia

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Avian influenza virus (AIV) subtype A/H7 viruses are of particular concern as they have been a leading cause of zoonotic infections over the past two decades, with human cases due to independent H7-lineages detected across multiple continents. Here we report genomic diversity of A/H7 low pathogenic avian influenza (LPAI) viruses identified through active surveillance in high-risk sites in Cambodia between 2015-2018. A predominant number of A/H7 viruses originated from duck samples, and none of their genes were directly related to the A/Anhui/1/2013-H7N9 lineage prevalent in poultry in China since 2013; however, some A/H7N4 viruses from 2018 showed a significant temporal and phylogenetic similarity to the A/H7N4 virus that caused a serious, but non-fatal infection in a 68-year-old woman in Jiangsu province, China in December 2017. Whole genome sequencing and phylogenetic analysis showed that the surface genes of Cambodian H7N4 viruses were most closely related to A/Jiangsu/1/2018-like H7N4, however not all internal genes shared the same source suggesting continued reassortment of this H7N4 lineage in live-bird markets. The Cambodia/Jiangsu-like HA genes shared a common ancestor during 2017, immediately prior to their detection, and were derived from A/H7N7 and A/H7N2 viruses previously detected in wild aquatic birds in east Asian countries, whereas the N4-NA genes were derived from A/H10N4 and A/H8N4 viruses previously detected in Georgia, Russia and Mongolia. Detection of A/H7N4 viruses in live-bird markets in Cambodia in such a short span of time and at such a large spatial distance, highlights the risk and high potential for rapid spread of this, and other, virus lineages throughout poultry in the region with the subsequent risk of further human infections.

*Keywords: subtype A/H7N4; zoonotic infection; live poultry markets; influenza surveillance; whole genome sequencing*



Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 10554

## **PATHOLOGY OF CLADE 2.3.4.4 H5N2 HPAIV IN EXPERIMENTALLY INFECTED COMMERCIAL BROAD BREASTED WHITE TURKEYS**

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Introduction: Between December 2014 and June 2015, the U.S. experienced the largest series of outbreaks of highly pathogenic avian influenza virus (HPAIV) in recent history, seriously impacting mostly turkey and layer commercial operations in the U.S. The virus was generated from the reassortment between the Eurasian A/goose/Guangdong (Gs/GD) H5 lineage clade 2.3.4.4 H5N8 HPAIV and a North American wild bird lineage influenza virus.

Objective: To characterize age-related differences in terms of pathology in commercial white broad breasted turkeys inoculated with A/turkey/Minnesota/12582/2015 (H5N2) HPAIV clade 2.3.4.4.

Methods: Turkeys were divided in groups and inoculated intranasally with  $10^{6.5}$  EID<sub>50</sub> of HPAIV H5N2 at either 6 or 16 weeks of age and monitored daily for disease signs and mortality.

Results: Turkeys infected at 6-weeks of age showed inapparent to little clinical signs with rapid disease progression, reaching 100% mortality at 3 days post infection (dpi). In contrast, turkeys infected at 16-weeks of age developed ataxia, lethargy and neurological signs and reached 100% mortality by 5 dpi. Both age groups showed microscopic lesions in vital organs associated with the presence of avian influenza virus (AIV) nucleoprotein (NP) in multiple cell types including neurons, glial cells, ependymal cells, respiratory epithelial cells, air capillary epithelium and pulmonary macrophages, cardiac myocytes, smooth muscle fibers, cells of the vascular walls, and pancreatic acini and ductal cells. Infection in the 6-weeks old turkeys resulted in peracute lesions consistent of extensive hemorrhages, edema and fibrinonecrotizing pneumonia in lung, mild to moderate heterophilic meningoencephalitis and enteritis, but inflammation was not prominent. In the 16-weeks old turkeys, necrosis and hemorrhages in tissues were accompanied by a more prominent subacute inflammatory infiltrate.

Conclusion: Age is a determinant factor in the progression of the disease and delay of mortality during infection with the H5N2 clade 2.3.4.4 HPAI virus in naïve white broad breasted turkeys.

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 10558

**FLEXIBILITY *IN VITRO* OF AMINO ACID 226 IN THE RECEPTOR-BINDING SITE OF AN H9 SUBTYPE INFLUENZA A VIRUS AND ITS EFFECT *IN VIVO* ON VIRUS REPLICATION, TROPISM, AND TRANSMISSION.**

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Introduction: Influenza A viruses remain a significant public health threat causing more than 300,000 hospitalizations in the United States during 2015-2016 season alone. While only few IAVs of avian origin have been associated with human infections, the ability of these viruses to cause zoonotic infections further increases the public health risk of influenza. Of these, H9N2 viruses in Asia are of particular importance as they have contributed internal gene segments to other emerging zoonotic IAVs. Notably, recent H9N2 viruses have acquired molecular markers that allow for a transition from “avian-like” to “human-like” terminal sialic acid (SA) receptor recognition via a single amino acid change at position 226 (H3 numbering), from glutamine (Q226) to leucine (L226), within the HA receptor-binding site (RBS).

Objective: We sought to determine the plasticity of amino acid 226 and the biological effects of alternative amino acids on variant viruses.

Methods: We created a library of viruses with the potential of having any of the 20 amino acids at position 226 on a prototypic H9 HA subtype IAV and performed *in vitro* and *in vivo* characterizations.

Results: We isolated H9 viruses that carried naturally occurring amino acids, variants found in other subtypes and variants not found in any subtype at position 226. Fitness studies in quails revealed that some natural amino acids conferred an *in vivo* replication advantage.

Conclusions: This study shows the flexibility of position 226 of the HA of H9 influenza viruses and the resulting effect of single amino acid changes on the phenotype of variants *in vivo* and *in vitro*.

**Keywords:** H9N2; glycan array; glycopolymer, host range, avian, quail

Topic: Virology and Pathogenesis: Emerging Influenza Viruses

Abstract No: 10596

## EVIDENCE OF A FIXED INTERNAL GENE CONSTELLATION IN INFLUENZA A VIRUSES ISOLATED FROM WILD BIRDS IN ARGENTINA (2006-2016).

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Introduction: Historically, long-term active influenza A virus (IAV) surveillance in wild birds has been circumscribed to North America, Europe and parts of Asia. Elsewhere, surveillance efforts have been mostly in response to disease outbreaks. Our group has been conducting active IAV surveillance in wild birds in Argentina (South America) as part of efforts to establish early warning systems for the potential introduction of IAVs from Asia into the Americas and/or from wild birds into commercial poultry.

Objectives: To understand the ecology of IAVs in wild birds in Argentina.

Methods: Cloacal swabs and feces samples (n=6595) were collected from 65 bird species in Argentina from 2006 to 2016, screened for influenza A virus and full virus genome characterization.

Results: IAVs from 6 waterfowl species revealed subtypes combinations of the H1N1, H4N2, H4N6, H4N8, H5N3, H6N2, H7N7, H7N9, and H10N7. Notably, the internal gene segments of the Argentine isolates belonged to the South American lineage, showing a divergent evolution of these viruses in the Southern Hemisphere. Time-scaled phylogenies indicated that South American gene segments diverged between ~30 and ~140 years ago from the most closely related influenza lineages, which include the avian North American, Eurasian and the equine H3N8 lineage viruses. Phylogenetic analyses of the hemagglutinin and neuraminidase gene segments of the H4, H6, and N8 subtypes revealed recent introductions and reassortment between viruses from the Northern and Southern Hemispheres in the Americas.

Conclusions: Remarkably and despite evidence of recent hemagglutinin and neuraminidase subtype introductions, the phylogenetic composition of internal gene constellation of these influenza A viruses has remained unchanged. Considering the extended time and the number of sampled species of the current study, and the paucity of previously available data, our results contribute to a better understanding of the ecology and evolution of influenza virus in South America.

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
 Abstract No: 10656

## A NOBEL H7N3 REASSORTANT THAT HAS ADAPTED TO DUCKS

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**Introduction and objectives:** The H7N9 avian influenza virus (AIV) had been epizootic in poultry in China since 2013. Through continuous monitoring at airports in Japan, a novel H7N3 reassortant of the zoonotic H7N9 HPAIVs, A/duck/Japan/AQ-HE30-1/2018 (HE30-1), was detected in a poultry meat product illegally brought by a passenger from China into Japan in 2018. In this study, we analyzed the genetic, pathogenic, and antigenic characteristics of HE30-1 by comparing HE30-1 with the previous zoonotic H7N9 AIVs isolated from human in China and their reassortant H7N2 AIV.

**Methods:** Complete genomic sequence of HE30-1 was phylogenetically analyzed to trace the genetic origins. Pathogenicity of HE30-1 was evaluated with chickens, domestic and mallard ducks. Antigenic characteristics of HE30-1 was analyzed by hemagglutination-inhibition (HI) assay followed by antigenic cartography and putative amino acid comparison with previous zoonotic H7N9 AIVs and their reassortants.

**Results:** The entire HE30-1 genomic sequence revealed that it comprised at least three different sources; the H7 HA, PB1, PA, NP, M, and NS segments of HE30-1 were directly derived from the zoonotic H7N9 AIVs, whereas the N3 NA and PB2 segments evolved from isolates circulating in poultry and wild birds, respectively, both of which had no direct relationship with zoonotic H7N9 AIVs. In experimental infection, HE30-1 was lethal in chickens but not in domestic or mallard ducks while it replicated and was shed in those birds. The antigenic analysis indicated that antigenic drift has occurred among the zoonotic H7N9 AIVs and their reassortants.

**Conclusion:** Findings suggested that HE30-1 has become adapted to domestic and mallard ducks as a result of multiple reassortments. Such adaptation to ducks might lead to the dissemination of the viruses possessing HA of zoonotic H7N9 AIV origin into other host species, such as mallard ducks, and might allow them to be transmitted by wild bird migration.

**Keywords:** zoonotic H7N9 avian influenza viruses, novel H7N3 reassortant, adaptation to ducks

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 10669

## IN VITRO GROWTH KINETICS TRAITS THAT DIFFER BETWEEN HUMAN AND AVIAN INFLUENZA STRAINS

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### Introduction and Objectives

We aim to identify predictors for human adaptation which are quantitatively comparable between strains and can be measured using high-throughput assays, for use in routine surveillance for pandemic risk assessment.

### Methods

We focused on the in vitro equivalent of three epidemiological parameters: the basic reproduction number, the mean generation time, and the initial growth rate. Using a mechanistic model, previously published data from growth kinetics experiments in human lung cells, and newly generated data, we compared estimates of these parameters for six influenza A strains.

### Results

Using previously published data, we found that the two human-adapted strains (pre-2009 seasonal H1N1, and pandemic H1N1) had a lower basic reproduction number, shorter mean generation time and slower growth rate than the two avian-adapted strains (H5N1 and H7N9). These same differences were then observed in data from new experiments where two strains were engineered to have different internal proteins (pandemic H1N1 and H5N1), but the same surface proteins (PR8), confirming our initial findings and implying that differences between strains were driven by internal genes. Also, the model predicted that the human-adapted strains underwent more replication cycles than the avian-adapted strains by the time of peak viral load, potentially accumulating mutations more quickly.

### Conclusion

The in vitro reproduction number, generation time and growth rate differ between human-adapted and avian-adapted influenza strains, and thus could potentially be used to assess host adaptation of internal proteins to inform pandemic risk assessment.

*Keywords: mathematical model; generation time; viral kinetics; host shift*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses

Abstract No: 10681

## COMPARISON THE PATHOGENICITY OF RECENT H5N6 AND H5N8 VIRUSES ISOLATED DURING THE WINTER SEASON 2016-2017 IN SOUTH KOREA

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### 1. Introduction

Highly pathogenic avian influenza (HPAI) H5 viruses have been continuously isolated from wild and domestic birds since the first detection in 1996 in Southeast Asia. HPAI H5Nx viruses can cause high mortality in poultry resulting in serious economic losses for the industry. Recently the two different H5N6 and H5N8 viruses were isolated in South Korea and the infections resulted in the culling of more than 37 million during the 2016/17 winter season.

### 2. Method

To compare the pathogenic potentials of A/Environment/Korea/W541/2016(H5N6) and A/Common Teal/Korea/W555/2017(H5N8), we infected the virus into chicken, ducks, mouse, and ferrets, and compared their growth kinetic mortality and transmission ability with those of previous HPAI H5N1 viruses. The virus titers were determined as log<sub>10</sub>EID<sub>50</sub> or as log<sub>10</sub>TCID<sub>50</sub>. Further, immunohistochemistry staining, and receptor binding assay were also evaluated.

### 3. Result

Animal experiments revealed that Em/W541(H5N6) was found to be highly pathogenic in both chickens and ducks, while CT/W555(H5N8) caused lethal infections in chickens but did not induce remarkable clinical illness in ducks. In mice, both viruses appeared to be moderately pathogenic and displayed limited tissue tropism relative to HPAI H5N1 viruses. Em/W541(H5N6) replicated to moderate levels in the upper respiratory tract of ferrets and was detected in the lungs, brain, spleen, liver, and colon. Although no transmission was detected in CT/W555(H5N8) infected ferrets, two of three ferrets in direct contact with Em/W541(H5N6)-infected animals shed virus and seroconverted at 14 dpi.

### 4. Conclusion

Although both Em/W541(H5N6) and CT/W555(H5N8) were highly pathogenic in chickens but they showed different pathogenic features in ducks. Further, Em/W541(H5N6) virus showed more severe virulence and transmission features in ferrets compared than CT/W555(H5N8) virus. Given the co-circulation of different, phenotypically distinct, subtypes of HPAI H5Nx viruses, detailed virologic investigations are imperative given the capacity of these viruses to evolve and cause human infections.

*Keywords: HPAI; H5N6; H5N8; reassortment; animal model*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 10717

## Pathogenesis of Influenza C virus in Mice and Ferrets

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Influenza C virus is known to infect human and swine and causes mild cold-like symptom only in pediatric patients infected by upper respiratory tracts. In this study, we tried to genetically detect influenza C virus in influenza-like illness patients and finally isolated the virus in eggs. The virus was challenged to mice and ferrets for pathogenesis study.

The 5,533 upper respiratory specimens of influenza-like illness patients through Korea Influenza and Respiratory Viruses Surveillance System (KINRESS) were genetically screened influenza C virus with real-time RT-PCR targeted HEF gene. Influenza C positive specimens were inoculated in amniotic cavity of embryonated eggs to isolate the virus. For pathogenesis study of influenza C virus, mice and ferrets were challenged intranasally and monitored lethality, body weight and especially body temperature and clinical symptoms in ferrets.

The 76 influenza C viruses were detected mostly in 0 to 6 years of age. Only one influenza C virus was isolated in eggs and it was identified Sao Paulo/82 lineage by HEF gene analysis. None of mice showed mortality, emaciation and ruffled fur. However, the virus was genetically detected in lungs of mice till 5 days of infection. Mild pneumonia was detected only in local alveoli and bronchiole after 3 and 5 days of infection. Influenza C virus infected ferrets did not showed any respiratory symptom without mild fever.

This is the first pathogenesis study of influenza C virus in mammalian models and the study finally help to understand characterization of influenza C virus.

This study was supported by intramural funds (4800-4845-300) of Korea Centers for Disease Control and Prevention.

*Keywords: Influenza C virus, Pathogenesis, Mice and ferrets*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 10731

## RISK ASSESSMENT OF THE TROPISM AND PATHOGENESIS OF CANINE INFLUENZA A/H3N2 VIRUSES IN THE HUMAN RESPIRATORY TRACT

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### Introduction and Objectives

After being isolated in 2007, canine influenza A/H3N2 virus (H3N2 CIV) has quickly expanded its geographical distribution causing substantial outbreaks among dogs. Various natural reassortants of H3N2 CIV with human, avian and swine influenza A viruses (IAVs) have also been identified. With the close proximity between humans and dogs, there is a potential risk of zoonotic transmission. In this study, we aim to risk assess the tropism and pathogenesis of H3N2 CIV and its reassortant in humans using ex-vivo explants and in-vitro cultures of human respiratory tract.

### Methods

Ex-vivo explant cultures of human bronchus and lung and in-vitro cultures of human type-I-like alveolar epithelial cells (AECs) and monocyte-derived macrophages (M $\phi$ ) were used to study the replication kinetics and innate host response of H3N2 CIVs and the reassortant between H3N2 CIV and UW-PR8 (H1N1) virus in comparison to human seasonal H3N2 virus.

### Results

There was limited viral replication of the canine influenza viruses in the human ex-vivo bronchus and lung explants, AECs and M $\phi$ . The replication of the reassortant virus was significantly higher than all other canine and human H3N2 viruses in human lung explants at 24 hpi. Although the canine influenza viruses were low cytokine and chemokine inducers, one of them induced significantly higher mRNA levels of IFN $\beta$ , CXCL10 and CCL5 than human seasonal H3N2 virus in M $\phi$ .

### Conclusion

Consistent with the absence of human cases, our results showed that the canine influenza viruses tested have low preference for replication in the human respiratory tract. However, continuous surveillance and risk assessment is required in the context of the continuous reassortment between H3N2 CIVs and other IAVs which poses a risk of a highly pathogenic reassortant with pandemic potential.

*Keywords: canine H3N2; tropism; risk assessment; ex-vivo; human respiratory tract*



Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 10781

## CANINE INFLUENZA VIRUS A(H3N2) CLADE WITH ANTIGENIC VARIATION, CHINA, 2016-2018.

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Avian-origin H3N2 canine influenza virus (CIV) emerged in Asia around 2005 and is now epidemic in dogs in China, South Korea and USA. It is noteworthy that CIVs do not only cause respiratory disease in dogs, but also provide the opportunities for interspecies transmission. To investigate the evolution of H3N2 CIV among dogs in China, we have collected 1058 throat swabs from dogs with respiratory symptoms since 2016, and isolated 77 H3N2 strains (7.28%). The full genomes of these isolates were sequenced. Phylogenetic analysis indicated that each genome segment of the H3N2 isolates after 2016 in China formed a separate clade, distinct from previous isolates from China, but grouped with isolates of South Korea and the United States. This clade had a new antigenicity different from previous isolates from China and formed a new antigenic group. Moreover, this new clade also possessed 4 amino acid substitutions 251R and 590S in polymerase basic 2 and 146S and 242I in hemagglutinin, which have frequently been identified in human influenza viruses. Of note, 251R and 590S in polymerase basic 2 are known determinants of adaptation to growth in mammals. Our results suggested that a new genetically and antigenically distinct CIV H3N2 clade possessing mutations associated with mammalian adaptation emerged in 2016 and replaced previously circulating strains in China. This clade probably poses a risk for zoonotic infection.

*Keywords: H3N2 canine influenza virus; surveillance; evolution; antigenicity; mammalian adaptations*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 10878

## GENOMIC SURVEILLANCE OF AVIAN INFLUENZA VIRUSES IN WETLANDS SEDIMENT FROM WILD BIRD HABITATS

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**Introduction and Objectives:** Avian influenza viruses (AIVs) cause severe disease in poultry and dangerous zoonotic infections in humans. Outbreaks disrupt trade, threaten food security, jeopardize public health, and encourage the adaptation of novel viruses to human hosts. Consequently, surveillance of AIVs in wild waterfowl, their natural animal reservoir, is a cornerstone of pandemic preparedness and disease prevention in livestock and humans.

Current surveillance efforts focus on testing individual birds, but are limited in their capacity to collect large, representative samples of the wild waterfowl community. We are developing a complementary community-level surveillance strategy: targeted genomic sequencing of environmental specimens from waterfowl habitats, focusing on wetlands sediment where virus-laden waterfowl feces accumulates.

**Methods:** Sediment was collected from 6 wetlands near Vancouver, Canada between September and December 2016. Total RNA was extracted from these sediments and prepared into metagenomic sequencing libraries. Hemagglutinin, neuraminidase, and matrix segments of the influenza A virus genome were enriched using 600 custom hybridization probes, then sequenced on an Illumina MiSeq. qPCR was used to estimate the fold-enrichment provided by the probes. Consensus sequences of hemagglutinin and neuraminidase genome segments were generated by mapping sequencing reads to subtype-specific references using the Burrow-Wheeler Aligner. Reference sequences were selected by querying the reads from each library against a BLAST database containing 8,555 AIV sequences.

**Results:** Influenza genome abundance in the samples increased 19 million-fold following two sequential captures with our custom hybridization probe enrichment method. Seven nearly-complete haemagglutinin and neuraminidase sequences (over 75% coverage) were recovered from 4 different wetland locations. These sequences allowed subtyping and phylogenetic characterization, revealing the presence of H3, H6, H11, N2, N8, and N9 subtypes in wetlands surrounding Vancouver, Canada during fall/winter of 2016.

**Conclusion:** Targeted genomic sequencing of wetlands sediment has demonstrated the potential to be a viable complement to current AIV surveillance practices.

*Keywords: avian influenza; environmental surveillance; genomics; targeted sequencing*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 10949

## MONITORING CHANGES IN AVIAN INFLUENZA: RELEVANCE FOR HUMAN HEALTH

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### Introduction and objectives

The continued evolution and diversification of goose/Guangdong lineage H5 viruses threatens global poultry health. Changes in epidemiology and evolution of the virus potentially impact the risk for zoonotic transmission. In this study we present approaches used across Europe to rapidly track new virus incursion and evolution with analysis to underpin and support risk assessments in relation to veterinary public health. In the period between 2014 and 2019 there were several waves of H5 highly pathogenic avian influenza (HPAI) virus in Europe which presented with different epidemiological characteristics and threat.

### Methods

Epidemiological data was captured for all H5 HPAI events in both poultry and wild birds across the EU. Data was analysed using a number of algorithms to present the time courses/demographics for virus spread but with underlying viral genetic characteristics using gene sequencing methodology. Genetic analysis included examination for markers known to confer increased threat of zoonotic transmission.

### Results

All incursions in the period under review involved clade 2.3.4.4 H5 HPAI. Minor incursion events occurred in 2014/15, 2017/18 and 2018/19. In 2016/17 the largest epizootic with HPAI ever recorded in Europe occurred with in excess of 1100 poultry outbreaks and 1500 wild bird detections. Virus subtypes were predominantly H5N8 and H5N6 and did not carry any specific additional genetic motifs that had been associated with increased transmissibility for humans. Data arising from these studies was used to inform risk assessments and managing occupational health risk in dealing with these events.

### Conclusion

Timely tracking and detailed analysis of H5 HPAI viruses in avian populations provides a valuable early warning system for veterinary public health. This analyses of viruses and related events can directly inform veterinary and public health risk assessments. With anticipated continued virus evolution and transcontinental spread such rapid approaches are vital for protecting veterinary public health.

*Keywords: H5; avian; evolution; risk assessment*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 10953

## VIS410, A BROAD-SPECTRUM, ANTI-INFLUENZA A MONOCLONAL ANTIBODY NEUTRALIZES BALOXAVIR-RESISTANT INFLUENZA A VIRUS *IN VITRO*

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**Introduction:** Current antiviral therapies for influenza include the neuraminidase inhibitors (NAIs) and, since 2018, baloxavir marboxil, which targets the PA subunit of the viral RNA polymerase. NAI resistant viruses have circulated in the last decade, and viruses resistant to baloxavir has been isolated from baloxavir-treated patients. Thus, there is a need for antivirals that target diverse influenza virus proteins and possess different mechanisms of action. VIS410 is a hemagglutinin (HA) stem binding, broadly active anti-influenza A monoclonal antibody (mAb) currently in clinical development for treatment of patients hospitalized with influenza A. VIS410 was previously shown to be synergistic with baloxavir *in vitro*, and effective against oseltamivir-resistant influenza A strains both *in vitro* and *in vivo*. Here, we assessed the antiviral activity of VIS410 against baloxavir-resistant influenza virus *in vitro*.

**Methods:** Reverse-genetically engineered wild-type (WT) influenza A/PR/8/1934 (H1N1) virus and a baloxavir-resistant counterpart carrying the PA I38T substitution were generated. Virus stocks were sequence-confirmed and tested for susceptibility to baloxavir and VIS410 using microneutralization assay in MDCK cells. Data were normalized to infected virus controls.

**Results:** The baloxavir-resistant PA I38T mutant exhibited ~100-fold lower susceptibility to baloxavir as compared to the WT virus. The observed EC<sub>50</sub> for baloxavir was consistent with other studies (WT EC<sub>50</sub> ~1 nM and PA I38T virus ~130 nM). In contrast, both WT and PA I38T viruses showed comparable susceptibility to VIS410 (with EC<sub>50</sub> ~1.1-2.2 µg/mL). Notably, in previous studies VIS410 was found to both neutralize A/PR/8/1934 (H1N1) virus and protect against lethal challenge, indicating that *in vitro* results may predict *in vivo* efficacy.

**Conclusions:** VIS410 demonstrated antiviral activity against baloxavir-resistant influenza A virus *in vitro*. These data support the clinical development of VIS410 that is active against influenza viruses resistant to currently licensed antiviral therapies.

**Keywords:** Monoclonal Antibody; Antiviral; PA Mutant

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 11059

## AN INFLUENZA A/H5N1 REASSORTANT ISOLATED FROM APPARENTLY HEALTHY CHICKEN AT A LIVE-POULTRY MARKET IN EAST JAVA, INDONESIA

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### Introduction and Objectives

Avian influenza A/H5N1 virus has become endemic in Indonesia since 2003. We have conducted surveillances to assess prevalence of avian A/H5N1 viruses in sick poultry at live-poultry markets. The present study was aimed to know the circulation of A/H5N1 viruses in apparently healthy chickens in the markets.

### Methods

Totally 133 cloacal swabs were collected from apparently healthy chickens during January and February in 2016 - 2019. After inoculated into specific antibody negative embryonated chicken eggs for virus isolation, hemagglutination (HA) test was performed. The virus isolates were then tested by RT-PCR for influenza type A and subtypes. The positive isolates were subjected to whole genome sequencing with a next generation sequencer, MiSeq, to analyse the 8 segment genes.

### Results

Nine egg harvests tested positive for HA activity, and all were confirmed as influenza type A virus by RT-PCR. Seven isolates were identified as A/H9N2, and 1 isolate as A/H5N1. Phylogenetic analyses of the H5N1 isolate revealed that the HA gene belonged to clade 2.3.2.1c Eurasian lineage. In BLAST analysis, the M gene was most closely related to our 2014-isolate of A/H5N1 clade 2.1.3.2b Indonesian lineage from sick chicken, while the PB2 gene to our 2018-isolate of H3N6 from asymptomatic duck; the remaining 6 genes were most closely related to our 2013-isolate of clade 2.3.2.1c Eurasian lineage from sick turkey. These indicated that the virus was a reassortant of subtype H5N1, in genesis of which three parental viruses, H5N1 Eurasian and Indonesian lineages and H3N6, were involved.

### Conclusion

Currently prevalent H5N1 virus in sick chickens at East Java was not isolated from apparently healthy chickens, and this indicated that the virus does not cause asymptomatic infection in chicken. Instead, an H5N1 reassortant was isolated; which inherited PB2 gene from avirulent H3N6 virus and possibly attenuated in chicken.

*Keywords: avian influenza A/H5N1 virus; reassortant; asymptomatic chicken; avirulent H3N6*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 11060

## THE GROWTH EVALUATION OF TRIVALENT OR QUADRIVALENT LIVE ATTENUATED INFLUENZA VACCINE *IN VITRO*

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### Introduction and Objectives

The method of real-time PCR (RT-PCR) is widely applied in detecting influenza viruses. The seasonal live attenuated influenza vaccines (LAIVs) include three viruses strains - A(H1N1), A(H3N2) and influenza B virus based on cold-adapted master donor. We developed virus titration method based on Taqman RT-PCR technology for polyvalent LAIVs.

### Methods

Reassortant vaccine strains recommended by the WHO for use in the 2015-2016 and 2017-2018 epidemic seasons were used as the standard samples to establish the RT-PCR assay. Growth characteristics of commercial lots of the trivalent LAIV were determined in MDCK cells using RT-PCR assay or ELISA. Supernatants were collected after 20 hours for the RT-PCR assay. Fixed monolayer was treated with mouse monoclonal antibodies to hemagglutinin of the H1, H3 and B influenza viruses at a concentration of 0.1 µg/ml for ELISA test.

### Results

Trivalent LAIVs virus RT-PCR titers after 20 hours of incubation in MDCK cells were comparable to those obtained by the traditional EID<sub>50</sub> assay. The ELISA titers were reduced by 2.0-2.4 log<sub>10</sub> times compared to RT-PCR titers. The reproduction of influenza viruses as part of the trivalent LAIV was reduced by 1.3-2.0 log<sub>10</sub> according to both tests. As part of the trivaccine, B/60/Phuket/2013/26 (Yamagata) reproduced better than as monovaccine. In regard to B/60/Brisbane/08/83 (Victoria) this effect has not been obtained. No suppression of B/Victoria and B/Yamagata viruses was observed under conditions of equality of infecting doses.

### Conclusion

The method based on Taqman RT-PCR technology is applicable to estimate the growth characteristics of trivalent LAIVs virus titers. RT-PCR assay is more sensitive in comparison with virus detection by ELISA. No inhibition of influenza B/Yamagata virus in the trivaccine is consistent with the previously obtained data, when in vitro experiments had shown that influenza B strains in heterogeneous mixtures were stronger competitors than influenza A strains.

*Keywords: live attenuated influenza vaccine; real-time PCR; ELISA*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 11071

## ISOLATION AND IDENTIFICATION OF AVIAN INFLUENZA VIRUSES FROM ASYMPTOMATIC DUCKS IN EAST JAVA, INDONESIA DURING 2016 – 2019

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### Introduction and Objectives

Avian influenza viruses occasionally infect human, raising concerns of co-infection with seasonal human influenza viruses and emergence of a novel virus with pandemic potential. Live-poultry markets have been shown to be significant locations for avian-to-human transmission. This study was aimed to assess infection of avian influenza viruses in ducks in Live-poultry markets.

### Methods

We collected 911 cloacae swabs from apparently healthy ducks at live-poultry markets in East Java during 2016, 2017, 2018 and 2019. All samples were inoculated into chicken eggs for virus isolation. The isolated viruses were examined for influenza virus type A and subtypes H3N6, H4N6 and H5N1 by RT-PCR. The subtype-undetermined isolates were subjected to whole genome sequencing with a next generation sequencer, MiSeq, to identify subtypes.

### Results

Egg harvests tested positive for hemagglutination were 112 (12%) and 92 (10%) of them were identified as influenza type A. Among the 92 type A isolates, 41 (4.5%) appeared as H3N6 subtype, 17 (1.9%) as H4N6, and 13 (1.5%) as H5N1. Furthermore, we identified by MiSeq H2N1, H2N2, H3N2, H4N2, H5N2, and H6N8 subtypes for the remaining 20 isolates. Phylogenetic analysis indicated that majority of these isolates were reassortant viruses and the source of genes for internal proteins was most frequently from H4N6 virus.

### Conclusion

We have previously isolated H5N1, H3N6, and H4N6 subtypes in sick ducks. In the present study, we additionally isolated H2N1, H2N2, H3N2, H4N2, H5N2, and H6N8 from asymptomatic ducks for the first time in Indonesia. It was revealed that 12% of apparently healthy ducks in the live-poultry market were infected with avian type A influenza viruses, mainly H3N6 and H4N6. Phylogenetic analysis revealed that inter- or intra-subtype reassortment frequently occurred among the East Java. This could be a potential risk for emerging of a new virulent virus.

*Keywords: asymptomatic ducks; live-poultry market; inter- or intra-subtype reassortment; avian influenza viruses*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses

Abstract No: 11078

## AVIAN INFLUENZA A/H9N2 REASSORTANT ISOLATED FROM SICK CHICKENS AT LIVE-POULTRY MARKETS IN EAST JAVA, INDONESIA

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### Introduction and Objectives

Avian influenza A/H9N2 virus has become one of the dominant subtypes of avian influenza viruses in domestic poultry and wild birds around the world. In Indonesia, there were A/H9N2 outbreaks in chicken causing dropped egg production in early 2017. Several cases of human infections from avian were also reported. The purpose of this study was to assess infection of A/H9N2 viruses in chickens at live-poultry markets, where known to be important place for avian-human transmission.

### Methods

We collected 649 cloacal swabs from 133 apparently healthy and 516 sick chickens at live-poultry markets during January and February from 2016 to 2019. For virus isolation, all samples were inoculated into chicken eggs followed by examination for hemagglutination activity. The isolated viruses were examined by RT-PCR for influenza virus type A and subtypes. The positive isolates were subjected to whole-genome sequencing with next-generation sequencer, MiSeq.

### Results

Egg harvests tested positive for hemagglutination activity were 140, and 35 were confirmed as influenza type A virus by RT-PCR. Seven isolates were identified as A/H9N2 (4 from healthy and 3 from sick chickens), and 26 isolates as A/H5N1 (1 from healthy and 25 from sick chickens). Whole-genome sequence was determined for 4 H9N2 isolates in 2018, 2 from healthy and 2 from sick chickens. BLAST and phylogenetic analyses revealed that all genes of the 4 isolates were derived from H9N2 viruses that were circulating in Vietnam in 2014 except PB2 gene of 2 isolates from sick chicken, which is most closely related to an A/H5N1 reassortant isolated at West Java in 2016 by other group.

### Conclusion

In our surveillance, 3% of apparently healthy chickens brought in live-poultry markets were infected with A/H9N2 virus. We isolated H9N2 reassortants from sick chickens, which inherited PB2 gene from A/H5N1 reassortant virus and possibly acquired enhanced virulency.

*Keywords: avian influenza H9N2; reassortant; chicken; enhanced virulency*



Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 11121

## Can we predict the severity of inflammatory disease by newly emerging influenza viruses encoding the PB1-F2 virulence protein?

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**Introduction & Objectives:** Avian H7N9 and H9N2 influenza A viruses (IAV) sporadically infect humans and have vastly different impacts on disease severity. Of the 41 confirmed H9N2 cases over the last 20 years, the majority experienced mild disease. Conversely, since its emergence in 2013, the H7N9 IAV has infected 1558 people and caused a 36% case fatality rate. The H7N9 IAV was identified as a reassortant virus, where six genes including the PB1 gene segment, which encodes PB1-F2, were provided by an H9N2 virus circulating in chickens. All 20<sup>th</sup> century human pandemic IAVs contained a PB1 gene derived directly from the avian IAV reservoir and we have shown these PB1-F2s can potently induce inflammation. We have linked PB1-F2 induction of the nod-like receptor 3 (NLRP3)-inflammasome pathway during IAV infection, which contributes to disease severity. However, as the pandemic viruses became endemic in swine and man, the gene encoding PB1-F2 mutated and many isolates encoded truncated PB1-F2. It is currently unknown what impact PB1-F2 has on illness in humans infected with newly emerging avian IAVs.

**Methods & Results:** We have performed bioinformatics analyses on the available sequences of PB1-F2 of avian and human H7N9 and H9N2 IAV isolates. This revealed the majority of avian and human H7N9 viruses contain the predicted inflammatory markers. In contrast, the avian H9N2 PB1-F2s had greater sequence variability and the 2013 isolate, which likely donated the PB1 gene segment to form the H7N9 IAV reassortant, contained all 4 inflammatory markers. Using our peptide challenge models, we found that the 2013 H9N2 PB1-F2 potently induces the NLRP3-inflammasome complex, while the 2009 H9N2 peptide could not.

**Conclusion:** The H9N2 PB1 gene segment that reassorted to form the H7N9 virus likely encoded an inflammatory PB1-F2 and may account for the differences in disease severity between the two virus subtypes.

*Keywords: PB1-F2, H7N9, H9N2, inflammasome*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 11199

## ISOLATION OF AVIAN INFLUENZA A(H4N6) VIRUSES FROM DUCKS AT A LIVE-POULTRY MARKET IN EAST JAVA, INDONESIA

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### Introduction and Objectives

Avian influenza viruses occasionally infect human, indicating the possibility of co-infection of avian and seasonal influenza viruses in human and emergence of a novel virus with pandemic potential. Recently we isolated type A influenza viruses possessing non-H5 non-H3 HA from apparently sick ducks. The aim of this study is to identify the subtype of the viruses by whole genome sequencing and to assess the prevalence in live-poultry markets in Indonesia.

### Methods

We collected 1.337 cloacae swabs from sick poultry at a live-poultry market in East Java from 2013 to 2019. All samples were inoculated into chicken eggs for virus isolation. The isolated viruses were subjected to RT-PCR to identify influenza virus type A and subtypes H5N1 and H3N6. The non-H5 non-H3 isolates were then subjected to whole genome sequencing with a next generation sequencer, MiSeq.

### Results

Egg harvests tested positive for hemagglutination activity were 383 and 130 were identified as influenza type A. Among them, 114 appeared as H5N1 and 8 as H3N6 subtype. There were 8 type A isolates that possessed non-H5 non-H3 HA gene from ducks. The whole genome sequencing revealed that these 8 isolate belonged to H4N6 subtype and 4 of them were reassortants having several genes derived from H3N6. We also found 4 cases of double infections of H4N6 and H5N1 viruses. The HA nucleotide sequences were most closely related to A/duck/Hunan/S2046/2011 (H4N2) (identity: 97%) and the NA to A/pochard/Buryatiya/1941/2000 (H4N6) (identity: 91%) by BLAST searches.

### Conclusion

Eight of our isolates from ducks were found to be the subtype H4N6. This is the first report of avian A(H4N6) virus isolation in Indonesia. Phylogenetic analysis indicated that our A(H4N6) viruses acquired HA and NA genes from different groups of viruses and the half of the isolates were reassortants with H3N6 virus.

*Keywords: avian influenza A(H4N6) virus; whole genome sequence; reassortant; live-poultry market*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 11288

## PRESENCE OF INFLUENZA A H9N2 VIRUSES AMONG POULTRY IN GHANA

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### Introduction

Avian influenza viruses such as highly pathogenic avian influenza A H5N1 (HPAIV H5N1) and low pathogenic H9N2 viruses pose a threat to both animals and humans. Ghana recorded its first cases of HPAIV H5N1 viruses among poultry in 2007. This outbreak was contained by the Veterinary Services Directorate of Ghana through various control measures such as culling of poultry. Since 2015, further outbreaks of HPAIV H5N1 have continued to occur and the disease appears endemic among poultry in the country. As a result of the outbreak, surveillance programs such as the collaboration between the Ghana Armed Forces (GAF), U. S. Naval Medical Research Unit #No.3 and the National Influenza Center was established.

### Methods

Screening of backyard animals in military barracks in Ghana is conducted at least once a year to identify circulating avian influenza viruses. Tracheal and cloacal swabs were collected from chickens, ducks and pigs throughout GAF installations nationwide. The presence of influenza viruses was detected using molecular methods and whole genome sequences were obtained by next generation sequencing. Sequences were aligned and phylogenetic analyses performed to characterize the influenza viruses identified.

### Results/Conclusion

We confirm that influenza A H9N2 viruses continue to prevail among poultry in Ghana. Phylogenetic analysis showed that these H9N2 viruses from Ghana clustered closely with viruses from Burkina Faso, which were further related to viruses from Morocco. Our finding confirmed reports of importation of H9N2 viruses from North Africa into West Africa. Co-circulation of low pathogenic H9N2 with HPAIV H5N1 viruses among poultry in Ghana represents a significant food security and public health threat. Surveillance programs and education on biosecurity and biocontainment measures should be prioritized to prevent catastrophic outcomes such as massive poultry deaths and fatal human infections.

*Keywords: avian influenza; H9N2; Ghana*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 11709

## Recombinant hemagglutinin produced from Chinese hamster ovary (CHO) stable cell clones and a PELC/CpG combination adjuvant for H7N9 subunit vaccine development

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The novel H7N9 avian influenza A virus has caused human infections in China since 2013; some isolates from the fifth wave of infections have emerged as highly pathogenic avian influenza viruses. Recombinant hemagglutinin proteins of H7N9 viruses can be rapidly and efficiently produced with low-level biocontainment facilities. In this study, recombinant H7 antigen was obtained from engineered stable clones of Chinese hamster ovary (CHO) cells for subsequent large-scale production. The stable CHO cell clones were also adapted to grow in serum-free suspension cultures. To improve the immunogenicity of the recombinant H7 antigens, we evaluated the use of a novel combination adjuvant of PELC and CpG (PELC/CpG) to augment the anti-H7N9 immune responses in mice. We compared the effects with other adjuvants such as alum, AddaVax (MF59-like), and several Toll-like receptor ligands such as R848, CpG, and poly (I:C). With the PELC/CpG combination adjuvant, CHO cell-expressed rH7 antigens containing terminally sialylated complex type N-glycans were able to induce high titers of neutralizing antibodies in sera and conferred protection following live virus challenges. These data indicate that the CHO cell-expressed recombinant H7 antigens and a PELC/CpG combination adjuvant can be used for H7N9 subunit vaccine development.

*Keywords: CHO cells, hemagglutinin, H7N9 vaccine, PELC/CpG adjuvant*

Topic: Virology and Pathogenesis: Emerging Influenza Viruses  
Abstract No: 11710

## DEVELOPMENT OF A SUSPENDED MDCK CELL LINE FOR INFLUENZA VACCINE PRODUCTION

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### Introduction and Objectives

Madine Darby Canine Kidney (MDCK) cells are currently considered for influenza vaccine manufacturing. The disadvantage of these cells is their anchorage dependent growth, which greatly complicates the processing steps at industrial scale. In this study, we decide to develop a suspended cell line for flu vaccine production.

### Methods

We adapted an adherent MDCK cell line (ATCC, CCL-34) to grow in suspension (sMDCK) in serum free medium by two-step process and evaluated the cell growth stability, flu virus productivity, tumorigenicity and scalability in 5L and 50L pilot-scale production.

### Results

The cell growth rate and achieved cell concentration of sMDCK cells were comparable with the adherent MDCK cells in previous study. The cells infected with the H7N9 virus (NIBRG-268, derived from A/Anhui/1/2013), H5N1 virus (NIBRG-14, derived from A/Vietnam/1194/2004) and B strains (B/Brisbane/9/2014 and B/ Brisbane/63/2014) resulted in high virus titer of 3.00, 3.00, 3.03 and 3.14 log HA units/50 $\mu$ l in 125mL spinner flask, respectively. 5L and 50L bioreactor productions were further evaluated, the  $2 \times 10^6$  cells/ml were obtained in both 5L and 50L bioreactors. The virus productivities were similar from 125mL spinner flask and 5L bioreactor, but for the 50L bioreactor, the peak HA titer of H7N9 showed lower than 5L bioreactor (2.47 HA units/50 $\mu$ l and 3.1 HA units/50 $\mu$ l, respectively), the operation parameters or accessories needed to further improved. The tumorigenicity test for sMDCK cells were performed following FDA guideline, and the result was negative.

### Conclusion

In conclusion, a new sMDCK cell line was generated which remain the similar growth rate compared with adherent MDCK cells. No tumorigenicity was found, and higher productivity of influenza virus was shown, this sMDCK represents a new cell substrate candidate for influenza vaccine production.

*Keywords: MDCK, flu, suspension, vaccine*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 10327

## Study of host lipid rafts in Influenza A Virus (IAV) host binding and endocytosis with subsequent identification of Hemagglutinin interacting host raft proteins.

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Binding of the Influenza A Virus (IAV) to the host cell followed by entry inside host cell are rather complex events. The binding of IAV to the host cell requires multiple simultaneous interactions between viral hemagglutinin (HA) and its host receptor sialic acid (SIA). However, the exact mechanism to form multiple HA-SIA interactions is poorly understood. Similarly, IAV enters the host cell via multiple endocytic routes making the process even more complicated. Also, a cellular endocytic pathway regulated by lipid rafts termed 'raft-dependent endocytosis', has so far been poorly investigated for IAV host cell entry. In this study, we observed the co-localization of IAV with the host lipid raft (GM1). Subsequently, host rafts disruption using methyl-beta-cyclodextrin showed significant reduction in the ability of IAV to bind the host cells. Interestingly, cyclodextrin mediated inhibition of raft-dependent endocytosis also showed significantly reduced IAV host internalization. However, exposure of cells to cyclodextrin, two hours post-IAV binding showed no such reduction. In summary, our data collectively demonstrates that host lipid rafts are selected by IAV as a host attachment factor for multivalent binding and IAV utilizes these micro-domains to exploit raft-dependent endocytosis for host internalization, a virus entry route previously unknown for IAV. Since IAV hemagglutinin (HA) is known to regulate two crucial events in IAV life cycle, receptor binding followed by endocytosis and membrane fusion; we further attempted to identify possible raft protein/s interacting with IAV HA. During our preliminary investigation, we have identified few raft proteins as potential interactors of IAV HA. Currently, we are further validating their interactions and functional roles in IAV life cycle. Through this study, we aim to enhance the way we understand IAV host binding, entry via endocytosis and membrane fusion inside the host.

*Keywords: Lipid rafts, raft-dependent endocytosis, MBCD, GM1*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 10358

## ANALYSES OF RECOMBINANT INFLUENZA VIRUSES REVEAL DISTINCT IMMUNE ANTAGONISTIC PROFILES INDUCED BY HUMAN VERSUS AVIAN NS1 PROTEINS IN PRIMARY HUMAN SYSTEMS

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### Introduction and Objectives

The influenza A virus (IAV) non-structural protein 1 (NS1) contributes to disease pathogenesis through the inhibition of host innate immune response. Dendritic cells (DCs) release interferons (IFN), pro-inflammatory cytokines and promote the adaptive immunity upon viral infection. We used recombinant IAV expressing NS1 proteins from human or avian origin to characterize the strain-specific effects in human DC. Also, we analyzed the effect that these different NS1 proteins could have during the infection of Normal Human Bronchial Epithelial cells (NHBEs) as a surrogate system of the human upper respiratory epithelium. Finally, we study the intercellular localization kinetics of these NS1 proteins in infected DCs.

### Methods

Recombinant IAV were generated by reverse-genetics techniques being rescued in the A/Puerto Rico/08/1934 background and expressing different NS1 proteins. DCs were differentiated from blood and NHBEs were cultured until a pseudostratified epithelium was developed. DCs and HNBEs were infected and gene expression, cytokine release and replication kinetics were measured by RTqPCR, Multiplex ELISA and plaque assay respectively. Additionally, we analyze the localization dynamics of these different NS1 proteins during DCs infection using Imagine Flow Cytometry.

### Results and Conclusion

IAV bearing human NS1 proteins induced higher levels of expression of IFNs than IAV with avian NS1 proteins in DCs and NHBEs. This pattern of IFNs responses was not due to differences in NS1 expression levels or virus replication (vRNA). Also, no significant differences were found in the expression of pro-inflammatory cytokines. Using Imaging Flow Cytometry, we found that NS1 from human have faster kinetics of nuclear export than avian NS1 in infected DCs, which could account for the distinct pattern of the IFN responses observed. Our data suggest that human and avian NS1 segregate based on their subcellular trafficking feature that might be responsible for differences in innate immune responses of DCs to IAV.

*Keywords: NS1 protein; immune response; interferon; dendritic cells; normal human bronchial epithelial cells*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 10458

## Influenza NS1 protein epigenetically upregulates microRNA-146a to suppress antiviral responses and promotes viral infection

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### Introduction:

Interaction between viruses and miRNAs is defined by a plethora of complex mechanisms, though not yet fully understood. Expression of miRNAs can be involved in antiviral responses, and/or promoting infection through complex regulatory pathways. miR-146a-5p, a host miRNAs that primarily involved in the regulation of IFN, NF-κB, and other antiviral pathways, was described as a pro-viral factor of viral infection for many viruses, including influenza viruses.

### Method and results:

We found that miR146a-5p promotes influenza virus via modulation of the IFN/STAT1 signaling pathway: depletion of miR-146a-5p by inhibitors or CRISPR-knockout attenuated viral replication in cells while introducing miR146a-5p mimic in mouse respiratory organs alleviated pathogenic outcomes of the HAPI viruses-infected animals. Transcriptome analysis of influenza virus-infected lung epithelial cells identified that miR146a-5p is one of the most upregulated miRNAs upon infection, and most significantly, such induction was observed only in the presence of the viral non-structural protein 1 (NS1) in the nucleus of infected cells. Notably, ectopic expression of nuclear NS1 alone, but not the NS1 mutant that exclusively expressed in the cytoplasm, was enough to activate mature and premature miR146a-5p expression in cells. Mechanistically, we found that F103/M106 residues of the NS1 protein are important for the induction of miR-146a-5p, in addition to functionally interact with cellular CSFP30. Further analysis revealed that the enhancement of miR-146a-5p expression by NS1 is contributed through its interaction with an epigenetic regulator, Bromodomain protein 4 (BRD4). Surprisingly, treatment of JQ1, a BRD4 inhibitor, significantly enhances miRNA-146a-5p expression, only in the presence of NS1, suggesting a novel function of BRD4 on modulating transcriptional activity during influenza virus replication.

### Conclusion:

Our study reveals an unrecognized function of NS1 to regulate host immune response by epigenetically upregulation of specific host miRNAs for viral replication and provides targets for the development of miRNA-based anti-influenza drugs.

*Keywords: Nonstructural protein 1 (NS1), miR-146a, epigenetic, immune response, viral replication*



Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 10474

## NOVEL ROLE FOR MIR-1290 IN HOST SPECIES SPECIFICITY OF INFLUENZA A VIRUS

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To better understand how influenza viruses can cross species barriers and adapt to human hosts, it is important to gain insight into the factors involved with influenza virus host species specificity. Although the roles of various cellular receptors and proteins in host species specificity have been well-characterized, at present there is little research available regarding the role of microRNAs (miRNAs). Here we describe a novel role for miR-1290, a host miRNA that is expressed in human lung cells and ferret animal models, but not chicken cells or mouse animal models. We show that miR-1290 is induced through the extracellular signal-regulated kinase (ERK) pathway in the early stages of influenza A virus (IAV) infection, and is associated with increased viral titers. We observed that miR-1290 was able to target and reduce expression of the host vimentin gene, the product of which can bind with the PB2 subunit of IAV ribonucleoprotein (vRNP). Knockdown of vimentin expression was found to significantly increase vRNP nuclear translocation and viral polymerase activity. Intranasal application of a miR-1290 antagonist in IAV-infected ferret animal models subsequently reduced miR-1290 expression, as well as viral titers and viral protein levels, in ferret lung tissue. Interestingly, miR-1290 could not be detected in either chicken cells or mouse animal models, and the 3' untranslated region (UTR) of the chicken vimentin gene contains no binding site for miR-1290. However, the introduction of a miR-1290 binding site into the chicken vimentin 3'UTR allowed miR-1290 to target and reduce expression of chicken vimentin as well. These findings point to a host species-specific mechanism by which IAV can upregulate miR-1290 to disrupt host vimentin expression and increase vRNP levels in the nucleus, thereby enhancing viral polymerase activity and viral replication.

**Keywords:** Influenza A virus; microRNA; Host-species specificity; Vimentin; Ferret

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 10551

## THE IMPORTIN- $\alpha$ 7 INTERACTOME AS A PLATFORM TO STUDY MOLECULAR MECHANISMS OF INFLUENZA A VIRUS HOST ADAPTATION AND PATHOGENESIS

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Influenza A virus (IAV) adaptation from animal-to-man is orchestrated by a complex interplay of viral proteins with various cellular factors. We have shown in the past that importin- $\alpha$ 7, a component of the cellular nuclear import machinery, acts as a positive regulator of human-type polymerase activity, thereby promoting IAV interspecies transmission along with increased pathogenicity in the mammalian host. However, the underlying molecular mechanisms are currently unknown.

In order to elucidate importin- $\alpha$ 7 interacting cellular networks that might support IAV replicative fitness in human cells, here, we determined the importin- $\alpha$ 7 interactome using an unbiased proteomic approach. We identified several importin- $\alpha$ 7 interacting cellular proteins that have been previously linked to IAV infections, including DDX21, NCBP1 and ANP32A. For ANP32A, it was recently shown to be involved in IAV host switch. Therefore, we investigated this particular interaction in more detail. Using co-immunoprecipitation and fluorescence microscopy analyses, we could show that ANP32A is imported into the nucleus via its C-terminal nuclear localization signal (NLS) in an importin- $\alpha$ 5/ $\alpha$ 7 dependent manner.

In order to determine whether the positive-regulatory function of importin- $\alpha$ 7 is mediated by ANP32A, we further established a complex importin- $\alpha$  overexpressing viral polymerase activity assay in mammalian cells. First, we show that overexpression of importin- $\alpha$ 7 increases viral polymerase activity in dependency of a balanced nuclear transport machinery. Importantly, importin- $\alpha$ 7 function is maintained in human cells deficient for ANP32A. Finally, we demonstrate that overexpression of ANP32A also increases viral polymerase activity. However, this positive regulatory function of ANP32A is independent of its NLS motif or the presence of importin- $\alpha$ 7.

Thus, our findings suggest that importin- $\alpha$ 7 and ANP32A promote viral polymerase activity in different cellular compartments by functionally-independent mechanisms. This knowledge will help to increase our current understanding of viral adaptation and to identify novel targets for therapeutic intervention.

*Keywords: Host factors; Importin- $\alpha$ 7; ANP32A; Nuclear transport; Influenza A Virus*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 10557

**PLASTICITY OF AMINO ACID RESIDUE 145 NEAR THE RECEPTOR BINDING SITE OF H3 SWINE INFLUENZA A VIRUSES AND ITS IMPACT ON RECEPTOR BINDING AND ANTIBODY RECOGNITION.**

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**Introduction:** The hemagglutinin (HA), a glycoprotein on the surface of influenza A virus (IAV), initiates the virus life cycle by binding to terminal sialic acid (SA) residues on host cells. The HA gradually accumulates amino acid (aa) substitutions that allow IAV to escape immunity through a mechanism known as antigenic drift. We recently confirmed that a small set of aa residues are largely responsible for driving antigenic drift in swine-origin H3 IAV. All identified residues are located adjacent to the HA receptor binding site (RBS), suggesting that substitutions associated with antigenic drift may also influence receptor binding. Among those substitutions, residue 145 was shown to be a major determinant of antigenic evolution.

**Objective:** To determine whether there are functional constraints to substitutions near the RBS and their impact on receptor binding and antigenic properties, we carried out site-directed mutagenesis experiments at the single aa level.

**Methods:** We generated a panel of viruses carrying substitutions at residue 145 representing all 20 amino acids. In vitro characterization studies were performed.

**Results:** Despite limited amino acid usage in nature, most substitutions at residue 145 were well tolerated without major impact on virus replication *in vitro*. All substitutions retained receptor binding specificity, but frequently led to decreased receptor binding. Glycan microarray analysis showed that substitutions at residue 145 modulate binding to a broad range of glycans. Furthermore, antigenic characterization identified specific substitutions at residue 145 that altered antibody recognition.

**Conclusions:** This work provides a better understanding of the functional effects of aa substitutions near the RBS in the H3 HA and the interplay between receptor binding and antigenic drift.

**Keywords:** glycan array; host range; H3N2, swine influenza; receptor binding

Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 10723

## HIGHLY PATHOGENIC AVIAN INFLUENZA H5N8 REASSORTANT VIRUSES COULD ALTER VIRULENCE IN MAMMALIAN MODELS

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### Introduction and objectives

Recently identified highly pathogenic avian influenza (HPAI) H5N8 viruses (clade 2.3.4.4) are relatively low to moderately pathogenic in mammalian hosts compared to HPAI H5N1 viruses. Due to the potential worldwide spread of the H5N8 virus and its co-circulation with other HPAI avian influenza viruses, we investigated HPAI H5N8 virulence factors through comparison with recent HPAI H5N1 and evaluated the pathogenic potentials of possible H5N8 reassortants in mouse and ferret models.

### Methods

Reassortant viruses were generated by coinfection comprised of A/MD/Korea/W452/2014(H5N8) with substitution of individual genes from A/EM/Korea/W149/2006(H5N1). To identify the pathogenicity in mice, each virus was infected and evaluated mortality and morbidity. Additionally, the viruses (W452<sub>W149PB2</sub>, W452<sub>W149HA</sub>, and W452<sub>W149NA</sub>) that demonstrated increased virulence compared with W452 viruses were infected in ferrets to investigate whether these viruses efficiently replicate and be pathogenic in this model.

### Results

Substituting the PB2 gene segment or the NA gene segment of the H5N8 virus by that from the H5N1 virus resulted in significantly enhanced pathogenicity compared with the parental H5N8 virus in mice. Of note, substitution of the PB2 gene segment of the H5N8 virus by that from the H5N1 virus resulted in a 1000-fold increase in virulence for mice compared to the parental virus (MLD50 decreased from 105.8 to 102.5 EID50). Further, the W452<sub>W149PB2</sub> virus also induced the highest virus titers in lungs at all time points and the highest levels of inflammatory cytokine responses among all viruses tested. This high virulence phenotype was also confirmed by high viral titers in the respiratory tracts of infected ferrets.

### Conclusions

Our study demonstrates that a single gene substitution from other avian influenza viruses can alter the pathogenicity of recent H5N8 viruses, and therefore emphasizes the need for intensive monitoring of reassortment events among co-circulating avian and mammalian viruses.

*Keywords: HPAI influenza virus, H5N8, reassortment, virulence, PB2*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
 Abstract No: 10764

## Degradation of interferon signaling factor DDX3 by PB1-F2 as a basis for virulence of 1918 pandemic influenza

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**Introduction and Objectives:** The Spanish influenza of 1918 remains the deadliest infectious disease in human history. Compared to the PB1-F2 proteins of influenza epidemic viruses, that of the 1918 pandemic (1918 PB1-F2) enhances the viral pathogenesis to a much greater extent. However, the host target(s) and the molecular mechanism underlying this high pathogenicity remain elusive.

**Methods:** Isogenic viruses of PR/8 strains, differing only in PB1-F2, and mutants unique to 1918 PB1-F2 cytotoxic sequence were generated. Type 1 IFN responses were analyzed in vitro and in vivo mouse infection model. Host proteins interacting with 1918 PB1-F2 were identified by mass spectrometry and the functional pathway by comparative interactome analyses. Rescue from lethal infection was tested by infusing mice with recombinant host target protein.

**Results:** As compared to PR8 PB1-F2, 1918 PB1-F2 exhibited greatly enhanced proteasome-dependent degradation resulting in physical instability in vitro and in vivo. The low stability was closely linked with a potent inhibition of type I IFN induction and enhanced pathogenicity in mice. The structural prediction showed that 1918 PB1-F2 acquired an internal intrinsically disordered region (IDR), which closely mapped with previously known cytotoxic sequences. The interactome analysis revealed that DDX3, an essential protein in type I IFN signaling pathway, bound and co-degraded 1918 PB1-F2, but not PR8 counterpart. The cell-permeable recombinant DDX3 protein rescued mice from lethal infection from 1918 PB1-F2 containing virus. Recent influenza isolates of swine origin that caused severe respiratory infection in human acquired 1918 unique sequence in PB1-F2.

**Conclusion:** The structure disorder/physical instability of 1918 PB1-F2 is associated with a new gain-of-function toward increased virulence. As a molecular basis of the severe pathogenicity of 1918 pandemic, the report will help guide future design of novel antivirals against influenza pandemics. The present report also warrants close monitoring of the PB1-F2 for global pandemic planning.

**Keywords:** Influenza; PB1-F2; DDX3; internal intrinsically disordered region; 1918 Spanish influenza

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 10807

## Antibodies directed towards influenza neuraminidase restrict influenza virus replication in primary human bronchial epithelial cells

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### Introduction and Objectives:

Influenza neuraminidase (NA) is implicated in various aspects of the virus replication cycle and therefore an attractive target for vaccination and antiviral strategies. Here we investigate the potential for NA-specific antibodies to interfere with A(H1N1)pdm09 replication in primary human airway epithelial (HAE) cells.

### Methods:

Monoclonal antibodies and polyclonal sera directed to the NA of A(H1N1)pdm09 were used to investigate the possible role of NA antibodies during virus entry and replication in HAE cultures using microscopy techniques.

### Results and conclusions:

Mouse polyclonal anti-NA sera and monoclonal antibodies were capable of blocking initial viral entry into HAE cells and the egress from the cell surface after release. However, only NA-specific polyclonal serum was able to reduce replication across multiple rounds of infection. The ability to restrict virus entry correlated with the ability of the serum or monoclonal antibody to mediate neuraminidase inhibition (NI). Finally, human sera with NI activity against the N1 of A(H1N1)pdm09 could decrease H6N1 virus infection of HAE cells. The work presented highlights the role of anti-NA antibodies to control influenza virus infection in a human setting.

*Keywords: Influenza; neuraminidase; antibodies; human airway epithelium*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 10820

## IMPACT OF VIRUS SUBTYPE AND AGE ON INFLUENZA A VIRUS-INDUCED OXIDATIVE STRESS-SPECIFIC GENE EXPRESSION IN THE LUNGS

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### Introduction and Objectives:

Host response to influenza A virus infection involves activation of several cellular processes including oxidative stress. Previously, various studies addressed the induction of different types of free radicals and the impact of key free radical generating enzymes and anti-oxidants on the outcomes of influenza infection. However, the influence of virus subtypes and host factors on oxidative stress is not well established.

### Methods:

In this study, using mouse model of influenza infection, we performed transcriptome analysis for oxidative stress-specific gene expression in the lung tissue of young and aged mice infected with influenza A virus subtypes A/PR8/H1N1 (PR8) or A/HK68/H3N2 (HK68).

### Results:

We found significant differences in the expression of antioxidants and genes involved in free radical metabolism. Specifically, at days 3 and 6 post-infection, HK68-infected mice showed increase in the number of oxidative stress transcripts as well as variations when compared to PR8 infection. In addition, a significant decrease was seen in expression of anti-oxidants especially with HK68 infection. Furthermore, aged mice showed significant reduction in the level of anti-oxidant genes when compared to young adult mice.

### Conclusion:

Taken together, our results establish differences in virus-induced oxidative stress gene expression in response to different influenza virus subtypes as well as the age of the host. These findings have implications for the extent to which the virus strains as well as host response contribute to severity of disease caused by influenza infection. More importantly, these results support the potential for supplementing anti-oxidants as adjunct therapeutics for improving immunity to influenza.

*Keywords: influenza A virus; lungs; oxidative stress; gene expression; transcriptome*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 10839

## IFITM3 and type I interferons are important for the control of influenza A virus replication in murine macrophages

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### Introduction and Objectives:

Seasonal influenza A virus (IAV) infection of airway epithelial cells results in productive replication and release of newly-synthesised virions, however infection of macrophages (MΦ) is generally abortive. IAV infection of both cell types induces a unique spectrum of host defence genes, including interferon (IFN)-stimulated genes (ISGs) regulated by production of type I IFN. IFN-induced transmembrane protein 3 (IFITM3) is an ISG that restricts the early stages of IAV replication in epithelial cells. The role of IFITM3 in restricting IAV replication in MΦ is unknown. Herein, we investigate the importance of IFITM3 and type I IFN in controlling seasonal IAV replication in MΦ.

**Methods:** We used MΦ isolated from mice with deficiencies in IFN regulatory factors (IRF3/7<sup>-/-</sup>), IFN signaling (IFNAR2<sup>-/-</sup>) and IFITM3 (IFITM3<sup>-/-</sup>) to assess effects on early (8h post-infection) and late-stage IAV replication (multicycle growth 48h post-infection). We also investigated IFITM3 expression in WT, IFITM3<sup>-/-</sup>, IRF3/7<sup>-/-</sup> and IFNAR2<sup>-/-</sup> MΦ after treatment with exogenous IFN or following IAV infection.

**Results:** MΦ isolated from IFITM3<sup>-/-</sup> and IRF3/7<sup>-/-</sup> mice were more susceptible to IAV infection, but late-stage replication was controlled through abortive infection. Treatment of IRF3/7<sup>-/-</sup> MΦ with exogenous IFN elicited potent induction of IFITM3, and also reduced the susceptibility of IFN-treated IRF3/7<sup>-/-</sup> MΦ to subsequent IAV infection. MΦ isolated from IFNAR2<sup>-/-</sup> MΦ were more susceptible to the early stages of IAV infection, and strikingly, did support a degree of productive replication (> 20-fold increase in viral titre between 2 and 48 h post-infection).

**Conclusion:** IFITM3 is a potent IAV restriction factor in MΦ, however it does not contribute to maintenance of the abortive replication phenotype. Productive IAV replication in IFNAR2<sup>-/-</sup> MΦ suggests type I IFN signalling through the IFNAR receptor is essential to control late-stage replication. Current studies are investigating cellular mechanisms that control late-stage IAV replication in MΦ.

*Keywords: macrophages; IFITM; viral replication; innate immunity*



Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 10840

## CHARACTERIZATION OF H5N8 HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUSES ISOLATED IN THE DEMOCRATIC REPUBLIC OF CONGO IN 2017

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### Introduction and Objectives

In May 2017, high mortality of chickens and Muscovy ducks caused by H5N8 highly pathogenic avian influenza viruses (HPAIVs) was reported in the Democratic Republic of Congo (DR Congo). We assessed the genetic and the antigenic characteristics, as well as the pathogenicity in poultry for better understanding the features of these HPAIVs newly detected.

### Methods

Twelve viruses were isolated from swab samples collected on 22 Muscovy ducks during the outbreaks. Genome sequences of these isolates were analyzed together with other H5 viruses. For antigenic analysis, the hemagglutinin-inhibition (HI) test was performed with a panel of reference antisera. For the pathogenicity assessment, a representative DR Congo isolate was intranasally inoculated to chickens, Muscovy ducks, and domestic ducks; at three days post inoculation (dpi), organ samples were collected from half of the birds in each species group for virus titration; the remaining birds were monitored for clinical observation until 14 dpi.

### Results and Discussion

The DR Congo HPAIVs were closely related to those from Cameroon and Uganda, altogether clustered in the clade 2.3.4.4 group B with the other African and Eurasian viruses, suggesting the same origin of the viruses. No big antigenic difference was observed between DR Congo isolates and representative viruses in the group icA, C, and D of clade 2.3.4.4; implying no antigenic drift in eastern-central Africa. All chickens and Muscovy ducks challenged with DR Congo isolate died between 3 and 4 dpi, while all domestic ducks survived. The neurotropism was pronounced in Muscovy duck because of high virus titer in the brain than in other organs.

### Conclusion

Our data confirmed the pathogenicity of the DR Congo virus in Muscovy ducks as observed in the field. National and regional awareness, as well as effective surveillance, are needed for better control of the HPAIVs.

*Keywords: H5N8; HPAIV; RDCongo; Characterization; Pathogenicity*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 10849

## Induction of PGRN by influenza virus inhibits the antiviral immune responses through downregulation of type I interferons signaling

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Type I interferons (IFNs) play a critical role in host defense against influenza virus infection, and the mechanism of influenza virus to evade type I IFNs responses remains to be fully understood. Here, we found that progranulin (PGRN) was significantly increased both *in vitro* and *in vivo* during influenza virus infection. Using a PGRN knockdown assay and PGRN-deficient mice model, we demonstrated that influenza virus-inducing PGRN negatively regulated type I IFNs production by inhibiting the activation of NF- $\kappa$ B and IRF3 signaling. Furthermore, we showed that PGRN directly interacted with NF- $\kappa$ B essential modulator (NEMO) via its Grn CDE domains. We also verified that PGRN recruited A20 to deubiquitinate K63-linked polyubiquitin chains on NEMO at K264. In addition, we found that macrophage played a major source of PGRN during influenza virus infection, and PGRN neutralizing antibodies could protect against influenza virus-induced lethality in mice. Our data reveal a negative regulation of type I IFNs signaling and identify a PGRN-mediated immune evasion pathway exploited by influenza virus with implication in antiviral applications. These findings also provide insights into the functions and crosstalk of PGRN in innate immunity.

**Keywords:** Influenza virus; PGRN; NF- $\kappa$ B; IRF3; type I interferons

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 10886

## NATURALLY LOSS OF THREONINE 37 PHOSPHORYLATION ON M1 PROTEIN ENHANCES THE INTER-SPECIES INFECTIVITY OF H9N2 AVIAN INFLUENZA VIRUS IN MAMMALS

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### Introduction

H9N2 avian influenza virus (AIV) caused an increasing number of human infection cases in recent years, but potential mechanism for that remains unclear. We noted that threonine 37 (T37) on M1 protein was gradually replaced by alanine 37 (A37) since 2010, which could be a putative phosphorylated M1 residues. We speculated that loss of the potential phosphorylation site may confer a replication advantage to H9N2 AIV that contributes to their increasing adaptation in mammals.

### Method

WT and mutant viruses were generated by reverse genetics. Viral replication and pathogenicity in A549 cells or mice were evaluated. Western blot was used to detect protein expression. Co-immunoprecipitation and *In Vitro* Kinase Assay were performed to determine the phosphorylation and kinase of T37 on M1 protein. siRNA was used to knock down the cellular kinase PKG.

### Result

Our findings showed that T37 replaced by A37 on M1 protein has become predominant in natural human isolates of H9N2 and reassortant viruses. T37 of H9N2 M1 protein can be phosphorylated by kinase PKG, while A37 on M1 protein cannot. However, this natural mutation from T37 to A37, as loss of this phosphorylation site, increased the stability of H9N2 M1 protein. We further demonstrated that loss of T37 phosphorylation protected M1 protein from proteasomal degradation directed by PKG through a phosphorylation-dependent manner, which resulted in the increased stability of M1 protein and avoided the host defenses. Notably, H9N2 virus carrying A37 on M1 protein obtained enhanced replication and adaptation in mammals *in vivo* and *in vitro*.

### Conclusion

The prevailing H9N2 AIV takes the way of losing the T37 phosphorylation to maintain the stability of viral protein, which ensures the virus to escape from host cellular defenses and obtain enhanced the replication ability of H9N2 AIV in the mammalian host *in vivo* and *in vitro*.

**Keywords:** H9N2 avian influenza virus; M1; phosphorylation; stability; mammalian infectivity

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 10888

## LONG-TERM CULTURED HUMAN LUNG ADENOCARCINOMA A549 CELLS SHOW ENHANCED SUSCEPTIBILITY TO HUMAN INFLUENZA A VIRUSES

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### Introduction and objectives

The human lung adenocarcinoma cell line A549, which is derived from alveolar type II cells, is widely used for influenza research. Long-term culture of A549 cells is reported to lead to the differentiation of these cells toward an alveolar type II cell phenotype. Here, we evaluated the susceptibility of long-term cultured A549 cells to human influenza viruses.

### Methods

A549 cells were cultured for 25 days (D25-A549) or one day (D1-A549) in Ham's F12K medium. Eight human influenza viruses [2 A/H1N1 2009 pandemic (A/H1N1pdm), 3 A/H3N2, and 3 type B] were inoculated into D25- and D1-A549 cells at a multiplicity of infection of 0.01. Infected cell supernatant was collected, and viral titers were determined by means of plaque assay on Madin-Darby canine kidney cells.

### Results

Type II alveolar epithelial cells contain characteristic electron-dense multilamellar bodies. We confirmed multilamellar body development in D25-A549 cells by transmission electron microscopic observation of ultrathin sections. Both A/H1N1pdm and all three A/H3N2 viruses grew much faster and to higher titers (0.6 to 2.6 log units higher at 48 h post-infection) in D25-A549 cells than in D1-A549 cells. However, all three influenza B viruses replicated poorly both in D25-A549 and D1-A549 cells. These findings indicate that D25-A549 cells more efficiently support the replication of human influenza A viruses than do D1-A549 cells.

### Conclusion

Our data suggest that long-term cultured A549 cells will be useful for influenza virus research, particularly for studies involving human influenza A viruses.

*Keywords: Influenza A virus, Influenza B virus, alveolar type II pneumocyte, long-term culture, electron microscopy*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 10891

## Alveolar Regeneration Upon Highly Pathogenic Influenza A/ H5N1 virus Induced Acute Lung Injury

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**Introduction and Objectives:** Highly pathogenic avian influenza (HPAI) H5N1 virus cause high mortality rate because of the development of ARDS in infected patients. Alveolar epithelium have the ability to regenerate from injury. Previous studies showed that alveolar regeneration involve the p63+Krt5+ lung progenitor cell. However, it is still not clear how these cells differentiate and contribute to regeneration of alveolar epithelium upon injury induced by H5N1 virus. In this study, an in vivo and in vitro model were used to investigate the role of lung progenitor cell upon influenza virus induced acute lung injury (ALI).

**Method:** C57/b6 mice and in vitro culture of human lung progenitor cells were infected with influenza H1N1pdm and H5N1 virus. RNA were extracted and analyzed with qPCR and RNA-seq. Cytokines and chemokines were detected by CBA and the Krt5 p63 were monitored by immuno-histochemical staining.

**Result:** Mice infected with H1N1pdm showed lower mortality rate than H5N1 with similar clinical symptoms and viral titre in lungs. Both H1N1pdm and H5N1 infected mice recovered, but H1N1pdm infected mice recovered significantly faster than H5N1 infected mice. Krt5 expression was significantly up-regulated and widely spread from the basal layer of bronchioles in H1N1pdm infected mice which migrate to the injury site and differentiate into alveolar type 1 cells, while H5N1 infected mice showed very limited expression of Krt5+ cells. Furthermore, HPAI H5N1 virus infection showed a significantly down regulation of Krt5 expression and up-regulation of apoptosis related gene in in vitro culture of lung progenitor cells.

**Conclusion:** The differential regeneration upon ALI induced by H1N1pdm and H5N1 virus infection suggested that alternative pathway and population of progenitor cell may be involved in the regeneration of H5N1 virus induced ALI. These differential regeneration between low pathogenic and HPAI upon injury play a critical role in the disease severity and recovery.

**Keywords:** HPAI; Alveolar Regeneration; Lung progenitor cell; Acute Lung Injury

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 10892

## ATTENUATING INFLUENZA A VIRUS INFECTION BY HEPARIN BINDING EGF-LIKE GROWTH FACTOR

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Influenza A virus (IAV) enter the host cells by receptor-mediated endocytosis and replicate in the nucleus. Uptake of IAV into the cells was reported to be promoted by epidermal growth factor receptor (EGFR). On the other hand, heparin-binding EGF-like growth factor (HB-EGF) as one of the ligands of EGFR was reported to induce internalization and degradation of EGFR. Therefore we hypothesized that HB-EGF may attenuate IAV entry and replication in host cells through internalization and degradation of EGFR. Our study demonstrated that HB-EGF-induced internalization of EGFR in a concentration- and time-dependent manner, and prolonged exposure to HB-EGF led to significant degradation of EGFR. Concordantly, the induced internalization and degradation of EGFR led to significant reduction of viral matrix 1 protein in the infected A549 cells. Using plaque assay, suppressed virion production in infected cells was also observed. Interferons which induce their effect responses via the Jak/Stat pathway play critical role as a first line of defence against viral infection. However, uncontrolled and exaggerated inflammatory response can result in “cytokine storm” which is used to describe the overproduction of immune cells and cytokines in response to virus infection. We found that HB-EGF regulates the nuclear localization of STAT1 and inhibit its phosphorylation induced by IAV in the early stage of the infection. Together these findings attest the potential of HB-EGF mediated endocytosis and degradation of EGFR as a novel anti-IAV strategy.

*Keywords: Influenza A virus; HB-EGF; EGFR; antiviral; JAK/STAT*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 10926

## INFLUENZA A VIRUS NUCLEOPROTEIN AND HOST CELLULAR NUCLEOLIN INTERACTION IN VIRUS REPLICATION

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A successful viral infection in a host depends on temporarily regulated interactions between virus and host factors. Influenza A virus is a common respiratory pathogen and remains as a public health threat worldwide. Recently, we reported host cellular nucleolin (NCL) as a novel interacting partner to nucleoprotein (NP), a major structural protein of influenza A virus. NP protein is 498aa long polypeptide encoded by viral RNA segment 5. NP is not merely a structural protein but functions as a key adaptor molecule between virus and host cellular processes. NCL, a multifunctional eukaryotic protein; ubiquitously distributed in a cell. The interaction between NCL-NP proteins during early phase of infection was observed to have an adverse effect on late phase of infection. In the current study, we sought to identify the functional domains and critical amino acid residues of viral NP responsible for this interaction. For which, EGFP fused deletion mutants of NP encoding RNA binding, homo oligomerization and homo oligomerization NP1-NP2 domains were generated. The interaction of EGFP fused deletion mutants with NCL was evaluated using mCherry fused NCL construct. Co-localization studies demonstrated interaction between NCL and RNA binding domain of NP. Further, amino acid residues of NP engaged in interaction were identified using crystal structure of viral NP and solution structure of RNA binding domains 1, 2 of human NCL by protein-protein docking tools; HADDOCK and PATCHDOCK. In concordance with above results, critical interacting residues were found in RNA binding domain. The significance of these residues was validated *in vitro* by generating mutants of NP RNA binding domain with residues disrupted by SDM and confirmed by co-IP and co-localization studies. Our study demonstrated that NP interacts with NCL through its RNA binding domain. The mechanistic insights of this interaction may help for the development of effective anti influenza viral strategies.

*Keywords: NUCLEOLIN (NCL); NUCLEOPROTEIN (NP); SITE DIRECTED MUTAGENESIS (SDM)*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
 Abstract No: 11021

## Sphingomyelin Plays a Role in Influenza Virus Infection

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**Introduction and objectives:** Influenza A virus (IAV) is a major human respiratory pathogen that poses significant threat to human health. Sphingomyelin (SM) is the most abundant membrane sphingolipid. SM preferentially associates with cholesterol to form distinct domains named lipid rafts. Hydrolysis of SM is catalyzed by lysosomal acid sphingomyelinase (ASMase). Lipid rafts and their cholesterol component were shown to play a role during IAV infection. In this study, we aimed to better understand the role of SM, another key component of lipid rafts, and its hydrolyzing enzyme ASMase during IAV infection.

**Methods:** First, the effect of pharmacological ASMase inhibition using desipramine and ASMase-genetic deficiency using Niemann–Pick diseases cells (NPD) on IAV infection was investigated. ASMase activity was assayed in mock-infected and IAV-infected cells at different time points. The effect of plasma membrane-SM depletion using exogenous bacterial sphingomyelinase (SMase) on IAV infection was examined. The impact of exogenous SM on replication of IAV was also examined. Additionally, the effect of SM depletion of viral envelope was determined. Finally, confocal microscopy was used to investigate the effect of SM depletion on IAV entry step. Viral titers were determined by plaque assay.

**Results:** We found that inhibition of ASMase by desipramine had no effect on IAV replication. Similarly, virus replication was not attenuated in NPD cells compared to normal fibroblasts. In addition, ASMase activity was significantly reduced in presence of IAV infection in a time-dependent manner. Depletion of plasma membrane SM by exogenous SMase impaired infection and reduced virus entry. Addition of exogenous SM induced minor enhancement of viral infection. Furthermore, treatment of IAV particles with SMase impaired viral infection and reduced virus infectivity.

**Conclusion:** Our data reveals that intact SM at the plasma membrane and in virus envelope is required for efficient IAV entry and subsequent replication.

*Keywords: Influenza A virus, lipid rafts, sphingomyelin, acid sphingomyelinase.*



Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 11027

## HYPERGLYCAEMIA INDUCES EPITHELIAL-ENDOTHELIAL BARRIER DAMAGE DURING INFLUENZA A INFECTION

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**INTRODUCTION:** Diabetes mellitus is on the rise globally and is a known susceptibility factor for severe influenza virus infections. However, the mechanism by which diabetes increases the severity of influenza is yet to be fully defined. Here, the effects of high glucose levels on influenza severity were investigated.

**METHODS:** To mimic the pulmonary epithelial-endothelial barrier, human epithelial cells were seeded on the top side of a permeable membrane and primary human pulmonary endothelial cells were seeded on the underside. Once epithelial cells reached confluency, the media in both upper and lower compartments was refreshed every 12 hours. The medium of the lower compartment was refreshed with either 7mmol/L (to represent normal glucose conditions) or 12mmol/L (to represent the hyperglycaemia seen in diabetes). After four days in differential glucose conditions, influenza A/Solomon Islands/03/2006 (H1N1) was added to the upper compartment of the trans-well system. After infection, various assays, qPCR and RNA-Seq was performed to measure barrier integrity and inflammation. Additionally, epithelial cells were fixed and stained using IHC to investigate tight junction integrity.

**RESULTS:** Using an *in vitro* co-culture model of the pulmonary epithelial-endothelial barrier, we show that, compared to normal glucose levels, high glucose conditions (a hallmark of diabetes mellitus) prior to influenza A virus infection increases barrier damage. Increased barrier damage was not associated with increased cell death, but rather an increased endothelial cell pro-inflammatory response and subsequent degradation of epithelial cell tight junctions.

**CONCLUSIONS:** This study demonstrated for the first time that hyperglycaemia may increase influenza severity by damaging the pulmonary epithelial-endothelial barrier and increasing pulmonary oedema. This understanding is imperative for the development of therapeutic approaches tailored for vulnerable patient groups infected with influenza virus.

**Keywords:** *diabetes mellitus, hyperglycaemia, pulmonary barrier, tight junctions, in vitro*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 11039

## The positive charge of Arg-201 on hemagglutinin is required for H6N1 avian influenza virus to bind with host cell

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### Introduction and Objectives:

H6N1 avian influenza virus (AIV) was found in Taiwan for more than four decades. In our previous study, we produced a monoclonal antibody EB2 that recognized an epitope in the HA1 domain on the hemagglutinin (HA) of a low-pathogenic H6N1 (A/chicken/Taiwan/2838V/00). The residue Arg-201 (R201) on this epitope was protected by a glycan at Asn-167 (N167) from tryptic digestion; therefore, the infectivity of the virus was retained. R201 was extremely conserved in various subtypes of AIV.

### Methods:

To study the role of R201 in the viral infection mechanism, the gene encoding for the surface antigen HA1 of H6N1 was expressed in bi-cistronic baculovirus expression system.

### Results:

We propose a two-step model for binding the influenza virus with a host cell. The first step involved the specific recognition of the receptor binding site on HA to the sialylated glycan on the host cell. After the virus is engulfed by the acidic endosome, R201 could bind to the cell surface with stronger interactions and trigger the fusion process.

### Conclusion:

This two-step binding model may lead to a new strategy for blocking the binding of influenza virus to the host cell and preventing the invasion of the virus.

*Keywords: Avian influenza virus; Charged amino acid; Receptor binding site; Two-step binding process.*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 11041

## Mapping the nuclear localization signal on influenza C virus nucleoprotein

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### Introduction and Objectives

The influenza C virus (ICV) is a human pathogen which can lead to lower respiratory infections especially in children. Although there is a high seroprevalence of ICV worldwide, knowledge on its nucleoprotein (NP) is limited. Compared to influenza A and B, ICV NP has a more extended C-terminal domain. This work attempted to identify unique structural features of ICV NP and to delineate the functional significance of the extended C-terminal domain, especially with respect to nuclear import.

### Methods

Multiple sequence alignment was employed to identify special features on ICV NP. Mutagenesis and fluorescence microscopy were employed to investigate putative nuclear localization signals (NLSs) and to identify important residues. Importin-NP binding was tested using co-immunoprecipitation and microscale thermophoresis.

### Results

We identified a bipartite nuclear localization signal on the C-terminal domain of ICV NP, and reported that a KKMK motif was crucial for nuclear import. The nuclear import of ICV NP was importin alpha dependent. We also characterized the binding of this bipartite NLS to importin alpha 1.

### Conclusion

We established that the C-terminal domain (CTD) of ICV NP is responsible for its nuclear import and also defined residues important for binding to importin alpha.

*Keywords: Influenza C virus; nucleoprotein; nuclear localization signal; importin alpha*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 11077

## SINGLE CELL SEQUENCING IDENTIFIES VARIABILITY IN HOST RESPONSE AMONG DIFFERENT GENERA OF INFLUENZA VIRUSES

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### Introduction

Seroprevalence and surveillance studies indicate that influenza C virus (ICV) infection is common among humans, and initial exposure occurs early in life. ICV often causes milder disease than influenza A and B viruses, but the mechanisms underlying differences in pathogenicity remain poorly understood.

### Methods

To compare early events of infection in natural target sites, we cultured primary human tracheal/bronchial epithelial cells under air-liquid interface conditions to allow differentiation. We infected these cells with human strains of influenza A, B or C virus. Cells were infected at low MOI (0.1) to ensure populations of directly infected cells and uninfected neighboring cells. To compare the early immune response and cell tropism among these viruses, we performed single-cell RNA sequencing of mock- and influenza-infected cells. In parallel, we infected cells pre-treated with interferon to mimic later rounds of infection after an early immune response is initiated.

### Results

Infection of primary cells by all three viruses was confirmed by RT-qPCR of bulk cell lysates. As expected, prior exposure to interferon  $\beta$  results resulted in reduced levels of viral transcripts. At single-cell level, we identified expression of genes associated with specific cell types, including basal, ciliated and secretory cells. We also identified expression of interferon stimulated genes, but these genes were not homogeneously expressed among all cell subpopulations and varied among cultures infected with different influenza viruses. We also found different patterns in gene expression in cells previously exposed to interferon, suggesting that host environment varies over subsequent rounds of infection.

### Conclusion

Single cell sequencing is an important tool for studying the host response to influenza infection in complex cellular environments such as the respiratory tract, in which cells vary in their susceptibility to infection and antiviral response. Further analysis will characterize differences among directly infected vs. neighboring cells and correlate responses with pathogenicity.

*Keywords: influenza C; single-cell sequencing; innate immunity; host-pathogen interactions; viral pathogenesis*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 11132

## Host innate factors regulating inflammatory disease resulting from influenza infection: a critical role is played at the cell-surface.

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**Introduction & objectives:** Respiratory pathogens, such as influenza A virus (IAV), efficiently circumvent the gel mucus layer to infect underlying epithelial cells, yet little is known about innate barrier defense mechanisms mounted by these cells. Cell surface mucins (cs-mucins) are the likely first point of contact by IAV due to their dominating structure and presentation of sialic acids, a major target for the receptor-binding glycoproteins of the virus. In addition to the heavily glycosylated extracellular domain that towers above other receptors expressed at the epithelial cell surface, cs-mucins contain a transmembrane domain that enables extracellular domain shedding and a cytoplasmic tail capable of triggering signalling cascades, implicating them as important molecules in host-protection from infection. We hypothesized that IAV interacts with the terminal sialic acids presented by cs-mucins and this results in modulating infection efficiency.

**Methods & Results:** Utilizing human lung epithelial cells, we found that IAV associates with the cs-mucin MUC1 but not MUC13 or MUC16. Over-expression of MUC1 by epithelial cells or the addition of sialylated synthetic MUC1 constructs, reduced IAV infection *in vitro*. In addition, *Muc1*<sup>-/-</sup> mice infected with IAV exhibited enhanced morbidity and mortality, as well as greater inflammatory mediator responses compared to wild-type mice.

**Conclusion:** This study implicates the cs-mucin MUC1 as a critical and dynamic component of the innate host response that limits the severity of influenza induced disease and provides the foundation for exploration of MUC1 in resolving inflammation.

**Keywords:** mucin, MUC1, mucosal barrier, influenza disease

Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 11135

## Host influenza history dictates vaccine responses suggesting a memory B cell mechanism

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### Introduction.

Influenza viruses are a recurrent and unsolved public health problem despite vaccination efforts. The well-known yearly cycling of influenza viruses is the result of the reciprocal relationship between the host and virus and leads to building a complex immune history in humans. It is now recognized that influenza history significantly influences current vaccine outcomes, but the mechanisms that regulate vaccine responses in the preimmune host have yet to be elucidated.

### Objectives.

Here we developed preimmune mouse and ferret models to investigate the influence of influenza history on vaccination outcomes by evaluating antibody responses and clinical disease at challenge.

### Methods.

Ferret and mice were imprinted with a sublethal dose of historical seasonal H1N1 strains A/USSR/90/1977 (USSR/77) or A/FortMonmouth/1/1947 (FM/47). Animals were allowed to recover over 60+ days to build immune memory prior to vaccination. The animals were vaccinated with an unadjuvanted or adjuvanted split virion vaccine. To evaluate protection, hosts were challenge with an antigenically distinct circulating 2009 H1N1 pandemic virus. Both models were monitored for signs of clinical disease including weight loss. Host responses were evaluated by Hemagglutinin Inhibition assays (HAI), antibody ELISAs, and cytokine ELISAs,

### Results.

At challenge the preimmune-vaccinated animals did not experience significant disease while control groups, naïve-vaccinated, lost a significant amount of weight. HAI assays showed significant increases in antibody titers in preimmune hosts post vaccination compared to naïve-vaccinated groups. Early time points post vaccination showed vaccine directed antibodies were present early after vaccination (Day 7). Investigation of the immunoglobulin isotype profiles showed higher IgG levels in preimmune-vaccinated groups suggesting B cell maturity.

### Conclusions

Together, results showed preimmune animals had greater responses to vaccination and the presence of IgG virus specific antibodies suggest plasticity in an existing memory B cell clone. These results are important and should be applied to influenza vaccine development.

*Keywords: Imprinting; Preimmunity; Vaccine; Split Virion; Memory B cell*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 11158

## INTEGRATIVE MULTIOMICS ANALYSIS OF INFLUENZA A VIRUS RESTRICTION MECHANISMS IDENTIFIES TBC1D5 AS A DRIVER OF VIRAL AUTOPHAGOSOMAL DEGRADATION

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### Introduction and Objectives

Efficient replication of influenza A virus (IAV) relies on its ability to recruit and exploit cellular factors, while evading innate immune surveillance and counteracting various cellular anti-viral mechanisms. To better understand cellular pathways that underlie the latter activity, we sought to generate a comprehensive map of key cellular components and viral-host interactions that restrict viral infection.

### Methods

We generated complementary orthogonal datasets that include genome-wide siRNA screens, proteomics and global transcriptomics datasets in human myeloid and/or primary epithelial cells challenged with seasonal H3N2 or highly pathogenic H5N1 IAVs. These datasets were integrated allowing us to build a comprehensive array of cellular factors that impact viral replication in a strain-specific or conserved manner.

### Results

Through these integrative analyses, we identified the Rab7 GTPase-activating protein TBC1D5 to negatively impact the replication of both H3N2 and H5N1 strains. TBC1D5 was found to be both necessary and sufficient to restrict viral replication in multiple cell types, including human tracheobronchial primary cells. In addition, mice depleted for TBC1D5 showed increased pathogenicity and higher viral loads in the lungs. TBC1D5 and IAV matrix 2 (M2) protein were found to interact in Rab7-positive late autophagosomes. Importantly, TBC1D5 depletion resulted in limited fusion between autophagosomes and lysosomes, resulting in diminished targeting of M2 to lysosomes for degradation and an enhancement of viral replication. Taken together, these data suggest a hypothesis for which M2 may bind to TBC1D5 to inhibit its activity and enable viral evasion of autophagosomal degradation.

### Conclusion

The integrative modeling of orthogonal systems-level datasets enabled the identification of cellular factors and pathways that negatively regulate IAV infection, including molecular mechanisms of IAV targeting for autophagosomal degradation.

*Keywords: OMICs, restriction mechanisms, macroautophagy*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 11159

## INFLAMMATORY MONOCYTE MATURATION PREDICTS THE SEVERITY OF SYMPTOMS FOLLOWING INFLUENZA INFECTION

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**Introduction and objectives:** The response to Influenza viruses varies substantially between individuals, and there are currently no known molecular predictors from the early stages of infection.

**Methods:** Multi-OMICS data sets that include transcriptional profiling and proteomics were used to define the mechanisms underpinning the differences in lung tissue integrity from mice infected with the highly infectious influenza strain H5N1 (defined by a high viral load that results in mice death post-infection) or the mild H1N1 strain. Bioinformatic methods including deconvolution of bulk tissue expression into immune-subset expression, network inference and mathematical modeling to identify cause-effect relations between distinct biological processes were used to develop models that predict the severity of symptoms following infection. These were then applied to human challenge transcriptomic data to confirm their relevance to human influenza infection.

**Results:** Deconvolution of the transcriptomic and proteomic data revealed that monocytes were the main subset that was differentially altered when comparing H5N1- and H1N1-infected mice. Gene set enrichment analysis suggested that this monocyte subset was recruited from the circulation and differentiated in situ into pro-inflammatory macrophages in infected lung tissue. Mathematical modeling suggested that H5N1-infection results in the induction of interferon-regulated chemokines (i.e. CCL2) that lead to the increased frequency of pro-inflammatory macrophage, and this, in turn, leads to the induction of the Fas/FasL apoptotic pathway. H5N1-infected Fas-/FasL- knockout mice show increased survival as compared to wild-type mice. A gene-signature of pro-inflammatory macrophage induction detected in the lung but also in blood correlated positively with the severity of symptoms when applied to human H1N1-challenge transcriptomic data.

**Conclusion:** Integration of OMICs data sets show that maturation of monocytes to FasL-expressing macrophages are critical for the pathogenesis of influenza virus in mice and humans.

**Keywords:** System Biology; Influenza; OMICs; Modeling



Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 11261

## UPR redefined in influenza virus replication

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**Introduction:** The Unfolded response (UPR) is a signalling cascade, represented by has three branches AFT6, IRE1 and PERK. Each branch undergoes differential stimulation depending upon the type and intensity of cellular stress; and regulates the expression of a different set of gene and related physiological events. We have performed a detailed *in vitro* and *in vivo* analysis of UPR pathways in influenza infection, and its association with replication of influenza virus.

### Methods:

A549 cells were infected with influenza virus and effect on the expression of UPR genes was estimated by qPCR and western blotting. To further validate, the virus-infected A549 cells were treated with UPR stimulator, Tunicamycin (1 ug/ml); and UPR genes expression in virus-infected with tunicamycin treatment and without treatment was compared. The effect of individual UPR branch on viral replication was estimated by treating specific inhibitors to virus-infected cells and performing real qPCR and western blotting for viral genes in each set. For *in vivo* experiment, Balb/c mice were infected with influenza virus and effect on UPR genes was estimated by real-time PCR from lung sample

### Results:

The expression of ATF6, CHOP and ATF4 gets downregulated upon influenza infection at transcriptional and protein level; with decreased ATF6 cleavage and expression of XBP1 spliced variant. The tunicamycin-induced activation of all the three branches of UPR was suppressed by influenza virus at the RNA and protein level. Finally, We observed that IRE1 and PERK inhibitors decrease viral gene expression while AFF6 inhibitor has no significant effect role in it

**Conclusion:** We concluded that influenza virus inhibits all the three branches of UPR. The IRE1 and PERK inhibitor can effectively suppress the viral replication hence could be considered as a therapeutic target. The suppression of UPR seems a host defence mechanism

*Keywords: UPR, influenza virus, host-pathogen interaction*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 11276

## **Deciphering the interaction between the host, the gut microbiota and the virus, following an infection with an H5N9 highly pathogenic Influenza virus in ducks**

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In contrast to chickens, which are very susceptible hosts for avian Influenza virus, ducks often show little or no clinical signs, even following infection with a highly pathogenic avian Influenza virus. Recent evidence suggests that the gut microbiota plays an important role in the regulation of viral infections, particularly with Influenza virus. The objectives were to decipher the interactions between the gut microbiota and an H5N9 highly pathogenic Influenza virus in ducks.

DNA from stools of infected and non-infected birds were extracted and the V1-V3 hypervariable region of the bacterial 16S ribosomal RNA gene was amplified and sequenced using an Illumina platform. Then, we compared virus replication, innate immune response and histopathological lesions between ducks with a gut microbiota depleted by a large spectrum antibiotic treatment to undepleted ducks, which received no antibiotic treatment.

Analysis following deep sequencing of the stool samples showed that the H5N9 infection induced significant dysbiosis in the gut microbiota. The microbial depletion after antibiotic treatment was confirmed by bacterial culture and 16S ribosomal RNA gene qPCR and FISH staining of the ileum. Virus shedding analysis showed that antibiotic-treated H5N9 virus infected ducks showed significantly higher cloacal shedding at day 3 and 5 post-infection, but no difference in oropharyngeal shedding at all-time points. The innate immune response was significantly decreased in the ileum of depleted and infected ducks and slightly increased in their lungs. Direct effects of the gut microbiota and its products on intestinal integrity and viral stability are still under investigation.

Our results illustrate the interplay between three different players: the host, its gut microbiota and the virus. The virus can modulate the structure and composition of the gut microbiota and the gut microbiota can modulate the viral replication, possibly through the regulation of the antiviral immune response.

Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 11312

## DEFINING THE EPITOPES OF HUMAN BROADLY-REACTIVE ANTI-INFLUENZA VIRUS NEURAMINIDASE MONOCLONAL ANTIBODIES

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**Content** \*(Abstract should be structured to include four separate sections. **Introduction and Objectives, Methods, Results and Conclusion.**)

**Introduction:** Anti-neuraminidase antibodies are an independent correlate of protection against influenza virus infection. These antibodies act by blocking enzymatic activity and can be neutralizing, neuraminidase inhibiting (NAI) or both. A fraction of human neuraminidase antibodies induced following natural infection display broad binding and NAI activity within N1 and N2. As we advance the development of universal influenza virus vaccines that incorporate standard amounts of neuraminidase antigen, it is important to identify the antigenic targets of broadly-reactive human monoclonal antibodies (mAbs).

**Methods:** Here, we describe escape mutants generated by serial passage of A/Netherlands/602/2009 (H1N1) or A/Switzerland/9715293/2013 (H3N2) viruses in the presence of either N1 or N2 targeting mAbs. Escape from neutralization was confirmed via plaque reduction neutralization assays while escape from NAI activity was confirmed via an enzyme-linked lectin assay (ELLA).

**Results:** We observed N1 escape mutations around the enzymatic site (S364N and N369T) and on the lateral surface (N88D and Q313K/R). For N2, escape mutations were observed near the enzymatic site (K199E/T), on the lateral surface (E258K) and on the side of the tetramer (A272D). All escape mutants showed resistance to mAb neutralization and NAI activity, aside from the N2 A272D mutant virus which showed a loss of mAb neutralization but remained sensitive to NAI.

**Conclusion:** ELLAs showed that some neuraminidase escape mutants have changes in their enzymatic activity, indicating that mutations outside of the enzymatic site can contribute to neuraminidase enzymatic activity. Currently, we are identifying additional mAb escape mutations and investigating the effect of escape mutations on viral fitness and transmission. This work will help define antigenic targets of the neuraminidase and can aid in designing better antigens for universal influenza virus vaccines. Additionally, this work addresses how the neuraminidase protein evolves under selective pressures and if this evolution alters viral fitness.

*Keywords: neuraminidase, antigenic drift, antigenic sites, escape mutants*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions

Abstract No: 11327

## Functions of host shut-off proteins of influenza A virus

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Virus infection induces a wide range of host defense responses, such as the innate immune response and inflammation. Some viruses express accessory proteins to induce general shutoff of host protein synthesis, which is one of the major viral strategies to counteract host antiviral activity and immune response. Influenza A virus expresses two viral proteins, PA-X and NS1, to induce host shutoff. Although both proteins are known to induce general shutoff, specificity of target genes and their functional interplay in mediating host shutoff are not fully elucidated. To analyze the effect of these shutoff proteins in viral pathogenicity and host responses, we generated four recombinant influenza A/California/04/2009 (pH1N1) viruses containing mutations affecting the expression of active PA-X and NS1. We analyzed viral growth, general shutoff activity, specificity of mRNA targets, and viral gene expressions. Our results showed that PA-X was the major contributor in reducing general host protein expression in the virus-infected cells. Intriguingly, our transcriptomic analysis from infected human airway A549 cells indicate that shutoff-active NS1 specifically targeted host mRNAs related to interferon signaling pathways and cytokine release. Specificity of target mRNAs was less evident in PA-X, although it preferentially degraded genes associated with cellular protein metabolism and protein repair. To further analyze the effect of viral shutoff activities in viral pathogenicity and immune responses *in vivo*, we infected C57BL/6 mouse with the recombinant viruses. Consistent with the transcriptomic analysis in human airway cells, the virus without shutoff activity induced strongest innate and cytokine responses, as well as inflammatory cell infiltration. These results suggest that both PA-X and NS1 shutoff activities have a strong impact on viral pathogenicity and host immune responses.

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 11729

## From birds to pigs: molecular determinants associated with the transmission potential of Eurasian avian-like swine influenza viruses in pigs

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### Introduction and Objectives

The Eurasian avian-like H1N1 swine influenza (EA-H1N1) virus represents one of the few successful interspecies adaptations of avian influenza viruses to mammalian hosts. We investigated if the molecular determinants associated with the successful interspecies transmission were intrinsically present in a precursor avian influenza virus prior to its introduction into pigs or if they were sequentially acquired during viral adaptation in pigs.

### Methods

We reconstructed the evolutionary path by phylogenetic analysis of the EA-H1N1 virus and generated three recombinant precursor viruses based on early ancestral nodal sequences. We characterized these precursor viruses via in vitro solid-phase binding assays, ex vivo swine lung and tracheal cultures and in vivo contact transmission experiments in pigs.

### Results

The precursor EA1 virus is genetically closely related to avian influenza viruses while the coding sequences of the precursor EA2 and EA3 resembles EAH1N1 viruses circulated in the early 1980s in swine. We observed increased HA receptor binding with step by step changes from  $\alpha$ 2,3-linked to  $\alpha$ 2,6-linked sialyl receptors as the precursor viruses became adapted in the swine host; however, all precursor viruses possessed comparable HA stability under a range of pH values and showed comparable replication efficiency in MDCK, embryonated chicken eggs, and newborn pig trachea cells. We confirmed that an avian H1N1 virus A/Duck/Bavaria/2/77 (DK2/77) lacked the ability to transmit among pigs by direct contact, while early EA-H1N1 swine influenza viruses A/Swine/Germany/2/81 (SW2/81) and A/Swine/Schleswig-Holstein/1/92 (SW1/92) were transmitted among pigs albeit with differing efficiencies. However, the EA1, EA2, or EA3 precursor viruses do not possess the ability to transmit efficiently among pigs.

### Conclusions

Our results suggest that the efficient transmissibility of the EA-H1N1 viruses among pigs was not intrinsically present in a precursor avian influenza virus prior to its introduction in pigs. Increased receptor binding affinity from  $\alpha$ 2,3-linked to  $\alpha$ 2,6-linked sialyl receptors can be observed early during the adaptation of the EA-H1N1 viruses in pigs.

*Keywords: H1N1; influenza virus; mammalian; avian; adaptation*

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 11751

## Functional roles of USP25 in innate immune response and during influenza A virus infection

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Ubiquitylation is a reversible post-translational modification implemented in a cascade of three enzymes: ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). These enzymatic activities work together to conjugate the conserved 76-residue polypeptide ubiquitin to protein substrates, which can be hydrolysed by deubiquitylating enzymes (DUBs). The ubiquitylation machinery is vitally important in regulating Influenza A virus (IAV) life cycle. Using a DUB specific activity-based probe, we identified and validated several that are activated upon IAV infection. Among others we identified USP25. To determine its functional implications in IAV infection, we generated cells with the gene deletion of USP25 using CRISPR/Cas9 strategy. Our results displayed a significant increase in virus production in USP25 knock-out cells compared to those in wild-type cells during IAV infection, suggesting an antiviral role of USP25. The increase of virus production in the knock-out cells was not a result of increased entry or replication, but correlated with increased autophagy as reflected by appearance of LC3 II. We therefore hypothesised that USP25 restricts autophagy dependent transport and release of IAV. Additionally, deletion of USP25 also resulted in decreased RIG-I protein expression, indicating that USP25 might stabilize RIG-I by removing K48-linked ubiquitin chains. Consistently, decreased RIG-I protein expression in the knock-out cells also resulted in attenuated interferon regulatory factor 3 (IRF3) and IRF7 protein expression. Taken together, our data implicates USP25 as a crucial restriction factor for IAV. Deletion of USP25 would not only dampen RIG-I dependent immune signaling but also induce autophagy to enhance viral release and virion stability.

Topic: Virology and Pathogenesis: Host-Pathogen Interactions  
Abstract No: 11785

## ANP32B BUT NOT ANP32A PROMOTES INFLUENZA A VIRUS REPLICATION AND PATHOGENICITY IN MICE

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Influenza A viruses (IAV) may cross species barriers from animal-to-man and give rise to large epidemics or even pandemics. Thus, understanding the complex interplay of viral and host factors is key for pandemic preparedness.

In this study, we investigated the role of recently identified host factors of the ANP32 family in influenza A virus pathogenesis. Therefore, we used ANP32A and ANP32B knockout mice (ANP32A<sup>-/-</sup> and ANP32B<sup>-/-</sup>, respectively). Infection of ANP32A<sup>-/-</sup> mice with a seasonal H3N2 influenza A virus or a highly pathogenic H5N1 human isolate revealed 100% lethality. In contrast, infection of ANP32B<sup>-/-</sup> mice with H3N2 IAV caused 20% lethality, while death of H5N1 infected ANP32B<sup>-/-</sup> mice was delayed. Genome-wide transcriptome analyses uncovered novel pathways that might be involved in the ANP32B mediated mode-of-action in IAV pathogenesis.

Thus, we here provide first evidence that ANP32B but not ANP32A acts as a cellular factor of influenza A virus pathogenesis in mice.

*Keywords: Host factors; ANP32A; ANP32B; Influenza A virus; Pathogenesis*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10377

## COULD TRIVALENT LAIV PROTECT AGAINST BOTH GENETIC LINEAGES OF INFLUENZA B VIRUS?

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**Introduction and Objectives.** Currently, two genetic lineages of influenza B virus are co-circulating in humans. This situation can lead to mismatch between the influenza B strain recommended for the seasonal vaccine and the circulating B virus. It is important to know whether vaccines containing a single influenza B strain can provide cross-protectivity against viruses of the antigenically distinct lineage. The aim of this study was to assess in ferrets the potential cross-protective activity of monovalent live attenuated influenza B vaccine (M-LAIV) and trivalent LAIV (T-LAIV) based on B/Victoria and/or B/Yamagata lineage virus against challenge with a heterologous wild-type influenza B virus.

**Methods.** Ferrets were given one dose of M-LAIV or T-LAIV and then were challenged with B/Victoria or B/Yamagata wild-type virus. Animals were monitored for clinical signs associated with morbidity. Samples of lung tissue and/or nasal washes were taken three days after vaccination/challenge. All samples were analyzed for the presence of challenge virus. Antibody response to vaccination was assessed by HI assay.

**Results.** All LAIVs tested were found to be safe and effective against wild-type influenza B viruses based on clinical signs, virological and histological data. Vaccination led to production of specific antihemagglutinating antibodies to vaccine virus, protected ferrets from homologous challenge infection, and significantly reduced clinical signs and replication of homologous challenge virus. Cross-lineage serum antibodies were not detected. However, ferrets vaccinated with M- or T-LAIV had a significantly lower level of heterologous challenge virus in the respiratory tract compare to control animals inoculated with challenge virus only. Notably, B/Victoria-based LAIVs were more protective compared to B/Yamagata-based LAIVs.

**Conclusion.** M- and T-LAIV were shown to have the potential to be cross-protective against infection with genetically different influenza lineages. Further studies are required to confirm this finding.

This work was supported by WHO Grant #TTI-LOA17-IEM-1 and RSF Grant #14-15-00034.

*Keywords: Influenza; live attenuated influenza vaccine; cross-protective activity*



Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10410

## Recombinant live attenuated influenza vaccine viruses carrying CD8 T-cell epitopes of respiratory syncytial virus protect mice against both infections without inflammatory disease

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**Introduction and objectives:** Influenza and respiratory syncytial virus (RSV) are the two major pathogens that cause serious lower respiratory tract infections in children, elderly and adults with co-morbidities. There is a number of licensed vaccines against influenza, whereas no RSV vaccine is yet commercially available. In 1960s, an alum-adjuvanted formalin inactivated (FI-RSV) vaccine caused exacerbation of RSV disease in children after natural infection due to the skewed Th-2 response, along with attenuation of CD8 T-cell responses. This obstacle can be overcome by delivering of RSV-specific CD8 T-cell epitopes to target cells using viral vectors. In this study, we designed a bivalent vaccine against influenza and RSV by insertion of RSV M2-1-specific CTL epitopes into genome of live attenuated influenza vaccine (LAIV) virus.

**Methods:** Recombinant H7N9 LAIV viruses carrying RSV-M2 specific epitopes within NA or truncated NS1 (126) genes were generated by reverse genetics, using A/Leningrad/134/17/57 master donor virus as a backbone. BALB/c mice were intranasally immunized with two LAIV-RSV doses or received intramuscularly one dose of FI-RSV, followed by live influenza or RSV challenge. Safety and protective efficacy was measured by viral loads in lungs, cytokine (IFN $\gamma$ , IL-5) response, eosinophilic infiltration and histopathology.

**Results:** Both LAIV-RSV vaccines induced functional RSV-specific CD8 T-cell immune responses that reduced RSV titers in mouse lungs without causing pulmonary eosinophilia and inflammatory disease, in contrast to the FI-RSV group. The LAIV-RSV also induced sterile immunity against virulent homologous influenza virus.

**Conclusion:** The bivalent LAIV-RSV vaccines were safe and protected mice against influenza and RSV. Such vaccines provide a unique opportunity to fight the two most dangerous respiratory viral infections using a single vaccine preparation.

This study was supported by the Grant of the Russian Science Fund 17-75-20054.

*Keywords: Respiratory syncytial virus; viral vector*

Topic: Virology and Pathogenesis: Immune Response to Infection

Abstract No: 10427

## Immunological profile in pregnant ferrets Infected With influenza virus

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### Introduction and Objective

Pregnancy has been associated with severe influenza, an association highlighted during the 2009 pandemic of influenza A(H1N1) virus (A[H1N1]pdm09) infection. To assess the underlying mechanism, we infected pregnant and non-pregnant ferrets with A(H1N1) pdm09 virus.

### Methods

In this study, we used 10 pregnant ferrets (6 infected with A[H1N1]pdm09 and 4 uninfected) and 8 nonpregnant ferrets and ferrets were infected intranasally with 500 µL of 10<sup>5</sup> 50% tissue culture infective doses (TCID<sub>50</sub>)/mL of the A(H1N1)pdm09 virus A/California/07/09. For immunological analysis, Production of inflammatory cytokines was detected in the pulmonary tract, bronchoalveolar lavage, and peripheral blood mononuclear cells (PBMCs) and The total number of peripheral CD8<sup>+</sup> T lymphocytes was enumerated in PBMCs by flow cytometry as well as the influenza virus-specific B-cell response was determined in PBMCs by ELOSPOT analysis

### Results and Conclusion

A(H1N1)pdm09-infected pregnant ferrets also had higher levels of inflammatory cytokines in their pulmonary tracts. Systemically, total CD8<sup>+</sup> T cell counts and A(H1N1)pdm09-specific B-cell responses in blood were significantly lower in pregnant ferrets. This model predicts that the poorer outcome for pregnant women during the A(H1N1)pdm09 pandemic was due to an elevated level of viral replication and to a cytokine imbalance that led to a less effective immune response.

*Keywords: Pregnancy, pandemic influenza A virus, immune response*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10443

## THE FUNCTION OF MAMMALIAN ARGONAUTE 2 IN INFLUENZA A VIRUS INFECTION

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**Introduction and Objectives:** Argonaute 2 protein (Ago2), is a key component of the RNA-induced silencing complex (RISC) that regulates cellular processes at a post-transcriptional level via miRNA and siRNA silencing machinery. Reports are divided on whether miRNA and siRNA play a significant role in influenza A virus infection. We found that Ago2 is associated with cellular processing bodies during influenza virus infection, implicating a previously uncharacterized role for this protein in mediating virus-host interactions.

**Methods:** The IFNs and viral protein were tested by western blot and real-time PCR. We use RNA sequencing, siRNA, CRISPR/Cas gene editing technologies, a luciferase reporter assay and CLIP to explore Ago2 functional role in IAV infection.

**Results:** Expression of type I interferons (IFNs) in response to influenza A virus infection was dramatically increased in both Ago2 knockdown and knockout HEK293 cells. Ago2 can associate with both viral mRNA and viral genomic RNA in virus infected cells. Ago2 was found to directly associate with 5'ppp RNAs, which suggests that Ago2 might interfere with RIG-I sensing of 5'ppp RNAs. In A549 Ago2-KO cells, viral protein levels increased in infected cells during early hours' infection, compared to A549 control cells, while viral RNA synthesis was not significantly changed. Notably, we observed that Ago2 suppressed virus replication in infected Drosha-KO, Dicer-KO and GW182-KD A549 cells, supporting a mechanism whereby Ago2 inhibition of influenza A virus replication is independent of microRNA or RNAi pathways. Furthermore, the suppression of Ago2 on virus replication and type I interferons (IFNs) are independent of each other in A549-RIG-KO cells.

**Conclusions:** Our data suggests an undefined role for Ago2 in suppression of the interferon response and influenza A virus replication that is enacted through non-miRNA regulatory machinery. Ago2 proteins may play dual roles in suppressing host antiviral innate immunity and restricting influenza virus infection.

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10662

## Improvement of influenza virus neutralizing antibody assay using pseudoviruses expressing HA and NA

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Influenza virus is a globally important respiratory pathogen which causes significant public health issue. Also, there have been increasing concerns about newly emerging influenza viruses. Prophylactic vaccine is likely to be the most efficient countermeasure against influenza virus infections when appropriate serological investigation system is available. Hemagglutination inhibition (HI) assay is a traditional measuring tool for efficacy evaluation and microneutralization (MN) assay provides more practical to assess protective humoral immune response following vaccination. However, HI results could not represent entire antibodies response rather than head domain of HA protein. Furthermore, MN assay is time consuming, laborious and required biosafety level 3 containment when live highly pathogenic influenza strain is required. To circumvent these limitations, we generated lentivirus-based influenza pseudoviruses harboring hemagglutinin and neuraminidase from H5N8, H3N2, swH3N2 and H1N1pdm09 viruses for pseudoviruses MN (PMN) assays which can be carried out at biosafety level 2 laboratory. Serum antibody titers of PMN were highly correlated with HI and MN and sensitivity of PMN was also higher than MN. In addition, PMN was able to quantify low level of HA stalk domain specific antibodies, while HI and MN failed to detect after HA stalk based immunization. In conclusion, PMN test will be applicable to measure elaborate vaccine efficacy formulated other than head domain of the hemagglutinin in the low level bio-safe containment and can be applied promptly even when highly pathogenic live viruses are yet to be available.

This work was supported by intramural funds ( #2019-NI071-00, #2016-NI43001-00).

*Keywords: Influenza, hemagglutination inhibition assays, microneutralization assays, pseudotypeviruses*

Topic: Virology and Pathogenesis: Immune Response to Infection

Abstract No: 10670

## EXPLOITING LENTIVIRAL PSEUDOTYPES FOR THE DETECTION OF NEURAMINIDASE INHIBITING ANTIBODIES.

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### Introduction and Objectives:

Lentiviral pseudotypes represent a safe alternative to wild type virus for serological assays, especially when considering highly pathogenic viruses. Influenza pseudotypes have been extensively used in microneutralisation assays, using easily quantifiable reporters such as GFP or luciferase to interrogate viral entry in the presence or absence of an inhibitor. These pseudotypes can also be used in the enzyme linked lectin assay to detect neuraminidase activity or its inhibition – representing a simple technique towards the generation of immunological data in the absence of live virus handling.

### Methods:

Lentiviral pseudotype production was optimised for NA activity in ELLA. Pseudotypes were produced bearing the HA and/or NA of A/California/7/2009 (H1N1) and A/Texas/50/2012 (H3N2) and the core from HIV-1 with a luciferase reporter gene incorporated. These were employed in downstream inhibition assays using a defined panel of sera.

### Results:

HA/NA and NA only lentiviral pseudotypes were successfully produced and optimised using the ELLA assay, and inhibition using a defined panel of sera functioned as predicted. Co-transfection of a heterologous avian HA (A/duck/Memphis/546/5/1974 (H11)) increased NA activity titres, most likely through an increase in virion budding. Early results indicate a strong correlation between HA only and NA only pseudotype neutralisation/inhibition data using the pandemic strain and post 2009 human sera.

### Conclusion:

Rapid generation of NA inhibition data against clean NA only pseudotypes was possible using the lentiviral pseudotype platform, also allowing for the separate assay of HA entry and NA activity using the same stock of virus. This highly adaptable system is readily available and work is possible in reduced BSL conditions even when using glycoproteins from highly pathogenic strains. New genes can be synthesised rapidly and fed into this pseudotype pipeline for rapid generation of immunological data.

Topic: Virology and Pathogenesis: Immune Response to Infection

Abstract No: 10699

## SEROLOGICAL DETECTION OF AVIAN INFLUENZA A/H7 IN NIGERIAN LIVE BIRD MARKETS

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Avian influenza has been reported in domestic birds in Nigeria since 2006 and subtype H5 has continued to be detected up till date. It has been suggested that waterfowls and local chickens sold in live bird markets may be natural reservoir and source of re-infection in poultry farms. This study aims at sero-detection of avian influenza virus in waterfowls and local birds at live bird markets in Plateau State, Nigeria. A total of 292 sera were collected over a period of three months and analysed by C-ELISA to screen for antibody response to influenza A nucleoprotein. Haemagglutination Inhibition (HI) specific for subtype H5, H9 and H7 was also carried out using standard protocols. The results showed seroprevalence of 5.1% (n=15) for influenza A. Serotype H7 was thereafter detected by HI in 5 of the 15 influenza A positive samples. The H7 positive sera also reacted with H7N3, H7N4, H7N1 and H7N7 virus strains with HI titre ranging between 1:32 to 1:512. This investigation for the first time showed serological evidence of influenza A subtype H7 in local birds and waterfowls sold at three live bird markets in Plateau State, Nigeria. Further virological surveillance to detect and isolate the virus is important in order to better understand influenza virus epidemiology in Nigeria and the potential risk that influenza poses to poultry production and public health.

*Keywords: Influenza A, subtype H7, serological detection, live bird market, Nigeria.*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10709

## Use of a biological assay to mitigate vaccine pyrogenicity

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**Background:** There were increased reports of fevers and febrile reactions in young children (particularly children aged < 5 years) receiving the Seqirus/CSL Southern Hemisphere 2010 trivalent inactivated influenza vaccine (IIV3); modifying the vaccine manufacturing process by increasing the minimum concentration of the splitting agent (sodium Taurodeoxycholate [TDOC]) to 1.5% w/v resolved this issue.

As part of scientific investigations, an assay measuring the Toll-like receptor (TLR)-3 and TLR-8 response to vaccine was developed for the assessment of potential vaccine pyrogenicity (NF-kB assay). The NF-kB assay is utilized to confirm suitability of manufacturing conditions as new strains are introduced for vaccine manufacture.

### Methods:

All new vaccine strains are screened via the NF-kB assay to determine potential vaccine pyrogenicity. In the event of a positive NF-kB response, the TDOC splitting agent may be increased up to 2.5% w/v TDOC, or an alternate vaccine strain may be selected.

### Results:

The NF-kB response to vaccine candidate viruses split using different levels of TDOC was assessed. Data from these splitting studies demonstrated that when the TDOC concentration was optimized, the NF-kB signal remained below the assay limit of detection (LOD) even when sensitivity of the assay was increased by testing samples at 6-fold the standard test concentration.

### Conclusion:

Data from this study confirm that the NF-kB Assay is a valuable tool for screening new virus strains to confirm suitability of manufacturing condition. This represents a practical example of the use of a biological assay to reduce potential vaccine pyrogenicity risk.

*Keywords:* Vaccine; Assay; NF-kB;

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10729

## H7-reactive memory B cell expansion by adjuvanted inactivated H7N9 influenza virus vaccination

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**Introduction and Objectives:** H7N9 influenza virus infections continue to occur in humans and concerns remain about the pandemic potential of the virus. Early H7N9 influenza virus vaccines, even in a 2-dose regimen, generated little protective antibody as measured by hemagglutination inhibition and neutralization assays, suggesting that the H7 hemagglutinin was poorly immunogenic. It is now understood that initial exposure to H7 in adult humans generates H7-specific antibodies that are mostly against the stalk domain, likely reflecting activation of preexisting memory B cells (MBCs) reactive to the conserved stalk in the relative absence of MBCs reactive to the novel H7 head. A strongly protective antibody response to H7 vaccination probably occurs only after an H7 head-reactive MBC population is established. In more recent studies, two doses of an adjuvanted H7 vaccine generated protective antibody levels in adult recipients. To relate H7 vaccine effectiveness to MBC population changes, we measured H7 head- and stalk-reactive MBCs in H7-naïve adults given an adjuvanted H7N9 inactivated influenza vaccine.

**Methods:** Subjects received two doses, 4 weeks apart, of an inactivated A/Shanghai/2/2013 (H7N9) influenza virus vaccine with adjuvant. Samples were collected at intervals after each dose. MBCs were measured by in vitro stimulation to induce antibody-secreting cell formation, with antigen-specific ELISpot assays as readouts for precursor MBCs.

**Results:** Circulating H7 head-specific MBCs were generated by a single dose of the vaccine and further expanded by boosting. In contrast, expansion of stalk-specific MBCs occurred in only a small number of subjects after the first vaccine dose, and in even fewer subjects after the second dose.

**Conclusion:** The presence of adjuvant with H7 vaccination quickly establishes patterns of H7-reactive MBC expansion that reflect the immunodominance of the head domain, perhaps by increasing naïve B cell activation and promoting germinal center formation and maintenance.

*Keywords: avian influenza; H7 hemagglutinin; memory B cells; vaccine; adjuvant*



Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10754

## INFLUENZA B VIRUS INFECTION IN HUMANS INDUCES BROADLY CROSS-REACTIVE AND PROTECTIVE NEURAMINIDASE-REACTIVE ANTIBODIES

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### Introduction and Objectives

Influenza remains a major global burden due to the absence of optimal vaccines and therapeutic options. Neuraminidase (NA) is an exosialidase that facilitates the release and migration of influenza viruses. Murine anti-influenza B virus NA antibodies are capable of protecting mice against lethal influenza virus challenge. Here, we describe novel human monoclonal antibodies (mAbs) targeting NA isolated from an influenza B virus infected patient.

### Methods

We generated a panel of recombinant human mAbs from single cell-sorted plasmablasts isolated from peripheral blood of an influenza B virus-infected patient. We characterized the binding breadth and functionality of the mAbs using different serological assays including enzyme-linked immunosorbent assays and neutralization assays. We also tested the prophylactic and therapeutic effects of the mAbs in mouse viral challenge experiments.

### Results

Approximately one third of the influenza B virus infection-induced plasmablasts targeted NA. Monoclonal antibodies generated from these cells displayed broad cross-reactivity to diverse influenza B strains from both antigenic lineages. These mAbs protect mice against lethal doses of recent influenza B strains from both the B/Victoria/2/87-like and B/Yamagata/16/88-like lineages in a prophylactic setting. Some of these mAbs are protective in a therapeutic setting when given 72 hours post infection.

### Conclusion

We have shown that influenza B virus infection can induce protective NA specific antibodies that are broadly cross-reactive. These findings are important for the development of novel vaccines and therapeutics that can broadly protect from influenza virus infection.

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10787

## A NOVEL INFLUENZA SPECIFIC ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY ASSAY

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**Background:** Antibody Dependent Cell-mediated Cytotoxicity (ADCC) is an assay to analyze Fc-mediated cytotoxic functions of effector cells against target cells. Detection of ADCC antibodies may predict vaccine efficacy. Influenza hemagglutinin (HA) and neuraminidase (NA) expressed on the infected host cell surface during infection induce antibodies including ADCC antibodies that mediate effector cell killing of influenza-infected cells. Prior studies of influenza ADCC antibody responses have assessed effector cell activation or response to antibody bound to influenza or influenza proteins. We developed a cell-killing ADCC assay with NK killer cells as effector cells and target cells expressing influenza HA, NA or HA+NA proteins.

**Method:** The assay has the following key components: 1) Target 293 cells that have been stably transfected with GFP and luciferase reporter genes are stably transfected with doxycycline-inducible HA, NA or HA+NA proteins from the 2014-2015 influenza H3N2 seasonal vaccine strain. 2) The effector cells are NK cells expressing high levels of CD16. 3) Killing is determined as luciferase activity compared to control treatment. Performance of the assay was then assessed through use of a panel of 11 influenza-naïve infant samples and 10 influenza-infected samples.

**Results:** A panel of influenza-infected adult serum and influenza-naïve infant serum samples were evaluated by this assay. Almost no target cell lysis (mean of 2.16% ± 3.14%) was observed with 11 influenza-naïve infant samples at the highest concentration of serum (1:100 dilution). In comparison, high-level target cell lysis occurred with 10 adult samples (86.89 ± 8.4%; range 73.41-94.27%) at the highest serum dilution.

**Conclusion:** This cell-based assay can measure antibodies that mediate cell lysis of target cells that present influenza HA and NA proteins on their surface. This assay is specific and sensitive and can be used to evaluate the ADCC functionality of anti-influenza antibodies. This could be useful in future influenza vaccine studies.

*Keywords: Antibody Dependent Cell-mediated Cytotoxicity (ADCC); Influenza hemagglutinin (HA); Influenza neuraminidase (NA); Target cells; NK-CD16 effector cells.*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10803

## SEROLOGICAL ASSAYS FOR DETECTION OF ANTIBODY LEVELS AGAINST SEASONAL INFLUENZA VIRUSES AND CORRELATES OF PROTECTION

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### INTRODUCTION AND OBJECTIVES

The immunological response to influenza vaccine and/or natural infection is evaluated by serological techniques such as the haemagglutination inhibition (HI), single radial haemolysis (SRH), virus neutralization (VN) and ELISA.

Although the traditional correlates of protection, as defined by HI titer  $\geq 40$  and SRH area  $\geq 25 \text{ mm}^2$ , have been withdrawn, they are still deeply debated.

The aim of this study was to establish the correlation among the four immunological assays and to evaluate the agreement on correlates of protection between HI and SRH assays.

### METHODS

The egg-derived viruses used for this study were seasonal influenza strains: A/California/07/2009 H1N1, A/Texas/50/2012 H3N2, B/Brisbane/60/2008 Victoria lineage and B/Massachusetts/02/2012 Yamagata lineage.

The human serum samples from adults were obtained from the laboratory serum bank and analyzed by HI, SRH, VN assays and ELISA.

### RESULTS

For influenza A and B strains, a strong positive correlation was found among the HI, SRH and VN assays. ELISA showed good correlation with the other three assays for both strains.

The assay agreement on protection was good for the A strains and very low for the B strains, particularly for Yamagata lineage.

### CONCLUSION

Overall, the data showed positive strong correlation among the four serological assays for both strains, especially for the HI and VN assays and highlighted the need for further investigation on the correlation between the SRH assay and ELISA.

Concerning the correlates of protection, the results suggested that the SRH is more sensitive than HI in detecting antibodies against the influenza B viruses and the protective HI level may be lower (HI threshold of 20).

The combination of all the assays could considerably improve the assessment of the immunogenicity of influenza vaccines and provide a more complete picture of antibody response.

*Keywords: Serological assays, seasonal influenza viruses, correlates of protection*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10865

## **MF59 increases the overall functional potency of vaccine-specific humoral immunity, but does not increase vaccine-specific antibody NK cell activation**

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### **Introduction and Objectives**

Seasonal and pandemic influenza infection remains a major public health concern worldwide. Driving robust humoral immunity in the face of pre-existing, often cross-reactive, immunity and particularly poorly immunogenic avian antigens has been a challenge. To overcome immune barriers, the adjuvant MF59 has been used in seasonal influenza vaccines to increase antibody titers and improve neutralizing activity, translating to a moderate increase in protection in vulnerable populations. However, its impact on stimulating antibody effector functions, including NK cell activation, monocyte phagocytosis, and complement activity, all of which have been implicated in protection against influenza, have yet to be defined.

### **Methods**

Using systems serology, a suite of assays that assess the capacity of antibodies to induce innate immune effector functions, changes in antibody functional profiles were assessed in healthy individuals who received experimental H5N1 avian influenza vaccine administered with MF59, with alum, or delivered unadjuvanted.

### **Results**

MF59 elicited antibody responses that stimulated robust monocyte and neutrophil phagocytosis, as well as complement activity. Conversely, vaccination with MF59 recruited NK cells poorly and did not augment FCGR3A binding. Vaccination with MF59 created a unique vaccine response profile that is distinct from unadjuvanted or alum-adjuvanted vaccination, and is specific for vaccine antigens.

### **Conclusion**

MF59 induces higher antibody titers, but does not increase antibody-dependent NK cell activation, which is a key component of the anti-influenza immune response. MF59 can also overcome pre-existing immune responses to influenza when delivered with a novel antigen. Collectively, defining the humoral antibody functions induced by distinct adjuvants may provide a path to design next generation vaccines able to selectively leverage the humoral immune functions, beyond binding and neutralization, resulting in better protection from infection.

*Keywords: antibody; innate immunity; adjuvant; vaccine; MF59*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10887

## SUBCLADE 2.2.1-SPECIFIC HUMAN MONOCLONAL ANTIBODIES THAT RECOGNIZE AN EPITOPE IN ANTIGENIC SITE A OF INFLUENZA A(H5) VIRUS HA DETECTED BETWEEN 2015 AND 2018

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**Introduction:** Highly pathogenic avian H5 influenza viruses persist among poultry and wild birds throughout the world. Sometimes, interspecies transmission occurs between avian and mammalian hosts. H5 viruses possessing the HA of subclade 2.3.4.4, 2.3.2.1, 2.2.1, or 7.2 were detected between 2015 and 2018. To understand the neutralizing epitopes of H5-HA, we characterized 15 human monoclonal antibodies (mAbs) against the HA of H5 viruses of subclade 2.2.1.

**Methods:** We examined 15 human mAbs against H5-HA, which were obtained from volunteers who received the inactivated, adjuvanted whole-virion pre-pandemic vaccine including A/Egypt/N03072/2010 (subclade 2.2.1) or A/Indonesia/5/2005 (subclade 2.1.3.2). We examined their reactivity, HI activity, and neutralizing capability against H5 viruses, belonging to subclade 2.3.4.4, 2.3.2.1, 2.2.1, or 7.2 that were detected between 2015 and 2018. To identify the epitope of the neutralizing mAbs, we generated escape mutant viruses. We also evaluated the antigenicity of the identified epitope by using human sera from volunteers who received the pre-pandemic H5 vaccine.

**Results:** Twelve mAbs were specific for the HA of subclade 2.2.1, 2 mAbs were specific for the HA of subclade 2.1.3.2, and 1 mAb was specific for the HA of both. Of the 15 mAbs analyzed, 9 that were specific for the HA of subclade 2.2.1 and shared the VH and VL genes, possessed hemagglutination inhibition and neutralizing activities, whereas the others did not. A single amino acid substitution or insertion at positions 144–147 in antigenic site A conferred resistance against these 9 mAbs to the subclade 2.2.1 viruses. The amino acids at positions 144–147 are highly conserved among subclade 2.2.1 but differ from those of other subclades.

**Conclusion:** The neutralizing epitope including amino acids at positions 144–147 in antigenic site A is targeted by human antibodies and plays a role in the antigenic difference between subclade 2.2.1 and other subclades.

**Keywords:** Influenza A virus; H5-HA; human monoclonal antibody; escape mutant virus

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10893

## Assessing antibody function in responses to twice-annual vaccination due to 2014/2015 H3N2 antigenic mismatch in Hong Kong.

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### Introduction and Objectives

Mis-matches can occur between selected inactivated influenza vaccines (IIV) strains and circulating strains resulting in reduced vaccine effectiveness and excess influenza-associated mortality, especially in the most susceptible age groups, older adults >65 years of age. Hong Kong has a subtropical climate and can experience both a winter and summer seasonal influenza epidemics. In winter 2014/15, Hong Kong experienced a substantial A(H3N2) winter epidemic with a mismatched Northern hemisphere (NH) vaccine. For the first time in 2015, the Hong Kong health authorities procured the southern hemisphere vaccine, which included an updated and matching A/Switzerland/9715293/2013(H3N2) strain, for a targeted summer vaccine campaign, resulting in some older adults being vaccinated twice-annually for the first time.

### Methods

We compared immune parameters in pre- and post-vaccination sera and PBMCs from older adults ≥75 years of age who received one versus two influenza vaccines per year.

### Results and Conclusion

We recently reported that one round of twice-annual vaccination resulted in elevated HAI titers in the second round of vaccination, but reduced H3N2-specific influenza-specific CD4<sup>+</sup>T cell responses. Here, we further report on once versus twice annual vaccination effects on recruitment of T follicular helper cell and plasmablast B cell responses, which are important drivers for high quality antibody responses. We further characterised influenza-specific antibody quality by IgG subclasses associated with ADCC effector functions. Twice-annual vaccination in subtropical regions for older adults may overcome waning immunity, prolonged influenza seasons, and antigenic drift in circulating strains.

*Keywords: Vaccine, antibody function, ADCC, T cells*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10947

## HA-stalk Reactive Secretory IgA Antibodies Exhibit Anti-viral Activity by Steric Hindrance of Viral HA and NA

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### Introduction

The intranasal inactivated influenza vaccine (IIV) could induce secretory IgA antibodies (SIgAs) in the upper respiratory tract mucosa, and therefore is expected to be more effective in preventing virus infection compared to the current injectable vaccine. Due to occasional antigenic mismatches between circulating virus and vaccine virus, whether influenza vaccine strategies could induce influenza virus broadly neutralizing antibodies (bnAbs) is an important issue. In this study, an influenza bnAb clone was obtained from an intranasal IIV recipient and the anti-viral functions of this bnAb were evaluated.

### Method

Plasmablasts were obtained from healthy human volunteers who were administered with A/Indonesia/5/05 (H5N1) whole virus intranasal IIVs. Antibody genes of each plasmablast were subcloned into IgG expression vectors and expressed. An influenza bnAb clone, F11 was selected on the basis of genetic characteristics, reactivity with HA, virus neutralization activity, and escape mutant analysis. Anti-viral activities: reactivity with HA, hemagglutination inhibition (HI) activity, and virus neutralization activity of F11 were measured in its IgG, monomeric IgA, and multimeric SIgA forms. Neuraminidase (NA) inhibition (NI) activity due to steric hindrance of NA by antibodies was measured by Enzyme-Linked Lectin Assay (ELLA).

### Result

An HA stalk reactive bnAb, F11 which could neutralize influenza virus subtypes H1N1 and H5N1 was obtained. IgA multimerization of F11 enhanced antibody reactivities with HA, HI activity, and virus neutralization activity of antibodies. Higher levels of interference with NA and HA RBS were measured in F11 SIgA multimers compared to monomers by ELLA and SPR analysis, respectively.

### Conclusion

The current study revealed that intranasal IIVs could induce influenza bnAbs in humans. Results indicated that IgA multimerization enhances anti-viral functions of anti-HA stalk antibodies not only by increasing the avidity of antibodies, but by various mechanisms of action, such as steric hindrance of HA RBS and NA.

*Keywords: intranasal inactivated vaccines, secretory IgA antibodies, broadly neutralizing antibodies*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10948

## NEURAMINIDASE ANTIBODY RESPONSES TO A(H1N1)pdm09 PANDEMIC VACCINE IN UK CHILDREN

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### INTRODUCTION AND OBJECTIVES

Neuraminidase-inhibiting (NAI) antibodies are an independent correlate of protection following natural influenza infection or vaccination. NAI antibodies generated by vaccination with inactivated influenza vaccines contribute to vaccine effectiveness by independently protecting from natural infection.

An enzyme-linked lectin assay (ELLA) was used to analyse NAI antibodies in sera from children vaccinated with two different pandemic vaccines in a head-to-head trial. Correlation between NAI and haemagglutination-inhibition (HAI) antibody titres pre- and post-vaccination within different age groups, by vaccine type, and with respect to pre-existing (baseline) antibody levels, were determined and compared to data from a similar trial in adults.

### METHODS

Paired residual sera were obtained from head-to-head trials conducted during 2009/10 of adjuvanted split-virion versus non-adjuvanted whole-virion A(H1N1)pdm09 influenza vaccines in UK children (6 months to 12 years) (ISRCTN89141709) and in adults (18 to >65 years) (ISRCTN92328241). Recombinant virus with NA from A(H1N1)pdm09 A/California/7/2009 and antigenically mismatched haemagglutinin (HA) (NIBRG-127, NIBSC, UK) was used for determination of NAI antibody by ELLA. Antibody titres to HA were measured by HAI using recombinant virus with HA from A(H1N1)pdm09 A/California/7/2009 (NIBRG-121, NIBSC, UK).

### RESULTS

Measurement of baseline NAI antibody titres found that younger children (<3 years) did not have pre-existing antibodies to A(H1N1)pdm09 NA and HA, in contrast to older children (3-12 years) where pre-existing NAI, but little HAI titres were found. Children (3-12years) showed a significantly higher increase in NAI antibody titres at 21 days following vaccination compared to adults (18-65 years). Increases in HAI and NAI titres were significantly correlated in all age groups.

### CONCLUSION

NAI antibody was detected in children vaccinated with pandemic A(H1N1)pdm09 vaccines with younger children (<3 years) showing a primary response, and older children a boosting response at 21 days post-vaccination. Optimisation and standardisation of neuraminidase content should be considered to improve performance of inactivated influenza vaccines.

*Keywords: neuraminidase; NAI; vaccine; ELLA; antibody*



Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 10987

## Gene regulation and antiviral activity of IL-36 in response to influenza virus infection.

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### INTRODUCTION

*Influenza virus-induced pneumonia and acute respiratory distress syndrome (ARDS) are associated with high morbidity and significant mortality in susceptible hosts, causing a profound health burden worldwide. The interleukin (IL)-36 including three agonists IL-36 $\alpha$ , IL-36 $\beta$ , IL-36 $\gamma$ , are novel members of IL-1 superfamily of cytokines, which stimulates inflammatory signals via binding to the IL-36 receptor (IL-36R). The function of IL-36 cytokines among specific influenza-induced ARDS population and mechanism during immune response in host defense against influenza infection remain largely unknown.*

### METHODS

*A total of 20 influenza-induced ARDS patients and 12 healthy individuals were enrolled. IL-36 expression was detected using enzyme-linked immunosorbent assays, real time-PCR and flow cytometry analysis. Cell models were subjected to clarify mechanisms associated with IL-36 expression and functions related to viral production and replication.*

### RESULTS

*Clinical finding demonstrates that up-regulation of IL-36 $\alpha$  and IL-36 $\gamma$  in plasma from influenza-induced ARDS patients. Moreover, the increased levels of IL-36R in PBMC from influenza-induced ARDS patients may imply a potential role of IL-36/IL-36R signaling system in the inflammatory pathogenesis of severe influenza infection. Furthermore, IL-36 is significantly up-regulated in various cell lines during influenza infection and COX-2 and iNOS may be important factors in influenza-induced IL-36 expression. In addition, we identified a potential anti-influenza activity of IL-36 $\alpha$  and IL-36 $\gamma$ , which may be depend on expression of interferon and its downstream effectors.*

### CONCLUSIONS

*In this study, we propose a hypothetical model of influenza induced IL-36 production and its biological. Influenza infection stimulated IL-36 expression via activation of COX-2 and iNOS pathway, then IL-36 activates interferon and interferon-stimulated genes to cause inhibition of influenza replication and production. In conclusion, this study identifies a critical role for IL-36 in the immune response to influenza infection and that could be useful as a prognostic predictor or biomarker of influenza-induced ARDS.*

**Keywords:** IL-36, influenza, ARDS, antiviral

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 11022

## Functional activity of M2e-specific antibodies induced by live attenuated influenza vaccine (LAIV) carrying four M2e tandem repeats embedded in the hemagglutinin molecule

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**Introduction and objectives.** The highly conserved ectodomain of influenza virus M2 protein (M2e) is a promising target for universal influenza vaccine development. Expression of M2e tandem repeats within the hemagglutinin (HA) molecule of LAIV virus is a new strategy of induction M2e-specific antibody. These antibodies neither bind efficiently to the free virus nor neutralize virus infection. Instead, they bind to M2 protein expressed on the surface of virus-infected cells and protect from natural infection. In this study we assessed functional activity of M2e-specific antibodies using two methods.

**Methods.** Recombinant LAIV viruses expressing chimeric HA+4M2e proteins were generated by the means of reverse genetics using A/Leningrad/17 LAIV backbone. The expression of M2e epitopes by chimeric viruses was confirmed by ELISA with M2e-specific antibody 14C2. BALB/c mice were immunized with two doses of chimeric LAIV+4M2e, control mice received either LAIV or PBS. ADCC was performed by co-culturing murine NK-cells with virus-infected MDCK cells in the presence of mouse immune sera. To assess cytotoxic degranulation flow cytometry was used (CD107a, CD49b, CD45 and CD3). Complement-dependent cytotoxicity (CDC) was carried out on MDCK-infected cells incubated with mice sera as objects and guinea pig serum as complement source, followed by flow cytometry with propidium iodide.

**Results.** Both cytotoxicity methods were successfully validated using high-titer mouse anti-HA antibody in MDCK-infected cells model. In the ADCC assay, significant levels of NK cells degranulation were observed in mice immunized with chimeric LAIV+4M2e viruses. Cytotoxic effect of M2e-specific antibody in CDC was too weak and none of the experimental groups had significant differences in CDC levels.

**Conclusions.** ADCC is preferable method for the assessment of functional activity of M2e-specific antibodies and it could be recommended for further studies of immunological mechanisms underlying the protective effect of LAIV+4M2e experimental vaccines.

**Funding.** This work was supported by RSF grant 19-15-00015.

**Keywords:** *Universal vaccine; ADCC; CDC*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 11030

## Assessing mechanisms of antibody neutralization: heterogeneity among broadly neutralizing antibodies

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Neutralizing antibodies against influenza have generally been classified according to their recognition sites, with antibodies against the head domain of hemagglutinin thought to inhibit attachment and antibodies against the stalk region thought to inhibit fusion. Here, we report the development of a microfluidic assay to measure neutralization of viral entry that can clearly differentiate between effects on attachment and fusion. Testing multiple broadly-neutralizing antibodies against the hemagglutinin stalk domain, we obtain a surprising result: some broadly-neutralizing antibodies inhibit fusion only, while others inhibit both fusion and viral attachment. As expected, antibodies tested against the globular head domain inhibit attachment alone. The assay is both microscale and parallel, permitting the assessment of multiple antibodies in small quantities such as from serosamples. These findings shed light on the unexpectedly heterogeneous mechanisms of antibody neutralization even within similar recognition sites. The assay we have developed also provides a tool to optimize vaccine design by permitting assessment of the elicited antibody response with greater mechanistic resolution.

*Keywords: broadly neutralizing antibodies, vaccine design, microfluidics, membrane fusion*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 11048

## Evaluating protein yield and immuno-efficacy for the recombinant HA of H6 avian influenza produced from a novel secretory bi-cistronic baculovirus expression system

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### Introduction

Glycosylation on HA of influenza virus has been shown to play a key role in triggering immune responses and a unique structural protection mechanism from tryptic cleavage. Eukaryotic protein expression system with post-modification is beneficial for the development of subunit vaccines to replace egg-based vaccines. In our previous research, we have established a novel secretory bi-cistronic baculovirus protein expression system to produce the eukaryotic recombinant HA fragment with *N*-glycans. This system can facilitate the expression, detection and isolation of recombinant HA fragment in an insect (*Spodoptera litura* larvae) and a culture of Sf21 cells (*Spodoptera frugiperda*). The recombinant HA fragment of H6 avian influenza had been verified to be glycosylated, and monomeric and trimeric forms were identified. In this study, we want to evaluate its protein yield and immuno-efficacy more deeply. In addition, we try to compare with traditional *Escherichia coli*-based protein expression system.

### Method

The secretory bi-cistronic baculovirus eukaryotic protein expression system and traditional *Escherichia coli*-based prokaryotic protein expression systems were used to produce the recombinant HA fragment of H6N1 avian influenza virus (2838V) in cell level. The products were purified by His-select nickel affinity and ion exchange chromatography. The protein yield was evaluated by H6 specific mAb, EB2. The products were used to immunize the BALB/c mice. During the immunization schedule, mouse sera were collected and analyzed for specificity using Western blotting. Finally the spleen cells were collected for enzyme-linked immunospot assay (ELISPOT) for immuno-efficacy evaluation.

### Result

The protein yield evaluation for the novel secretory bi-cistronic baculovirus protein expression system was established. In addition, the protein yield and immuno-efficacy comparison with traditional *Escherichia coli*-based system was discussed for the first time.

### Conclusion

In this study, the results suggest that the secretory bi-cistronic baculovirus protein expression system is beneficial for the development of next generation influenza subunit vaccines.

**Keywords:** Avian influenza virus (AIV); Hemagglutinin (HA); Bi-cistronic baculovirus expression system; Subunit vaccine; ELISPOT

Topic: Virology and Pathogenesis: Immune Response to Infection

Abstract No: 11069

## INFLUENZA VACCINE IMMUNITY IS MODULATED BY HUMAN-LIKE HEMAGGLUTININ (HA) T CELL EPITOPES

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### Introduction and Objectives

Sequence variation is one means by which influenza escapes effective immune response; another, described here, is to impede the development of effective B cell responses and to limit the development of high affinity antibodies through immune regulation. We previously discovered regulatory T cell (Treg) epitopes bearing homology to human sequences in H7N9 avian influenza. We hypothesized that similar human-like epitopes in the sequences of seasonal influenza viral proteins may modulate or limit seasonal influenza vaccine efficacy. Here we describe our method for identification and validation of Treg-inducing human-like Treg epitopes in seasonal influenza.

### Methods

We obtained the sequences of the WHO-recommended trivalent vaccine strains for 2008 and 2009 from GenBank (A/H1N1/Brisbane/59/2007, A/H3N2/Brisbane/10/2007, A/H1N1/California/07/2009, A/H3N2/Perth/16/ 2009, B/Brisbane/60/2008). We searched for human-like epitopes using the EpiMatrix, ClustiMer and JanusMatrix algorithms, and identified the corresponding peptides in the BEI influenza epitope resource (BEI, NIAID, NIH). T cells from vaccinated donors from these seasons were exposed to BEI peptide pools containing the human-like epitopes in long-term cultures, ex vivo, and then stained for CD4, CD127, and FoxP3 and sorted by flow cytometry.

### Results

The algorithms identified 16 distinct human-like peptides in the HA sequences of the seasonal vaccine strains; eight from H1, three from H3 and five from influenza B. Three of the H3 human-like epitopes were conserved in the 2008 and 2009 seasonal H3 strains. H1 sequences contained three different human-like peptides in each of the two seasons. Preliminary flow cytometry results from cell samples treated with peptide pools containing the human-like peptides showed T cells expressing higher levels of PD-1 and greater numbers of activated Treg than untreated cells.

### Conclusion

Treg-activating epitopes may reduce anti-influenza antibody titer and affinity, contributing to a mechanism of immune evasion that may as yet be underappreciated in season influenza vaccination.

*Keywords: Seasonal influenza vaccine; Regulatory T cell epitopes; Immunoinformatics; Flow cytometry staining*

Topic: Virology and Pathogenesis: Immune Response to Infection  
 Abstract No: 11097

## IMPACT OF PRE-EXISTING SPECIFIC INFLUENZA ANTIBODIES AS A DETERMINANT OF DISEASE SEVERITY AND CLINICAL OUTCOME ON INFLUENZA VIRUS-INFECTED PATIENTS

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**Introduction and Objectives:** It is unclear to what extent pre-existing antibody-mediated immunity shapes influenza virus infection in humans. Similarly, the influence of previous history of influenza vaccination on disease severity and clinical outcome is not well understood. We aimed to determine the balance and functionality of pre-existing antibodies against the surface proteins of the influenza virus that correlates with protection in humans.

**Methods:** We systematically evaluated the aggregate properties of circulating antibodies in serum by HI assay and ELISA (HA, stalk-specific and full-length, and NA) at the onset and convalesce of influenza infection on a cohort of H1N1/Cal09 influenza virus-infected patients (solid organ transplant recipients, SOTRs). Responses after TIV influenza vaccination were also evaluated in uninfected SOTRs. Antibody-mediated effector functions were also measured by readouts of multiple assays that evaluate specific activity against influenza surface proteins. Severe disease was computed as presence of lower respiratory symptoms (LRS) or viral pneumonia.

**Results:** Overall, humoral immune response was greater after natural influenza infection compared to uninfected TIV-vaccinated SOTRs. Meanwhile infection strongly induced antibodies for all the serological metrics, influenza vaccination failed to induce stalk-specific antibodies against H1N1/Cal09 protein. Frequency of seasonal influenza vaccination before the flu episode was 65%, however only 22.5% were seroprotected. Results demonstrated that presence of anti-HA (stalk and full-length) antibodies at the onset of the infection was inversely correlated against the development of LRS. Likewise, serum ADCC activity against the H1N1/Cal09-stalk was higher on SOTRs with mild disease. Interestingly, HI or NA antibodies did not significantly influence protection and clinical outcome on SOTRs.

### Conclusions:

This study provides a precise understanding of host immune responses and risk factors associated with clinical outcome and protective immunity in patients with influenza virus infection. Understanding which antibody feature correlate with protection in humans could accelerate discovery and provision of therapeutic strategies.

*Keywords: correlate of protection, stalk antibody, influenza severity, immune response to flu*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 11100

## NOVEL APPROACHES FOR STUDYING CELL-MEDIATED IMMUNE RESPONSES TO INFLUENZA VACCINATION IN HUMANS

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**Introduction:** Vaccination is the most cost-effective strategy for preventing influenza. However, a substantial proportion of individuals do not respond or respond poorly to vaccination. Hence, delineating the mechanisms and identifying molecular signatures associated with immunogenicity of influenza vaccines in different populations is crucial to improve the vaccine efficacy.

**Methods:** Comprehensive antibody panels were developed to identify the new paradigms in the innate priming environment, focusing on natural killer cells, innate lymphoid cells, mucosal-associated invariant T cells and g $\delta$  T cells by multiparametric flow cytometry of peripheral blood mononuclear cells from vaccine recipients. To probe the adaptive response, sialic acid receptor binding site mutant HA (H1, H3, B) and HA stem (group 1 and 2) probes were conjugated to fluorochromes to characterize HA-specific B cells. By combining cell sorting, single cell molecular analyses, and the Illumina MiSeq system, paired heavy and light chains of B cell receptors from isolated antigen specific B cells were sequenced.

**Results:** We observed a decreased number of the above cell subsets before vaccination, as well as a lack of expansion upon vaccination in PBMCs from older adults (>65 years), as compared to young adults (20-30 years). The specific functions of these cells are being analyzed using single cell sorting and high-throughput transcriptomic and proteomics analysis. HA-specific B cells from an individual vaccinated with 2012-13 trivalent inactivated influenza vaccine demonstrated increased level of class switched B cells, increased mutation level in variable Ig genes and clonal expansion.

**Conclusion:** Decreased number of NK, ILC, MAIT and g $\delta$  T cells and lack expansion of these cell subsets were detected in older vaccinees. Their function in response to influenza vaccination is under investigation. Work flow to examine antigen-specific B cells and acquire paired BCR sequences was set up to dissect the specificities, breadth and depth of antibody responses.

**Keywords:** cell-mediated immune response, influenza vaccines, innate immunity, antigen-specific B cells

Topic: Virology and Pathogenesis: Immune Response to Infection  
 Abstract No: 11105

## The Platelet Innate Immune Response to Influenza Virus Infection

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**Introduction and objectives** Platelets (thrombocytes) are key players in hemostasis; the process that protects the system from unnecessary blood loss. The emerging view of platelets seen as immune cells places the underappreciated influenza symptom of thrombocytopenia and cardiovascular complications into a different perspective, of which the cause(s) or underlying mechanism(s) remain to be elucidated.

**Methods** We show by flow cytometry and electron microscopy in influenza A/H1N1 patients and in a ferret infection model that platelets take up and clear influenza viruses from the lungs.

**Results and Discussion** We observe that virus load and platelet counts correlate inversely along the course of virus infection in patients and ferrets. Loss of platelets (thrombocytopenia) reaches 22% in A/H1N1 and up to 62% in ferrets infected with an highly-pathogenic H5N1 virus strain. This influenza symptom is the result of direct interaction between platelet and virus as we observe virus-containing platelets in the blood drawn from the infected ferrets. Uptake of these viruses is clathrin-mediated and requires binding to the sialic acid glycan receptor. Accordingly, surface sialic acids are removed from infected platelets by the virus neuraminidase designating them for degradation in the liver. We propose a paradigm for clearance of respiratory viruses via this route. Importantly, since the observed interaction causes platelets to become activated this disturbed platelet function should contribute to acute cardiovascular events. These new insights clarify the pathophysiology of influenza and for the first time shows how severe respiratory infections can propagate cardiovascular disease.

**Keywords:** influenza; platelets;



Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 11139

## THE IMMUNOMODULATORY EFFECTS OF FAK IN HIGHLY PATHOGENIC AVIAN INFLUENZA A/H5N1 VIRUS INFECTION

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**Introduction and Objectives:** The highly pathogenic avian influenza (HPAI) H5N1 virus is still considered a worldwide health threat. Since the first recorded human case of infection in 1997, the mortality rate has remained high at near 60 percent. Dysregulation of cytokine and chemokine induction, excessive inflammatory infiltrates, and viral-induced tissue destruction are the main characteristics of a severe H5N1 viral infection. Neuraminidase inhibitor (NAI) oseltamivir continues to be the most widely used drug for treating H5N1 viral infections. However, there is evidence that H5N1 viral neuraminidase mutations can lead to drug resistance against NAIs. Alternative influenza drug treatments have arisen which focus on targeting the hosts' intracellular signalling cascades. One of the targets will be focal adhesion kinase (FAK), which is activated during H5N1 infection.

**Methods:** Human PBMC-derived macrophages and primary alveolar epithelial cells were infected with influenza A/H5N1 virus and FAK activity were minimalised upon the treatment of an inhibitor. Cytokine and chemokine expression were measured by quantitative real-time polymerase chain reaction, signalling pathways involved were identified by Western blotting, and an intracellular signalling antibody array.

**Results:** We were able to significantly reduce both cytokine and chemokine mRNA levels in drug-treated, virus-infected cells when comparing to vehicle-treated, virus-infected cells. Additionally, activation of both IRF3 and p38 were also reduced in the presence of the inhibitor. Moreover, the use of an intracellular signalling array identified downstream proteins related to FAK activation.

**Conclusion:** FAK plays a role in immunomodulation during HPAI H5N1 infection. Signalling molecules downstream of FAK may be also involved in regulating the host immune response during HPAI virus infection and they are potential targets for development of novel therapeutic treatments for treating influenza illness.

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 11197

## Nasal cytokine profiles of patients hospitalised with rhinovirus Species C associated respiratory wheeze differ in children with and without pre-existing asthma

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Rhinovirus C (RV-C) is an important pathogen of children hospitalised with episodic wheeze. Here we determine the nasal cytokine profiles of these children and investigate their relationship with RV-C load and asthma diagnosis.

Flocked nasal swabs were collected from 604 children aged between 24 and 72 months presenting at a paediatric hospital emergency department a clinical diagnosis of acute wheeze. Respiratory virus testing was done using an in-house multiplex PCR for RSV, influenza virus, human metapneumovirus and parainfluenza viruses. RV testing was done using a separate endpoint RT-PCR. RV-C viral load was determined using a method that we have previously published. A subset (n=34) representative of the main study population and where RV-C was the only detected pathogen were chosen to characterise the cytokine response to RV-C associated wheeze. Ten nasal samples from otherwise healthy community children matched for age and sex were included as controls. A 34-plex cytokines and chemokines panel was used for this part of the study.

RV-C was the most commonly detected virus in pre-school aged children hospitalised with episodic wheeze, and viral load was the same in asthmatics and non-asthmatics. RV-C associated respiratory wheeze in hospitalized children was characterised by elevated levels of Th2 cytokines including IL-4 and IL-9. Similarly, the nasal cytokine profile of RV-C infected hospitalised children with asthma showed increased levels of IL-4 and IL-9 but also showed elevated levels of IL-13 and Th17 cytokines IL-17 and IL-1 $\beta$ . Th1 associated cytokines of both asthmatic and non-asthmatic children were either attenuated or unchanged compared to healthy controls.

Medically attended RV-C induced wheeze is characterised by a Th2 cytokine response that is independent of viral load. RV-C infected hospitalised children with asthma display a broader and greater cytokine response than RV-C infected children without asthma which suggests differing pathomechanisms in the genesis of wheeze.

*Keywords: Infection, Immunity, Rhinovirus, Wheeze, asthma*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 11235

## Different innate immune responses by infection with various influenza viruses in macrophages.

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**Introduction and Objectives** Compared to influenza A viruses (IAV), infection of influenza B viruses (IBV) generally is known to cause local mild symptoms but be occasionally lethal to individuals. Previously, it was reported that productions of interferons (IFNs), IFN-stimulated genes (ISG), and proinflammatory cytokines were induced by infection of IBV in epithelial cells rather than that of IAV. However, differences among innate responses in immune cells, especially macrophages by infection of IBV or IAV are still unknown. To clarify whether the differences by infection are induced, ISG/NF- $\kappa$ B activations in human monocyte/macrophages after infection with IAV/IBV were evaluated.

**Methods** PMA-differentiated THP-1 cells were co-cultured with several strains of IAV (H1N1, H3N2) or IBV (B/Yam, B/Vic) and then innate immune responses such as ISG or NF- $\kappa$ B/AP-1 were examined.

**Results** All IAV and IBV infection induced both ISG and NF- $\kappa$ B/AP-1 activations with virus titer dependent manner. In the case of H1N1 virus infection, these activations induced by infection with similar virus titers. On the other hands, these activations by either H3N2 or IBV infections were different among strains. In addition, infections of IBVs, including both B/Yam and B/Vic, induced strongest activations followed by those of H1N1, and these activations by infection of H3N2 viruses required highest virus titers.

**Conclusion** Taken together, infection of IBV induced stronger immune responses in macrophages than IAV infection. Further study will clarify relationship between these innate immune responses and severity during influenza virus infection.

*Keywords: ISG NF- $\kappa$ B macrophage infection*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 11304

**INFECTION-PERMISSIVE IMMUNITY AGAINST INFLUENZA VIRUS  
PROVIDED BY VACCINATION PREVENTS LOSS OF ALVEOLAR  
MACROPHAGES AND MODULATES VIRUS-INDUCED CROSS-REACTIVE  
IMMUNE RESPONSES DURING SUBSEQUENT INFLUENZA  
INFECTIONS.**

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Introduction/Objective:

Conventional influenza vaccines aim at the induction of virus-neutralizing antibodies. This requires vaccine efficiencies that are not always reported for vaccinees. We investigated to what extent infection-permissive immunity, in contrast to virus-neutralizing immunity, provided by a classical influenza virus vaccine (trivalent inactivated virus vaccine, TIV) could modulate disease and virus-induced host immune memory responses after homologous H1N1 infection.

Methods:

Balb/c mice were vaccinated intramuscularly once or twice with trivalent inactivated virus vaccine (TIV, equivalent of 3ug HA). Three weeks after immunization, mice were challenged sublethally with homologous H1N1 virus. Lung virus titers were quantified at different days post infection as well as dynamics of different pulmonary myeloid cell populations were monitored. For rechallenge experiments, mice were infected with H3N2 virus four weeks after primary challenge. The effect of vaccination on tissue resident memory CD8+ T cells, as well as on germinal center B cell responses in lung tissue and lymph nodes and cross-reactive sera was investigated and correlated with protection during secondary heterosubtypic infection.

Results:

More than one TIV vaccination is needed to induce a serum hemagglutinin inhibition titer and fully prevent onset of morbidity upon virus infection. However, single TIV administration correlated with lower viral lung titers and faster recovery after homologous challenge. Contrary to negative control mice, complete abolishment of alveolar macrophages was prevented in TIV-vaccinated animals. TIV vaccination also affects levels of pulmonary B and T cell levels after infection. In the absence of detectable virus neutralization antibodies, TIV vaccination shifts virus-induced broad heterosubtypic cross-protection from the cellular to the humoral branch of the immune system.

Discussion:

These results suggest that suboptimal vaccination with conventional influenza vaccines may still positively modulate disease outcome, allowing but modulating induction of heterosubtypic immunity by virus infection.

*Keywords: Vaccination, heterosubtypic immunity, cross protection, cellular and humoral immunity*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 11711

## HEMAGGLUTININ HEAD AND STALK BINDING ANTIBODY RESPONSES FOLLOWING THE 2009 PANDEMIC INFLUENZA INFECTION IN ETHNIC CHINESE.

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**Introduction:** During the 2009 pandemic, novel H1N1 influenza subtype with an antigenic shift emerged as the predominantly circulating virus and swiftly replaced the existing seasonal H1N1 virus. We utilized this situation as an opportunity to investigate and understand the kinetics of stalk antibody responses during natural infection.

**Methods:** Influenza stalk antibodies were measured in a well characterized cohort of ethnic Chinese individuals by ELISA and micro-neutralization assays.

**Results:** We observed a significant increase in group 1 stalk antibody responses in those who had 2009 pandemic infection(33.7%), as opposed to those who had seasonal H1N1(10.7%) or H3N2(5.5%) infections. But such was not observed for stalk antibody responses against group 2 viruses. These results were following a similar trend as the PCR confirmed individual data. The group 1 stalk antibody responses are significantly higher in the 61-70 age decade ( $p < 0.001$ ) compared to all the other age groups. We observed that the seasonal vaccination during 2009 did not boost stalk antibody responses.

**Conclusion:** The prevalence and magnitude of group 1 stalk antibody titers increased significantly after the 2009 pandemic. However, these titers remained relatively low compared to HAI titers. Therefore, novel vaccination strategies will be required to boost these highly cross reactive responses in order to increase the heterosubtypic protection level.

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 11744

## Mucosal immunization with the influenza A virus encoding truncated NS1 protein protects mice from heterologous challenge

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### Introduction:

Influenza viruses with truncated NS1 protein are highly immunogenic and are used for the development of live attenuated influenza vaccines and recombinant viral vectors. Here we investigated whether the immunogenic advantage of the virus expressing only the N-terminal part of NS1 protein (124 aa) can be translated into the induction of the protective immunity against heterologous influenza virus in mice.

### Methods:

Mice were immunized intraperitoneally with 7 log[TCID<sub>50</sub>]/mouse or intranasally with 2.5 and 6 lg[TCID<sub>50</sub>]/mouse of A/PR/8/34 (H1N1) or A/PR8/NS1-124 (H1N1) viruses. CD8 T-cellular immune response in spleens and lungs was analyzed 8 days post immunization (d.p.i). Immunized mice were infected with 10 LD<sub>50</sub> of influenza A/Aichi/2/68 (H3N2) virus in the challenge experiment 30 d.p.i. Viral load, innate and adaptive T-cellular immune responses were assessed in the lung tissue on days 2 and 4 after the challenge.

### Results:

T-cellular immune response after intraperitoneal immunization with A/PR8/NS1-full or A/PR8/NS1-124 was substantial in both cases, but it was higher for the virus with truncated NS1 protein. However, the formation of influenza-specific systemic humoral and T-cellular responses in both groups did not protect mice against a heterologous challenge. On the other hand, the intranasal immunization with A/PR8/NS1-124, but not with A/PR8/NS1-full virus prevented the lethality after A/Aichi/2/68 infection. In 4 days after the challenge, A/PR8/NS1-124-immunized mice demonstrated the increased level of polyfunctional influenza-specific CD8 T-cells in the lungs and the reduction in viral load compared to A/PR8/NS1-full and non-immunized groups. The concentration of IFN- $\gamma$ , TNF- $\alpha$ , MCP-1, IL-6, IL-1 $\alpha$  and the level of neutrophils in lung homogenates after the challenge were lower in A/PR8/NS1-124 group than in A/PR8/NS1-full or control group.

### Conclusion:

Mucosal immunization and the formation of local immunity in the lungs were shown to be crucial for cross-protection. Intranasal immunization with A/PR8/NS1-124 provides the increased level of influenza-specific CD8 T-cells in the lungs and reduces the severity of the infection after the heterologous challenge.

*Keywords: NS1 protein; influenza; immune response; cross-protection; polyfunctional T-cells*

Topic: Virology and Pathogenesis: Immune Response to Infection  
Abstract No: 11788

## The cross-reaction between seasonal influenza vaccine and H7N9 Virus in mice

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**Introduction and Objectives:** In February 2013, avian influenza A H7N9 viruses have caused sporadic outbreaks in China. As of September 2013, a total of 1533 laboratory-confirmed cases and 607 deaths have been officially reported. Vaccination is the most effective way to prevent influenza and its severe outcomes, however, it is not clear whether seasonal influenza vaccines can protect H7N9.

**Methods:** In order to get high-level serum antibodies against season influenza, mice were immunized with influenza vaccines from 2011 to 2015 separately. Then, the cross-reaction level between anti-serum of influenza vaccine and H7N9 recombinant influenza virus expressing A/Anhui/1/2013 (H7N9) HA and NA genes was detected by hemagglutination inhibition (HI) and microneutralization (MN) test.

**Results:** The results showed that, HI titers of mice receiving influenza vaccine against recombinant H7N9 influenza virus were less than 1:4, and the neutralizing antibodies detected by MN test were less than 1:10.

**Conclusion:** Mice experiments showed that seasonal influenza vaccine could not protect H7N9 virus, but further experiments needed to be verified by wild-type H7N9 virus challenge assay.

*Keywords: H7N9; seasonal influenza vaccine; cross-reaction*

Topic: Virology and Pathogenesis: Immune Response to Infection

Abstract No: 11789

## CEIRS REAGENTS AND RESOURCES TO ADVANCE INFLUENZA RESEARCH AND DISCOVERY

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### Introduction/Objectives:

The Centers of Excellence for Influenza Research and Surveillance (CEIRS) program is an integrated network of research centers funded by the National Institute of Allergy and Infectious Diseases (NIAID). The network is comprised of 5 prime institutions along with over 60 sites around the world. A key mission of the Network is to provide information and public health tools to control the impact of epidemic influenza and potential threat of pandemic influenza. The Data Processing and Coordinating Center (DPCC) supports CEIRS by providing a platform for data sharing and information exchange that enhances the accessibility of publicly available scientific resources generated by the Network.

### Methods:

CEIRS investigators perform basic and applied research on influenza viruses isolated from humans and animals to better understand host immune responses, viral pathogenesis, and factors enabling virus emergence and transmission. These studies have generated a large and varied inventory of research resources, which are made available to the broader scientific community.

### Results:

The DPCC deployed the CEIRS Reagents resource page on the CEIRS public website to increase the accessibility and dissemination of CEIRS-generated reagents. This web-based interface includes a searchable catalog of research reagents which can be requested directly from the webpage at no cost. Scientists can browse reagents by type and keyword, refine searches via sorting and filtering, and request information on specific resources. Available reagents include plasmids and reverse genetics viruses, viral isolates, monoclonal and polyclonal antibodies, antisera, tissues and cells from animal models, and cell lines from mice, ferrets, and swine. In addition to reagents, the CEIRS Cohort Studies page is a centralized and searchable catalog of human cohort study samples that can be leveraged for research related to the universal influenza vaccine strategic initiative.

### Conclusion:

In total, CEIRS research and surveillance activities have resulted in the development of over 9,000 reagents and several human cohort studies which can be used as essential tools to conduct influenza research projects. Further information about CEIRS-generated resources can be found on the CEIRS public website at <https://www.niaidceirs.org/resources>.

*Keywords: Reagents; Antibodies; Plasmids, Ferret; Swine; viral isolates*



Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10412

## Neuraminidase of H1N1pdm Viruses Exhibited Mixed Genetic Makeup in Taiwan in 2015-2016 Season

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**Introduction and objectives:** Neuraminidase (NA) is a surface protein constantly changing its genetic outfit along the influenza virus evolutionary course. Genetic variants mixed within a viral population were reported from time to time which can influence the emergence of pandemic viruses as well as jeopardize drug susceptibility.

**Methods:** 55 clinical specimens of H1N1pdm infections during 2015–2016 season in Taiwan were collected from human subjects. Whole genomes were obtained through next-generation sequencing and analyzed in searching for position-specific genetic variants on NA.

**Results:** A mixed population of two distinct variants at NA position 151 were revealed, not only in cell cultures but also directly from the collected specimens. Reverse genetics was implemented and found the mixture of the two was characterized by a higher virus replication rate compared to the recombinant viruses harboring either individual variant. One variant was further found reducing drug susceptibility to NA inhibitors.

**Conclusion:** Two distinct NA-151 variants were detectable in clinical specimens in the 2015-2016 H1N1pdm. Reverse genetics shows that the mixed population may aid the virus replication. One NA-151 variant was also found associated with NA drug susceptibility.

*Keywords: influenza virus; neuraminidase; genetic variants; reverse genetics; drug susceptibility*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10438

## THE ANTIGENIC CHANGE IN THE HEMAGGLUTININ OF A(H7N9) INFLUENZA VIRUS

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### Introduction and Objectives

Many cases of human infection with H7N9 virus were detected in China between 2013 and 2017. The H7N9 viruses were maintained in chickens and transmitted to human at live bird markets until 2017. During circulation in birds, H7N9 viruses accumulated amino acid substitutions in their hemagglutinin (HA) that resulted in an antigenic change in the recent H7N9 viruses. Here we characterized 46 mouse monoclonal antibodies against the HA of the prototype strain.

### Results and Conclusion

Sixteen H7-HA-specific mAbs possessed hemagglutination inhibition (HI) and neutralization activities by recognizing the major antigenic site A; 4 other H7-HA-specific clones showed HI and neutralizing activities via recognition of the major antigenic sites A and D. Seven of the mAbs reacted with several HA subtypes, possibly recognized the HA stem, and partially protected mice from lethal infection with the prototype H7N9 virus. The remaining 19 mAbs had neither HI nor neutralization activity. All human H7N9 viruses tested showed similar neutralization sensitivity to the first group of 16 mAbs, whereas human H7N9 viruses isolated in 2016–2017 were not neutralized by the second group of 4 mAbs. These results suggest that amino acid substitutions at the epitope of the second mAb group may be involved in the antigenic drift of the H7N9 viruses. Further analysis is required to fully understand the antigenic change in H7N9 viruses.

*Keywords: Antigenic change; HA; H7N9; monoclonal antibody*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10481

## Study of Amino Acid Mutation at Neuraminidase Residue 267 of A(H3N2) Influenza Viruses

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### Introduction and Objectives

Influenza viruses evolve to evade immune pressure via various mechanisms. Our recent study demonstrated that a novel *N*-linked glycosylation site at residue 245 and a mutation at residue 468 together resulted in significant NA antigenic drift of recently circulating A(H3N2) viruses. Since 2013, a threonine (T) to lysine (K) mutation at NA residue 267 has occurred and become fixed in the circulating A(H3N2) strains. In this study, we evaluated whether this mutation alters biological properties, especially the antigenicity, of NA.

### Methods

A/Hong Kong/4801/2014 virus NA (HK/14 NA; T267) and mutant HK/14 NA (K267) were used in our study. Glycans added at residue 267 were analyzed; the binding of mouse and human monoclonal antibodies (mAbs) specific to NA was examined by ELISA; ferret and human antisera were used to evaluate the impact of T267K mutation on NA antigenicity, using enzyme-linked lectin assay (ELLA), an NA inhibition assay that uses bovine fetuin as the substrate.

### Results

The T267K mutation resulted in the loss of *O*-linked glycans added to residue 267. This amino acid substitution did not impact the binding of NA by the tested mouse mAbs and most of the tested human mAbs; however, it significantly enhanced the binding of NA by a human mAb while abolished the binding by another. Ferret antisera raised against HK/14 virus inhibited wild-type and mutant HK/14 NAs at similar titers. The impact on NA inhibition by human antisera is being assessed.

### Conclusions

A T267K mutation has occurred and become dominant in the NA of circulating A(H3N2) viruses. This molecular change has profound impact on the binding of NA by some human mAbs, but not on NA antigenicity as measured by ferret antisera. Our findings highlight the significance of monitoring and assessing amino acid mutations in HA and NA of influenza viruses.

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10531

## Antigenic drift originating from changes to the lateral surface of the neuraminidase head of influenza A virus.

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### Introduction and Objectives

Recent studies indicate the importance of anti-NA antibodies for protection against influenza virus infection. Some NA-specific monoclonal antibodies (mAbs) recognize epitopes around the enzymatic center of the NA head and restrict virus spread through neuraminidase inhibition (NI) activity. Furthermore, a mAb possessing NI activity was found to require Fc-Fcγ receptor (FcγR) interactions, which activate effector immunological cells, for in vivo protection. To evade such NI antibodies, amino acid changes accumulate around the enzymatic active site. However, amino acid alterations also accumulate at the lateral surface of the NA head, which is far from the enzymatic center. The reason for this accumulation remains unknown.

### Methods

We isolated anti-NA mAbs from individuals infected with A(H1N1)pdm09 virus, and investigated their epitopes, NI activity, and in vivo protection activity.

### Results and Conclusion

We found that amino acid mutations on the lateral surface of the NA head abolished the binding of these mAbs. While some mAbs possessed NI activity, others did not. However, all mAbs protected mice from lethal challenge infection via their NI activity or FcγR-mediated antiviral activity. Serological analysis of individuals who were infected with A(H1N1)pdm09 virus revealed that some possessed or acquired the anti-NA lateral surface antibodies upon infection. Our results demonstrate that anti-NA lateral surface mAbs without NI activity can provide protection by activating FcγR-mediated antiviral activity and can drive antigenic drift at the lateral surface of the NA head. These findings have implications for NA antigenic characterization in that they demonstrate that traditional neuraminidase inhibition assays are inadequate to fully characterize NA antigenicity.

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
 Abstract No: 10532

## GENETIC AND ANTIGENIC CHARACTERISATION OF INFLUENZA A(H3N2) VIRUSES ISOLATED IN YOKOHAMA DURING THE 2016/17 AND 2017/18 INFLUENZA SEASONS

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### Background

Influenza A(H3N2) virus rapidly evolves to evade human immune responses, resulting in changes in the antigenicity of its haemagglutinin (HA). Continuous genetic and antigenic analyses of A(H3N2) virus are important to detect antigenic mutants as quickly as possible.

### Methods

We determined the HA sequences of A(H3N2) viruses detected in Yokohama, Japan during the 2016/17 and 2017/18 influenza seasons to identify amino acid substitutions and the loss or gain of potential N-glycosylation sites in HA, both of which can affect the antigenicity of HA. We also examined the antigenicity of isolates by using ferret antisera obtained from experimentally infected ferrets.

### Results

Influenza A(H3N2) viruses belonging to six clades (clades 3C.2A1, 3C.2A1a, 3C.2A1b, 3C.2A2, 3C.2A3, and 3C.2A4) were detected during the 2016/17 influenza season, whereas viruses belonging to two clades (clades 3C.2A1b and 3C.2A2) dominated during the 2017/18 season. Isolates in clades 3C.2A1a and 3C.2A3 lost one N-linked glycosylation site in HA relative to the other clades. Antigenic analysis revealed antigenic differences among clades, especially clade 3C.2A2 and 3C.2A4 viruses, which showed distinct antigenic differences from each other and from the other clades in an antigenic map.

### Conclusion:

Multiple clades, some of which differed antigenically from others, co-circulated in Yokohama, Japan during the 2016/17 and 2017/18 influenza seasons. The N-linked glycosylation sites of HA have frequently changed in recent years, which has likely affected the antigenicity of the viruses.

**Keywords:** H3N2; HA; antigenicity; glycosylation; epidemiology

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
 Abstract No: 10691

## GENETIC AND ANTIGENIC CHARACTERIZATION OF HIGHLY PATHOGENIC AVIAN INFLUENZA A(H5) VIRUSES COLLECTED IN VIETNAM, 2017-2018

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### Introduction

Highly pathogenic avian influenza (HPAI) A(H5) viruses continue to circulate in Vietnamese poultry. Viruses belonging to both clade 2.3.2.1c and 2.3.4.4 have continued to circulate in Vietnam since their first detection in 2012 and 2014, respectively. Sample collection and timely characterization of viruses are necessary to ensure production and use of effective vaccines in poultry and for recommendation of pre-pandemic candidate vaccine viruses (CVVs) by the World Health Organization (WHO).

### Methodology

Codon complete genome sequences were generated for 107 HPAI A(H5) viruses collected in live poultry markets and poultry outbreaks from January 2017 to August 2018. Phylogenetic analysis was conducted to identify the genetic diversity and genotypes of the viruses. Ferret antisera were raised to select viruses and hemagglutination inhibition assays were performed to assess antigenic variability.

### Results

Clade 2.3.2.1c viruses clustered with viruses previously detected in Vietnam. Many viruses had an increasing number of amino acid substitutions compared to the WHO CVV, A/duck/Vietnam/NCVD-1584/2012, with conserved changes found in predicted antigenic sites. Clade 2.3.4.4 viruses were the dominant clade detected during this time period and were identified across the country. Most viruses were closely related to previously described viruses and CVVs A/Hubei/29578/2016, A/duck/Hyogo/1/2016 and A/chicken/Vietnam/NCVD-15A59/2015. Antigenically, the majority of clade 2.3.2.1c viruses were well inhibited by antiserum raised to A/duck/Vietnam/NCVD-1584/2012. Clade 2.3.4.4 viruses were poorly inhibited by antiserum raised to the CVV, A/Sichuan/26221/2014, while other viruses were inhibited by antiserum against A/chicken/Vietnam/NCVD-15A59/2015. Antiserum raised to A/duck/Hyogo/1/2016 inhibited genetically related viruses, but not other clade 2.3.4.4 viruses. Antisera raised to A/Hubei/29578/2016 and other CVVs failed to inhibit several recently detected clade 2.3.4.4 viruses indicating emergence of a new antigenically distinct group.

### Conclusion

Given the overlapping geographic distribution of multiple, antigenically distinct clades of viruses in Vietnam, multivalent poultry vaccine formulations may be required, as well as continued development of CVVs.

*Keywords: Vietnam, influenza, H5N1, H5N6, surveillance, poultry*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10728

## THE CIRCULATION AND ORIGINS OF SEASONAL INFLUENZA VIRUSES IN SINGAPORE

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### Introduction

Understanding the spatiotemporal dynamics that govern the circulation of seasonal influenza viruses is crucial for the development of epidemic mitigation strategies. While the global patterns of seasonal influenza virus circulation are increasingly well-characterized, circulation dynamics at geographically localized scales remain relatively unexplored. The city-state of the Republic of Singapore is highly integrated into global and regional transport networks and located within a region that plays a crucial role in the global circulation of influenza viruses. Importantly, Singapore has high-resolution year-round influenza virus surveillance, which creates opportunities to explore spatiotemporal dynamics at a highly localized scale.

### Methods

In order to investigate the relative roles of external introductions of seasonal influenza viruses into Singapore and virus circulation within Singapore, we analyzed a high-resolution dataset consisting of 763 influenza virus genomic sequences, collected in Singapore from 2016-2017. Using statistical models, we clustered the virus sequences that were putatively linked by transmission events. We then used phylogeographic methods to infer the likely geographic origins of each cluster of Singaporean viruses.

### Results

Influenza virus circulation in Singapore was characterized by year-round viral activity, with multiple genetically distinct clusters of viruses circulating at any given time within all currently circulating influenza A subtypes and influenza B lineages. Virus introductions into Singapore were frequent and these external introductions appear to be the main drivers of epidemic activity. The majority of virus introductions to Singapore originated from Southeast Asia, South Asia, China, Japan and Australia with variation by virus type/subtype.

### Conclusions

Influenza virus circulation in Singapore is highly complex, exhibiting a large degree of intra- and inter-epidemic genetic diversity, and Singapore appears to be centrally located in the network of global influenza virus migration.

*Keywords: seasonal influenza; Singapore; phylogeography; persistence; migration*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10738

## Receptor binding characteristics of human A(H3N2) influenza variants circulating since 2013

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### Introduction and objectives

Currently circulating human influenza A(H3N2) viruses fall into two clades, 3C.2a or 3C.3a, both of which emerged in 2013. Here we compare receptor-binding characteristics of reverse genetic viruses from each clade and investigate the effects of introducing subclade-defining amino acid substitutions.

### Methods

Viruses, possessing the haemagglutinin (HA) genes of A/HK/4800/2014 (3C.2a) or A/Finland/438/2014 (3C.3a), including those with specific mutations, were generated by reverse genetics.

The concentration of each mutant virus was determined by ELISA using a HA stalk-specific cross-reacting monoclonal antibody or an anti-influenza A nucleoprotein antibody. This enabled standardisation of amounts of each virus used in assays.

Two receptor-binding assays were used. First, titres obtained in haemagglutination assays (HAA) using red blood cells (RBCs) from different species, known to have differing amounts of receptors on their surfaces, were compared. Secondly, a quantitative biophysical assay, surface biolayer interferometry (BLI), was used to measure the amounts of virus binding to a human receptor analogue (6'SLN).

### Results

For clade 3C.2a viruses, wild type did not haemagglutinate turkey or guinea pig RBCs, TRBCs and GPRBCs respectively, and showed no detectable binding by BLI assay. A glycosylation sequon at HA1 residues 158-160 is a defining feature of this clade. Only mutants lacking this were able to agglutinate both TRBCs and GPRBCs and this stronger binding was confirmed by BLI assay.

For clade 3C.3a viruses, wild type was able to agglutinate both TRBCs and GPRBCs and had detectable binding by BLI assay. HA1 substitutions A128T and S138A caused reduced binding. Moreover, the introduction of a glycosylation sequon at HA1 residues 158-160, as in clade 3C.2a viruses, resulted in no detectable receptor-binding by both HAA and BLI.

### Conclusions:

3C.3a viruses have higher binding avidities than 3C.2a viruses. Presence of the HA1 158-160 glycosylation sequon is the prime determinant of reduced avidity.

*Keywords: receptor-binding, glycosylation sequon, H3N2 viruses*



Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10751

## Epidemiological Dynamics and Molecular Evolution of Influenza B Virus from Influenza-like Illness in Lebanon.

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**Background:** Influenza B viruses are a major cause of serious acute respiratory infections in humans. Here, we investigated the epidemiology and genetic characteristics of influenza B in Lebanon.

**Methods:** Nasopharyngeal swab specimens were collected from patients presenting with influenza-like symptoms during 2016-2018. Specimens were screened for influenza A and B using one step multiplex real-time PCR. The hemagglutinin (HA) and neuraminidase (NA) genes of influenza B positive specimens with Cq-value <30 were amplified and sequenced for further genetic analysis. Phylogenetic analysis was performed to compare the Lebanese influenza B specimens with the vaccine strains and specimens from the Eastern Mediterranean Region and Europe.

**Results:** Influenza A and B co-circulated between October and May and peaked between January and March. Influenza A/H3N2 (33.4%; n=71) and B/Yamagata (29.7%; n=63) were the predominantly circulating viruses followed by B/Victoria (20%, n=20) and A/H1N1pdm09 (0.5%; n=1) during the 2016-2017 season. During the 2017-2018 season, A/H3N2 (31.5%; n=29) and A/H1N1pdm09 (29.3%; n=27) were most prevalent with co-circulation of B/Yamagata (17.4%, n=16) and B/Victoria (1%, n=1). The vaccination rate was low (28-30.7%) among our study population. Phylogenetic analysis showed that B/Yamagata strains belong to clade-3 while B/Victoria belonged to clade-1A. None of the analyzed specimens had a mutation known to confer resistance to NA inhibitors (NAIs).

**Conclusion:** Multiple subtypes of influenza co-circulate each year in Lebanon with a peak around January and March. All analyzed influenza B specimens were susceptible to NAIs. The trivalent vaccine included B/Victoria sequence which did not match the B/Yamagata lineage that predominated during the study period, highlighting the importance of quadrivalent vaccines.

*Keywords: influenza; surveillance; vaccination; Lebanon; phylogenetic analysis*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10762

## Dinucleotide Evolutionary Dynamics in Influenza A Virus

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**Introduction:** Significant biases of dinucleotide composition in influenza A viruses (IAV) were reported in recent years. Our previous study showed that a codon-usage-altered IAV mutant with elevated CpG usage attenuated in mammalian in vitro and in vivo models. However, the relationship between dinucleotide preference and codon usage bias remained unanswered, and the evolutionary dynamics of the dinucleotide usage at segment level are yet to be investigated.

**Methods:** A Monte Carlo type method was applied to access the level of under-/over- representation of various dinucleotides, among different segments and different groups, of 159,028 IAV mRNA sequences. The dinucleotide odds ratios time series were investigated to study the dinucleotide evolutionary dynamics. The yearly consensus sequences were obtained to trace the mutations of certain dinucleotides of interest.

**Results:** After excluding the potential bias from codon usage and amino acid sequences, CpG, UpA, CpA, UpG were found exceptional biased dinucleotides in all viral segments from all groups. We observed significant decrease of CpG frequency in segments 1, 3, 4 and 5 in seasonal H1 virus after its re-emergence in humans on 1977. The temporal variations of the CpG in seasonal H1 virus were mainly contributed by the dinucleotide changes at the codon position 3-1 and 2-3 where silent mutation played a major role. The depletion of CpG and UpA through silent mutations led to over-representation of UpG and CpA. We also found dinucleotide preference directly results in significant synonymous codon usage bias.

**Conclusion:** The dinucleotide evolutionary dynamics in IAV suggested close interplay between CpG, UpA, CpA and UpG, as well as interactive linkage between dinucleotide and codon usage bias. This work helps to understand the evolutionary history of IAV and complex selection pressures that shape the virus genome.

**Keywords:** Influenza; Dinucleotide composition; CpG; codon usage

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
 Abstract No: 10824

## COUNTRYWIDE TRANSMISSION PATTERNS OF INFLUENZA A(H1N1)pdm09 IN KENYA 2009-2016 IDENTIFIED THROUGH WHOLE- GENOME SEQUENCING

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### Introduction and Objectives

Mapping the transmission patterns of influenza A(H1N1)pdm09 from its introduction into Kenya using whole-genome sequencing (WGS) offers insight into the emergence, spread and persistence of this novel virus throughout Kenya. This transmission pattern may be useful for epidemic preparedness for future introductions of other highly pathogenic influenza viruses in Kenya.

### Methods

A(H1N1)pdm09 whole-genomes were generated directly from influenza positive clinical specimens from in-patients admitted to one national hospital and five County referral hospitals within the Centers for Disease Control (CDC) Surveillance and Kilifi Health and Demographic Surveillance System (KHDSS), Kenya, 2009-16. Full-length hemagglutinin (HA) and concatenated WGS were utilized to infer phylogenetic relationships of A(H1N1)pdm09 in Kenya. Contemporaneous global HA and WGS from other countries were also included to infer the global phylogeny of the Kenyan viruses. Spatio-temporal clustering of viruses was inferred using ClusterPicker.

### Results

A total of 330 A(H1N1)pdm09 whole genomes were assembled. Kenyan sequences clustered with globally distributed clades 6A, 6B, and 7. Clade 7 A(H1N1)pdm09 was first observed in Nairobi in 2009, disseminated countrywide, and circulated from 2009 to 2011. Clade 6B disseminated countrywide from 2011 to 2016 while clade 6A circulated in Kilifi, Siaya, and Nakuru from 2011 to 2014. WGS analysis also revealed spatio-temporal clustering of the Kenyan viruses with temporal clusters revealing re-introductions of A(H1N1)pdm09 into Kenya.

### Conclusion

Over the period 2009-16, Kenya experienced re-introductions of A(H1N1)pdm09 clades, with first observations primarily in Nairobi, dissemination of viruses countrywide and local reduction of virus frequency over time. Further analysis will clarify time and frequency of introductions into Kenya, resolve pathways of countrywide transmission, infer phylogenetic relationships with global contemporaneous A(H1N1)pdm09, and estimate time-resolved phylogenies.

*Keywords: Influenza A(H1N1)pdm09; surveillance; whole-genome sequencing; Kenya*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10825

## GENETIC CHARACTERISTIC OF THE HEMAGGLUTININ OF HUMAN INFLUENZA VIRUSES TYPE A ISOLATED IN 2017-2018 SEASON IN UKRAINE

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**Introduction.** A hemagglutinin (HA) of influenza A virus is determinant of virus infectivity, transmissibility and pathogenicity. Antigenic changes in the viruses preceded by changes in genes. Focusing on the genetic changes of the HA is important to detect new variant of each epidemic strain. The aim of work was to analyze hemagglutinin genes of human influenza A viruses which were isolated during 2017-2018 season in Ukraine.

**Methods.** Nasal-throat swabs analyzed using real-time RT-PCR. Influenza A viruses were isolated on MDCK and MDCK-SIAT cells. Sequencing performed in CC WHO centers (London and Atlanta). Other sequences obtained from web-site GISAID using BLAST analysis, and aligned using ClustalW. The influenza A sequences characterized in a neighbor-joining phylogenetic tree. Phylogenetic analysis performed using MEGA 6 software.

**Results.** All analyzed influenza A(H1N1)pdm09 viruses has belonged to the dominant hemagglutinin phylogenetic subgroup 6B.1, which also includes vaccine strain A/Michigan/45/2015. The S183P substitution was observed approximately in a half of tested viruses. Isolate A/Kyiv/316/2017 obtained I324V substitution that was found only in African strains as well. All another viruses, have acquired substitutions S74R, S164T and I295V (shown previous season in A/Odessa/166/2017). Mutation S74R is located in the Cb antigenic site of hemagglutinin, and may effect on its antigenic properties. Isolates A/Khmelnytskyi/363/2018 and A/Khmelnytskyi/364/2018 showed substitution D222N, that may be associated with severe clinical outcomes.

The majority of A(H3N2) viruses shared changes at residues N121K and R142G and located in dominant subclade 3C.2A1. Isolates A/Kyiv/7/2018, A/Kyiv/178/2018 and A/Dnipro/408/2018 selected some additional changes. They located in new emerged branch 3C.2A1b/135K, that continued circulate in 2018-2019. Other isolates within 3C.2A1 subclade shared les changes at residues (T131K, R142K and R261Q).

**Conclusions.** Despite the influenza A(H1N1)pdm09 viruses has obtained lot of amino acid (AA) changes, they belonged to dominant subgroup 6B.1. All of A(H3N2) viruses located within the dominant subcluster 3C.2A1.

*Keywords: Influenza viruses; genetic analysis; aminoacid substitutions*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10826

## RECONSTRUCTING THE ANTIGENIC EVOLUTION OF INFLUENZA A VIRUSES IN MULTIPLE HOSTS

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### Introduction:

Influenza A viruses have a remarkable capacity to transmit between species, presenting an ongoing pandemic threat. Influenza A viruses of the H3N2 and H1N1 subtype are particularly adept at host-switching, and currently circulate in birds, humans, swine, dogs, and horses. Additionally, these viruses rapidly evolve their surface antigens to evade host immune detection, requiring bi-annual updates to influenza vaccine strains in humans. Vaccines also have been formulated for lineages in swine, canines, and equines, although the processes for updating vaccine strains are ad hoc and infrequent, in part due to lower estimated rates of antigenic evolution in non-human hosts. However, the prohibitive cost of large-scale phenotypic testing of A subtype viruses in non-human hosts has impeded a rigorous comparison of rates of nucleotide and antigenic evolution of H3N2 and H1N1 viruses across species.

### Methods:

Here, we first attempt to validate whether antigenic characteristics of the hemagglutinin subunits can be effectively applied to predict antigenic evolution across diverse host species. This method harnesses the advantages of the recently developed mixed effects model implemented within the Bayesian genealogical framework.

### Results:

Nucleotide and antigenic rates of evolution were inferred, while accounting for the viral evolutionary trajectories, and superimposing the host transmission history onto the viral phylogeny. We then performed a comparative analysis of H3N2 and H1N1 evolutionary rates across host-specific and continent-specific lineages, at both a nucleotide and phenotypic level.

### Conclusion:

These findings further our basic understanding of how varying immune-driven selection pressures in different host species relate to host-specific rates of antigenic evolution. Critically, this knowledge advances efforts to predict evolutionary trajectories and evaluate pandemic risks, and informs the required timing of vaccine strain updates in different hosts.

*Keywords: Antigenic evolution; Influenza; Zoonosis; BEAST; Vaccines*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10831

### Predicting coevolving pairs of sites in influenza virus HA protein.

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#### Introduction and objectives

The function of a protein site may depend on context (i.e., depending on the genetic background, it may convey virulence or have no effect). Such context-dependence is an example of fitness being determined cooperatively among two or more sites in a protein. Finding pairs of sites with coordinated evolution, however, is difficult due to the vast number of pairs possible. Here, we present a computational approach to finding pairs of sites with statistically significant coordinated evolution.

#### Methods

We study pairs of sites within the human influenza A(H3N2) virus haemagglutinin. This protein has a densely sampled evolutionary history extending 50+ years. We assume that pairs of sites changing within an unexpectedly "short" time frame have elevated likelihood of coordinated evolution. We use maximum likelihood inference of ancestral amino acid changes to ascertain the timing (to the nearest branch) of residue changes at sites in the human HA(H3), using data from 5479 curated sequences spanning 1966 - 2016. Under the null model that all sites change independently, we use simulation to assess the evidence of non-independent changes. Because RNA viruses can exhibit short-lived mutations of low fitness, we analyze separately the changes on the tip branches of a phylogeny from the changes on internal branches.

#### Results

We find small numbers of pairs with statistically significant coordination. Interestingly, the significant pairs on the tip branches are almost non-overlapping with those on the internal branches. On the internal branches, the significant pairs included most of the sites known to be critical for antigenic evolution of human HA1(H3), however sometimes with different pairings. Additional significant pairs involved sites not previously implicated in coevolution.

#### Conclusion

The statistically significant pairs found on internal branches may be fruitful starting points for experimental analysis.

*Keywords: Coevolution; haemagglutinin; human; H3*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10854

## UTILITY OF WHOLE-GENOME SEQUENCE ANALYSIS IN TRACKING THE TRANSMISSION OF A(H3N2) VIRUS CIRCULATING IN COASTAL KENYA

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### Introduction

Genetic variation of hemagglutinin (HA) and neuraminidase (NA) genes based on gene sequencing has until recently been the mainstay in surveillance of circulating seasonal influenza viruses. With growing availability of whole-genome sequencing (WGS), our study aims to investigate the utility of WGS analysis in tracking the transmission pathways and monitoring evolution of A(H3N2) viruses in Kilifi, Kenya.

### Methods

Next-generation sequencing (NGS) on the Illumina MiSeq platform was used to generate A(H3N2) WGS directly from influenza positive clinical specimens from out-patients presenting with acute respiratory illness to health facilities in Kilifi, coastal Kenya, 2015-17. WGS and full-length HA and NA phylogenetic trees were constructed using IQ-TREE's maximum likelihood method. Local and global WGS, HA and NA phylogenies were compared to investigate the utility of WGS in tracking A(H3N2). Transmission clusters were identified using ClusterPicker.

### Results

Between December 2015 and March 2017, 66 A(H3N2) WGS were obtained from 71 out-patient influenza A positive specimens. Sequenced viruses formed 4 distinct clusters (3C.2a - 34; 3C.2a2 - 3; 3C.2a3 - 6; 3C.2a1b - 23), which showed spatio-temporal clustering in the WGS phylogeny comparing local and contemporaneous global viruses from other countries. Multiple introductions of viruses in Kilifi were evident from the temporal clustering analysis. The local phylogeny in the global tree revealed local diversification of Kilifi viruses whereby local strains formed 4 unique clusters. However, full-length HA and NA phylogenies provided little evidence for multiple A(H3N2) introductions, spatio-temporal clustering, and local diversification of Kilifi strains compared to WGS.

### Conclusion

Our results demonstrate advantages of WGS in monitoring the evolution and identification of transmission clusters of seasonal influenza viruses over short epidemic seasons. The routine use of WGS for influenza surveillance can provide timely information and identify changes that can aid in improving vaccine strain selection and public health control measures at population level.

*Keywords: Influenza A(H3N2); whole-genome sequencing; transmission; Kenya*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
 Abstract No: 10861

## ANTIGENIC AND GENETIC TRAJECTORY OF INFLUENZA A(H1N1)PDM09 VIRUSES SINCE 2009

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**INTRODUCTION:** The first pandemic of the 21st century was detected in April 2009 after a swine-origin A(H1N1) virus arose with gene segment from avian, swine and human lineages. The A(H1N1)pdm09 virus quickly supplanted the previously circulating H1N1 seasonal viruses in seasonal epidemics. Since its emergence in the human population, the A(H1N1)pdm09 viral genome has undergone considerable genetic evolution.

**METHOD:** A(H1N1)pdm09 genomic data and associated metadata for viruses collected between 04/01/2009 and 09/30/2018, was downloaded from GISAID EpiFlu. Antigenic characterization data were compiled from hemagglutination-inhibition assays. Maximum-likelihood phylogenetic trees were generated in IQ-Tree using a representative sample of the complete data set.

**RESULTS:** The hemagglutinin gene segments has followed similar genetic rates of evolution as the seasonal A(H1N1) virus which emerged in 1918 and a general step-wise branching pattern from 2009 to 2014. The emergence of 6B.1 clade viruses in 2015 with substitutions of S84N, S162N (additional glycosylation site) and I216T resulted in a selective sweep in the global diversity. This was followed in 2017 by a second sweep by viruses sharing substitutions S74R, S164T (change in the third position of the new glycosylation site) and I295V, in subclade 6B.1A. Since the spread of 6B.1A viruses, multiple third order subclades have co-circulated. Overall, the majority of these diverse subclades contain a convergent substitution at position S183P. Post-infection ferret antisera has had limited ability to discriminate antigenic change, while post-vaccination human sera in certain years has detected antigenic change among the circulating genetic clades.

**CONCLUSION:** After 10 years of circulation in the human population, evolutionary pressures have produced a multitude of different genetic clades and subclades of A(H1N1)pdm09. Traditional antigenic characterization with post-infection ferret antiserum has failed to detect discernable antigenic evolution in the multiple genetic clades. Additionally, there has been remarkably few reported reassortment events between human seasonal A(H1N1)pdm09 and A(H3N2).

*Keywords: H1N1pdm09, genetic evolution, 6B.1*



Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10872

## STOCHASTIC TRANSMISSION BOTTLENECKS CONSTRAIN ADAPTATION OF H7N9 INFLUENZA VIRUSES TO MAMMALIAN HOSTS

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**Introduction and objectives:** H7N9 influenza viruses have caused over 1,500 human spillover infections and can be transmitted by respiratory droplet in ferrets. Though these viruses seem poised to adapt to humans and cause widespread outbreaks, no such event has occurred.

**Methods:** We used deep sequencing to investigate evolutionary dynamics of H7N9 viruses over time in ferrets infected with biological isolate or recombinant H7N9 viruses. The study included 7 transmission events and an additional 16 infections without transmission.

**Results:** Both low-pathogenicity avian influenza (LPAI; A/Anhui/1/2013) and high-pathogenicity avian influenza (HPAI; A/Guangdong/17SF003/2016) viral isolates exhibit low levels of genetic diversity within hosts. This diversity appears to be subject to purifying selection and is further significantly reduced following transmission. Genetic diversity in recombinant viruses is very limited before and after transmission and is shaped by both weak purifying selection and genetic drift (randomness). We find no evidence of natural selection favoring new mutations in viral isolates or recombinant viruses. Strikingly, frequencies of specific mutations in index ferrets do not predict their frequencies post-transmission. We detect a handful of variants across the genome which steadily increase in frequency in index ferrets, but are lost during transmission. Likewise, some variants that decrease in frequency over time in index animals are nonetheless transmitted and become dominant in contacts immediately following transmission.

**Conclusions:** Together, these findings suggest that airborne H7N9 influenza virus transmission in mammals is defined by a stringent and stochastic bottleneck, consistent with an emerging picture of seasonal influenza virus transmission in humans. We speculate that purifying selection, randomness, and tight bottlenecks combine to severely constrain the ability of H7N9 viruses to effectively adapt to mammalian hosts in typical spillover infections, even with onward airborne transmission. Further analyses are ongoing, e.g., to determine the fitness impact of specific variants identified in these studies.

*Keywords: H7N9; transmission; bottlenecks; mammalian-adaptation; evolution*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10927

## A unified genotyping nomenclature for the internal genes of influenza A viruses

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### Introduction

Recent influenza viruses of pandemic concern, including H7N9, H10N8, and H5N6, acquired their internal genes by reassortment from a poultry-adapted H9N2 lineage termed G57. Investigation into the predominance of distinct internal sets like G57 in both poultry and human infections is limited by the lack of a unified nomenclature to describe the diversity of combinations of internal genes and quantify their distribution across HA-NA subtypes. We developed such a nomenclature for the internal genes of avian influenza viruses based on phylogenetic clustering with PhyCLIP, a statistically-principled clustering algorithm.

### Methods

We applied PhyCLIP to the global phylogeny reconstructed from publicly available sequence data for each internal gene segment, with global genotype designation determined by cluster-membership across all six segments. We then quantified the geographic, temporal and host-range distributions of the internal gene genotypes.

### Results

PhyCLIP-based genotyping resolves the substantial genotypic diversity in the global avian influenza sequence dataset into spatiotemporally consistent subpopulations, with cluster sizes following a clear power law. The major genotypes encompassed multiple HA-NA-defined subtypes, emphasising the high frequency of reassortment between viruses in both domestic and wild bird populations. The majority of the genotypes were from Asia owing to an overrepresentation of Asian sequences in public databases. Many rare genotypes came from regions underrepresented in global surveillance, suggesting that expanded surveillance could reveal previously unobserved diversity. Differences in the predominant genotypes from ducks and chickens indicates further investigation into the adaptation of viruses to gallinaceous poultry is required to understand differential selection pressures.

### Conclusion

A unified nomenclature for the genotypic diversity of internal gene combinations allows for inferences into the evolutionary history of viruses and the fitness of internal genotypes based on relative genotype abundance across hosts, geographic region, and time. These findings further our understanding of the ecological and evolutionary processes that structure virus reassortment patterns.

*Keywords: Influenza Evolution, Nomenclature, Genotypic Diversity, Reassortment*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10933

## WITHIN-HOST VIRUS EVOLUTION IN SEQUENTIALLY SAMPLED INFLUENZA-POSITIVE PATIENTS

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Due to high virus mutation rates, the influenza virus population within infected hosts consists of diverse but genetically related variants. Genetic variation in the virus population is fundamental to influenza virus evolution because it offers the opportunity to quickly adapt to changing environments. Current understanding of within-host genetic variation of seasonal human influenza viruses is almost entirely based on samples collected at single time-points during infection, thus merely providing a single evolutionary snapshot. Insights in evolution of the virus population in infected human hosts are mostly lacking.

To track within-host development of virus variants, we applied next generation sequencing to 420 sequentially obtained samples from 83 influenza A/H3N2 and influenza B positive patients enrolled in a large clinical study into antiviral drug dosing in Southeast Asia. Patients received oseltamivir for at least 5 days during sample collection. Plasma samples from the same patients were used to relate influenza virus-specific antibody immunity to emergence of antigenic variants during infection.

Variants with amino acid substitutions on hemagglutinin positions associated with antibody escape were detected in 17% of A/H3N2 virus patients. Variant proportion typically reached low proportions (2 - 10%) in the virus population, with substitutions occurring in antigenic sites B, C, and D. Antibody titers in these patients were below the detection limit of the assay used. Variants with substitutions associated with reduced inhibition (RI) by oseltamivir developed in 19% of A/H3N2 virus patients. In 7% of patients, variants associated with RI reached proportions of >50% in the virus population. Analysis of influenza B virus samples is ongoing.

Our results suggest that oseltamivir treatment regularly results in outgrowth of viruses with amino acid substitutions conferring high-level resistance to oseltamivir. This work also suggest that within-host outgrowth of antigenic variants is a rare event.

*Keywords: within-host evolution antibody escape oseltamivir resistance*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10939

## COMPARATIVE EVOLUTION AND PHYLOGEOGRAPHY OF INFLUENZA B/VICTORIA LINEAGE VIRUSES WITH DOUBLE AND TRIPLE AMINO ACID DELETIONS IN HEMAGGLUTININ

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**INTRODUCTION:** Influenza A and B viruses cause seasonal epidemics worldwide, but differ by evolutionary and global circulation patterns. The majority of observed genetic and antigenic drift has been through the accumulation of nucleotide substitutions in hemagglutinin and neuraminidase gene segments, with rare observances of insertions or deletions. Since 2016, several separate deletion events have occurred in the influenza B/Victoria hemagglutinin (V1A) resulting in either deletion of residues 163-164 or 162-164, which are in a major immune epitope.

**METHODS:** Time-scaled phylogenies of influenza B/Victoria lineage genomes collected since 2008 were estimated using BEAST, with an additional discrete sample geographic trait used in Bayesian stochastic search variable selection to assess phylogeographic spread. Antigenic characterization was assessed by hemagglutination inhibition assay using post-infection ferret antisera and human sera pre- and post- vaccination with seasonal influenza vaccine.

**RESULTS:** The first deletion lineage with substantial circulation was the 163-164 deletion (V1A.1) which emerged mid-2016. Initial geospatial patterns were limited to the Americas, however global spread was seen in subsequent seasons. In mid-2017, two 162-164 deletion lineages evolved independently in Asia, with only one resulting in sustained circulation, mainly in Asia. In early 2018, a third 162-164 deletion lineage was detected in several countries in West Africa. This 162-164 deletion lineage spread to Asia and beyond in a much shorter time span than other deletion variants. All deletion variant viruses showed significant antigenic drift from previous vaccine candidate B/Brisbane/60/2008 (V1A). Ferret antisera raised to current B/Victoria vaccine strain B/Colorado/06/2017 (V1A.1) well inhibited 163-164 deletion viruses and most 162-164 deletion viruses. Some antigenic differences were seen among the different 162-164 deletion lineage viruses.

**CONCLUSIONS:** Deletions in the hemagglutinin are an important evolutionary mechanism. Increased geospatial differences in virus diversity were observed during the 2018-19 northern hemisphere season, with circulation of V1A, V1A.1 and multiple 162-164 deletion lineages.

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10946

## Evolution of A/H1N1 2009 pandemic influenza virus in pigs in France from 2010 to 2018: divergence of a swine-specific lineage, reassortment events and bi-directional transmissions with humans

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The swine-origin A/H1N1 2009 pandemic virus (H1N1pdm) spread to pig populations worldwide, while it became seasonal in humans. As influenza viruses may evolve differently in swine and humans, and because pigs have the propensity to facilitate reassortments between viruses of different origins, H1N1pdm monitoring in swine is critically important for risk analysis.

In France, H1N1pdm was detected in pigs with respiratory clinical signs each year from 2010, counting for 5% of swine influenza A viruses (swIAVs) identified from 2010 to 2018, with some variations in annual frequencies. It was detected all year round, but numbers of H1N1pdm-infected herds were significantly higher during seasonal epidemics, suggesting *de novo* human-to-pig transmissions besides its enzootic circulation in pig population. Phylogenetic analyses confirmed that most H1N1pdm isolated in pigs were much close to contemporary seasonal H1N1pdm. These analyses also revealed the divergence of a swine-specific genogroup, thought to have occurred around 2011 following mutation accumulation, without any antigenic drift up to now. Over the study period, H1N1pdm-infected herds, including those affected by the swine-divergent strains, were preferentially located in regions with the smallest pig population sizes. In high pig density areas mixed infections with H1N1pdm and other swIAV lineages were evidenced, and reassortant swIAVs with HA or M or all internal gene(s) from H1N1pdm were described from 2014, including triple-reassortants that also acquired a gene from a human seasonal H3N2.

To date, neither the swine-divergent H1N1pdm strains, nor the novel swIAV reassortants with H1N1pdm genes have been responsible for zoonotic infections in France. However, a pig-to-human transmission of a seasonal-like H1N1pdm was evidenced in 2018, recalling that these events do occur. Appropriate biosecurity measures are needed inside holdings in order to limit interspecies transmissions and further emergence in pigs of variants or reassortants that would not be covered by human vaccines.

*Keywords: swine influenza, pandemic H1N1, pig, reassortment, interspecies transmission, zoonosis*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
 Abstract No: 10951

## H5N1 within-host diversity in humans and domestic ducks in Cambodia

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### Introduction

H5Nx viruses periodically cross species barriers and cause disease in humans. The likelihood that an avian influenza virus will acquire mammalian-adapting mutations and evolve enhanced transmissibility depends on its ability to acquire and select mutations within hosts during spillover.

### Methods

We use deep sequence data from infected humans and ducks in Cambodia to examine how H5N1 viruses evolve during natural spillover infection.

### Results

We find that viral populations in both species are characterized by predominantly low-frequency (<10%) variation shaped by a combination of purifying selection and genetic drift. Human samples had more within-host polymorphisms on average, although the distribution of single nucleotide polymorphism (SNP) frequencies and overall mean SNP frequency was similar in both host species. We detect a handful of mutations in humans at sites explicitly linked to H5N1 mammalian adaptation (PB2 627 Lys, HA 138 Val, and HA 226 Leu, H3 numbering), although these mutations were present at low frequencies, despite ≥8 days of infection. Half of the within-host variants identified in our dataset are never detected in a single H5N1 consensus sequence. Those that are were primarily found on branches leading to avian infections on the H5N1 phylogeny, suggesting they are unlikely to contribute to human spillover.

### Conclusions

Taken together, our data suggest that during human spillover, H5N1 viruses have the capacity to generate well-known markers of mammalian adaptation in multiple, independent hosts. However, our data also show that these mutations arise and evolve within a genetic background shaped heavily by purifying selection and randomness. We speculate that during spillover, a short duration of infection, randomness, and purifying selection may together severely limit the evolutionary capacity of H5N1 viruses to evolve extensively within human hosts. Future examination of H5N1 viruses from other clades will be necessary to confirm whether our findings are generalizable across genetic backgrounds.

*Keywords: within-host diversity; H5N1; spillover infection*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 10989

## SHORT-SIGHTED EVOLUTION OF INFLUENZA CELLULAR RECEPTOR BINDING IN HUMAN POPULATIONS

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### Introduction

Haemagglutinin (HA) cellular receptor binding avidity plays an important role in determining within-host fitness of influenza. Previous studies have demonstrated that many adsorptive mutants incur a cost for viral replication, suggesting a trade-off between immune escape (high binding trait) and viral replication (low binding trait) for binding adaptation. However, no studies to date have shown evidence for host adaptation of binding avidity in human influenza history.

### Methods

We developed and validated an influenza receptor binding adaptation model by comparing model simulation results to the viral phylogenies of binding trait between internal and external nodes using sequence-derived HA net-charge as a marker for binding avidity. We first obtained 686 full-length influenza A/H3N2 HA sequences to investigate how HA net-charge varies in hosts of different ages. To evaluate the type of selection, phylogenetic trees were reconstructed to compare the net-charge of isolates at internal and external nodes. Finally, we constructed an individual-based disease-dynamic model incorporating within-host binding avidity adaptation to investigate the impact of binding avidity changes on population-level dynamics.

### Results

Individuals aged 20-50 years had a lower proportion of high net-charge sequence isolates than others, similar to age-specific patterns of seroprevalence. Viruses on external branches demonstrated more variability in net-charge than viruses at internal nodes, suggesting that binding avidity is a phenotype under stabilizing selection. The individual-based model showed that although the average binding avidity of extant viruses changed over the course of a simulated epidemic, it was unable to adapt to values that maximized transmission between individuals.

### Conclusion

Differences in net-charge by age suggest that binding avidity is a trait under within-host selection. Furthermore, our results also suggest that receptor binding avidity is subject to short-sighted evolution: it decreases a virus's effective reproductive number from the optimum at the population level in favour of increasing within-host reproductive fitness.

*Keywords: Influenza evolution; Receptor binding; Short-sighted evolution; Age-specific net-charge; Disease model*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology

Abstract No: 10996

## IDENTIFICATION OF AMINO ACID SUBSTITUTIONS IN NEURAMINIDASE OF CURRENTLY CIRCULATING A(H3N2) VIRUSES RESULTING IN REDUCED SIALIDASE ACTIVITY

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**Introduction:** Neuraminidase (NA) inhibitors (NAI), oseltamivir and zanamivir, are the main antiviral medications for influenza and NAI susceptibility is routinely measured by IC<sub>50</sub> with MUNANA substrate. Some recent A(H3N2) human influenza viruses showed reduced inhibition (RI) by these NAIs which was associated with positively charged amino acid substitutions at residues 329, 331 or 334 of NA. Substitutions at N329 and S331 disrupt a glycosylation sequon (NDS).

**Methods:** During routine NAI susceptibility monitoring, viruses displaying RI were identified which carried positively charged amino acid substitutions at NA residues 329, 331 or 334. Enzyme kinetics of NAs with these substitutions were determined, their prevalence was assessed by phylogeny and glycosylation sequon usage by mass spectrometry.

**Results:** N329K was present in ~2% and ~0.2% of viruses circulating in 2015-2016 and 2017-2019, respectively while S329R was present in only ~0.1%. S331R occurred in ~8% and ~1% of viruses, respectively, over the same periods. S334R was detected at ~2% frequency in 2017-2019. These substitutions occurred in viruses of both HA clades, 3C.2a and 3C.3a.

RI phenotypes resulted from reduced sialidase activity compared to relevant wild-type (WT) viruses. Those containing: (i) S329R or N329K showed 5-10- or 10-20-fold higher K<sub>M</sub> values and 7-22- or 10-40-fold increased K<sub>i</sub> values with NAIs, respectively; (ii) S331R showed 7-29-fold higher K<sub>M</sub> values and 9-39-fold increased K<sub>i</sub> values with NAIs; (iii) S334R caused smaller effects, with K<sub>M</sub> values 1.5-12-fold higher and 1.3-13-fold increased K<sub>i</sub> values with NAIs.

Mass spectrometry showed glycosylation at sequons 329-331 (NDS) and nearby 367-369 (NET), which is associated with the secondary sialic acid binding site. Loss of glycosylation per se due to N329S yielded WT virus kinetics unlike N329K and S329R, and S329R did not affect glycosylation at N367.

**Conclusion:** Viruses with these NA amino acid substitutions cannot be assessed for susceptibility to NAIs in standard assays.

*Keywords: A(H3N2); Neuraminidase; Antivirals; Resistance*



Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 11015

## USING WHOLE-GENOME SEQUENCING TO INVESTIGATE THE WITHIN-SEASON GENETIC DIVERSITY OF INFLUENZA A(H3N2) VIRUSES IN A SCHOOL-ASSOCIATED COMMUNITY IN WISCONSIN, USA, 2017-2018

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**Introduction and Objectives:** The Oregon Child Absenteeism due to Respiratory Disease Study (ORCHARDS), located in Oregon, Wisconsin, seeks to evaluate the relationship between school absenteeism and influenza activity in the community. The framework of this study allows for unique insight into the genetic diversity of influenza within households of the Oregon School District.

**Methods:** During the 2017-2018 influenza season, pharyngeal swabs were collected from children who reported absences from school due to influenza-like illnesses. Additionally, swabs were collected from family members in the household. These swabs were collected on the day of home visitation (day 0) and 7 days later. Isolates were selected for whole genome sequencing if the child was positive for any influenza strain and at least one household member was positive for influenza either on day 0 or day 7.

**Results:** Within the selected households, there were 149 samples sequenced, with 14 positive for A(H1N1), 93 for A(H3N2), two with non-subtypable A, and 40 samples positive for influenza B. We observed three peaks in the incidence of A(H3N2) in the 2017-2018 influenza season, where each peak consisted of different viruses at the consensus level. The first peak consisted primarily of 3C2.A2 viruses that had largely identical hemagglutinin (HA) sequences. The second peak had more diversity, ranging from 0 to 12 single nucleotide variations in HA sequences. The third peak consisted primarily of 3C3.A viruses, indicating a shift in the consensus population. Interestingly, the peaks and changes in the influenza population occurred near breaks in the school year, suggesting the shifts in the influenza population may have been due to the introduction of influenza viruses from outside the community.

**Conclusions:** Viruses isolated from school-aged children and their families in a small community represented wide diversity within a single influenza season and demonstrated successive waves of genetically distinct viruses.

*Keywords: whole genome sequencing; influenza; influenza type A; H3N2; community-based surveillance*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 11033

## EVOLUTION OF THE POLYMERASE COMPLEX OF SEASONAL INFLUENZA A VIRUSES

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**Introduction:** The currently circulating seasonal influenza A viruses, subtypes A(H3N2) and A(H1N1pdm2009) emerged in 1968 and 2009 respectively after zoonotic reassortment events with influenza A viruses of avian and porcine origin. After the resulting pandemics, these viruses continued to circulate as seasonal influenza viruses. Although much is known on the antigenic evolution of these viruses, the genetic diversity and evolution of the polymerase complex is still poorly understood.

**Objective:** We characterized the evolution of the polymerase complex of seasonal influenza viruses A(H3N2) and A(H1N1pdm09) and its implications on polymerase activity, virus replication and possibly virulence.

**Methods:** Genome sequences of the polymerase genes for seasonal influenza viruses were downloaded from publicly available databases and phylogenetic analyses were used to investigate evolution and the fixation of specific substitutions. The polymerase complex of 13 A(H3N2) and 6 A(H1N1pdm2009) viruses were cloned into expression vectors and tested in the mini replicon assay. The replication kinetics of these isolates and reassortants thereof were tested in MDCK, A549 and primary differentiated human airway epithelium cells.

**Results:** A clear ongoing evolution of the polymerase complex was observed during circulation of seasonal influenza viruses in the human population. Interestingly, polymerase complex activity of seasonal influenza A(H3N2) gradually increases from 1968 until present day, especially in the first decade after introduction. The PB1 and NP segments contribute most to this effect and we identified several substitutions that were fixated during its evolution to have profound effects on polymerase complex activity.

**Conclusion:** Our results indicate that the evolution of the polymerase complex of seasonal influenza A viruses is an ongoing process since their introduction into the human population with distinct implications for polymerase activity and possibly replication capacity. We are currently investigating the effect of this enhanced polymerase activity on virus replication and possible co-evolution of different substitutions and segments.

*Keywords: seasonal influenza A, evolution, polymerase complex, replication, virulence*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology

Abstract No: 11052

## EVOLUTION OF AVIAN INFLUENZA A(H5N1) VIRUSES ISOLATED FROM POULTRY IN EAST JAVA, INDONESIA

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**Introduction and Objectives** In Indonesia, avian influenza A(H5N1) virus has become endemic in poultry since 2003. The virus of H5 HA clade 2.1, the Indonesian lineage, had been exclusively circulating until 2012. Detection of clade 2.3.2.1, the Eurasian lineage, was reported for the first time in Indonesia in September 2012. In this study we aimed to isolate A(H5N1) viruses from poultry in East Java and to trace the evolution of the both lineages.

**Methods** We collected 1,337 cloacae swabs from sick poultry at live-poultry markets and farms in East Java from May 2013 to February 2019. All samples were inoculated into chicken eggs for virus isolation. The isolated viruses were examined for influenza type A, subtype H5N1, and H5 HA lineages by RT-PCR. The HA sequences were determined using ABI BigDye terminator system or by next generation sequencer MiSeq.

**Results** Egg harvests tested positive for hemagglutination activity were 383. Among positive isolates, 130 were identified as influenza virus type A and 114 as H5N1 subtype. There were only 9 isolates of Indonesian lineage. The remaining 105 were Eurasian lineage. HA sequences of the Eurasian lineages isolated in 2013 showed 99%-nt identity with an isolate of Vietnam in 2012. Those HA were identified as clade 2.3.2.1c of Eurasian lineage. The phylogenetic analysis showed that the East Java isolates formed a group distinct from Vietnam isolates.

**Conclusion** Our results showed that the newly introduced A(H5N1) virus of clade 2.3.2.1c Eurasian lineage had become dominant in poultry after 2013 and evolved to form a unique group, while the Indonesian lineage seemed to be in the end of the endemic era of 10 years. Recently, one fatal case of human A(H5N1) infection was reported in September 2017 and the isolated virus had HA gene 98% identical with our Eurasian isolates in 2015.

**Keywords:** A(H5N1) Eurasian lineage; Indonesian lineage; clade 2.3.2.1c; East Java

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology

Abstract No: 11113

## Independent Introductions of clade 2.3.2 H5N1 Avian Influenza in Rural Villages in West Java, Indonesia 2013-2014.

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**Introduction:**In Indonesia, the circulating H5N1 clade is 2.1.3, which has dominated the 2.1.1 and 2.1.2 subclades since the introduction of all three in the mid-2000s. In late 2012, the Ministry of Agriculture in Indonesia identified a new, more virulent clade of H5N1, 2.3.2.1 responsible for the deaths of thousands of ducks

**Methods:**Human and animal outbreak field investigations were conducted within a radius of 200 m from index cases based on reports from local animal husbandry officers between October 2013 and November 2015 in 3 rural districts in West Java, Indonesia (Indramayu, Majalengka, and Kuningan).Cloacal swabs were collected from dead or sick poultry using a nylon-flocked swab and tested for H5N1 influenza virus using PCR. Whole genome sequencing of detected H5N1 and its analysis followed standard procedures.

**Results:**Consensus H5N1 circulating clade 2.1.3 sequences were aligned to all blast hits matching assembled contigs to provide phylogenetic context. The resulting trees (Fig) showed H5 sequences in two distinct clusters: 13 consensus sequences clustered with the circulating 2.1.3 H5 lineage endemic to Indonesia, but a second cluster with two consensus sequences in it corresponded to the 2.3.2 clade thought to have originated in China. These three isolates represent independent introductions of clade 2.3.2 virus into Indonesia, given their phylogenetic separation. An overlay of each of structural mappings with all predicted and experimentally verified H5 epitopes shows that several of the differences within isolates from each clade are antigenic changes (relative to their closest vaccine strains).

**Conclusions:**Fatal avian influenza outbreaks in chickens in Indonesia in 2014were due to infection with not only the circulating clade 2.1.3 H5N1 viruses, but also from clade 2.3.2 viruses; the result of independent introductions into West Java. These clades have distinct antigenic differences from recommended vaccine strains for the respective clades.

*Keywords: Avian Influenza, Clade 2.3.2, Indonesia, Vaccine*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 11128

## CHARACTERIZATION OF INFLUENZA A(H1N1)PDM09 VARIANTS SELECTED WITH HUMAN ANTISERA COLLECTED IN THE 2017/18 SEASON

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### Introduction and objectives

Influenza vaccines need to be constantly updated because viruses frequently alter their antigenicity. Prediction of viral antigenic evolution is useful for the selection of suitable vaccine strains. However, a technique for prediction has not yet been established. To develop an experimental approach for this prediction, we selected antigenic mutants from circulating A(H1N1)pdm09 influenza viruses under the pressure by using human sera and characterized these mutants.

### Methods

We generated two small viral libraries, which were pooled 9 A(H1N1)pdm09 viruses isolated in the 2017/18 season, to facilitate selection of antigenic mutants. Each library was mixed with individual human sera collected in the 2017/18 season. After neutralization, library-serum mixtures were inoculated onto cells and cultured for 2-4 days. The viral HA gene sequences in the supernatants were determined by using deep sequencing. Escaped mutants in the supernatants were plaque-purified and their reactivity with individual human sera was examined by neutralization (NT) assays.

### Results

By using deep sequencing, ten amino acid changes were detected in the antigenic site of HA in viruses that escaped neutralization by human sera. Among them, S190I and E235G changes have been reported in circulating viruses in nature. In antigenic analysis using human sera, eight and three of 24 human sera showed 4-fold reduction against 190I and 235G mutant viruses, respectively, in NT titers, compared with those against wild-type viruses.

### Conclusion

The amino acid changes in mutant viruses selected in our study have been reported in circulating viruses in nature, and these viruses showed reduced reactivity to some human sera compared with wild-type viruses. Thus, our approach may help the prediction of epidemic variants before these viruses spread in nature and also the selection of vaccine viruses.

*Keywords: A(H1N1)pdm09; escape mutant; evolution; human sera; prediction*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology

Abstract No: 11178

## Quantifying Antigenicity of Influenza A(H1N1) Virus using Mutations and Variations in Glycosylation of Hemagglutinin

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**Introduction and Objectives:** In addition to causing the pandemic influenza outbreaks of 1918 and 2009, subtype H1N1 influenza A viruses (IAVs) have caused seasonal epidemics since 1977. Both genetic mutations and *N*-linked glycosylation (hereafter referred to as *N*-glycosylation) of HA were shown to be associated with antigenic variations of these H1N1 viruses. In this study, we formulated the study of antigenicity as a multi-task sparse learning problem with the aim to identify gene sequence, proteome, and site-specific *N*-glycosylation as antigenicity determinants.

**Methods:** A multi-task learning sparse group least absolute shrinkage and selection operator (LASSO) (MTL-SGL) regression method was developed and applied to derive two types of predominant features including protein sequence and *N*-linked glycosylation in hemagglutinin (HA) affecting variations in serologic data for human and swine H1N1 IAVs.

**Results and Conclusion:** Results suggested that mutations and changes in *N*-linked glycosylation sites are associated with the rise of antigenic variants of H1N1 IAVs. Furthermore, the implicated mutations are predominantly located at five reported antibody-binding sites, and within or close to the HA receptor binding site. All of the three *N*-linked glycosylation sites (i.e. sequons NCSV at HA 54, NHTV at HA 125, and NLSK at HA 160) identified by MTL-SGL to determine antigenic changes were experimentally validated in the H1N1 antigenic variants using mass spectrometry analyses. Compared with conventional sparse learning methods, MTL-SGL achieved a lower prediction error and higher accuracy, indicating that grouped features and MTL in the MTL-SGL method are not only able to handle serologic data generated from multiple reagents, supplies, and protocols, but also perform better in genetic sequence-based antigenic quantification. In summary, the results of this study suggest that mutations and variations in *N*-glycosylation in HA caused antigenic variations in H1N1 IAVs and that the sequence-based antigenicity predictive model will be useful in understanding antigenic evolution of IAVs.

**Keywords:** H1N1, antigenic evolution, *N*-linked glycosylation, machine learning, mass spectrometry analyses

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 11187

## POPULATION DYNAMICS OF H5N1 VIRUSES IN CHINA AND THE EMERGENCE OF NOVEL H5NX VIRUSES

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**Introduction and Objectives.** Since 1997, highly pathogenic avian influenza (HPAI) H5N1 virus has evolved and subsequently diversified into multiple genetically distinct sublineages. These H5N1 viruses persisted primarily in domestic birds with frequent infection of wild birds at ecological interfaces. Since 2008, H5N1 viruses in the clade 2.3.4.4 sublineage underwent reassortment to acquire new NA subtypes, leading to the emergence of H5N6 and H5N8 viruses that have caused severe epidemics in poultry globally since 2014. While China has been viewed as the epicenter for novel GsGD viruses, how these new reassortant H5Nx viruses emerged and replaced their ancestral H5N1 strains remain unexplored.

**Methods.** Here, we studied the evolution of H5N1 viruses in China before the emergence of H5Nx viruses. Population dynamics of three major sublineages of H5N1 viruses (clades 2.3.2, 2.3.4, and 7) were analysed and compared. We also incorporated ecological states in the time-scaled phylogenetic analyses to infer the viral transmission between different ecological systems.

**Results.** Analysis of population dynamics showed that different H5N1 sublineages experienced a population decline in 2006. Dating analysis showed that the decrease in H5N1 population occurred prior to the emergence of H5Nx viruses, suggesting that the decline was not due to competition with the new reassortant strains. We further observed that clade 2.3.2 viruses were maintained in wild birds from 2005-2008 before re-emerging in poultry. A major shift from H5N1 to diverse NA subtypes in clade 2.3.4 was concurrent with the development of the wild bird-related lineage post-2007.

**Conclusion.** While multiple factors may explain the emergence of novel panzootic viruses, here we propose that H5Nx arose during a population bottleneck, possibly related to increased H5N1 control measures introduced just prior to the observed H5N1 population decline. Our study highlights the importance of ecological context in understanding the emergence of novel avian influenza viruses.

*Keywords: evolution; H5; avian*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
 Abstract No: 11192

## Ha – Na Epistasis contributed to the emergence of the seasonal oseltamivir-resistant influenza A (H1N1) virus (2006-2007).

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**Background** – In 2007, a H275Y mutation occurred in the neuraminidase of A(H1N1) viruses, leading to oseltamivir resistance. This emergence was reported to be sustainable through a restored viral fitness due to four N1 compensatory substitutions (R194G, R222Q, V234M, D344N), but the molecular basis of the viral fitness balance has remained unclear.

**Methods** –Using GISAID, we analysed all A(H1N1) viruses sequences available from 1999 until 2008. Multiple sequence alignments were performed for each segment separately and outlier sequences were excluded. Phylogenetic trees of HA and NA segments were inferred with a standard Maximum Likelihood inference. The identification of putative pairs of epistatically linked protein loci, within and across segments, was based on a phylogenetic method developed in [ref]. Additionally, we performed molecular modelling and 3D-models of HA and NA proteins.

**Results** – We identify two classes of epistatic mutations: driver mutations observed before the resistance mutation H275Y are likely to mitigate its effect, trailer mutations occurring after H275Y may compensate the fitness cost of resistance. Overall, we find about 20 changes in both HA and NA, yielding a 4-step evolution of the viruses. In addition to N1 R222Q and V234M mutations, the resistant cluster emerged with H1 (D35N, T82K, Y94H) and N1 (K78E, G249K, K329E, G354D) driver mutations. These mutations might have induced an antigenic change prior to the emergence of the resistant strain. Interestingly, HA trailer mutations are located around the binding site, with a possible impact on the HA-NA balance.

**Conclusions** – This analysis of the molecular evolution of HA and NA provides new data on the temporal co-occurrence of mutations genetically linked to the H275 substitution. It provides evidence for epistatic interactions within NA and between HA and NA, improves our understanding of this emergence, and may be used in real-time for the risk assessment for similar events in the future.



Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
 Abstract No: 11193

## SEROLOGIC EVIDENCE OF AVIAN INFLUENZA A (H9N2) VIRUS INFECTION AMONG POULTRY WORKERS, DOMESTIC ANIMALS AND MIGRATORY WATERFOWL IN PAKISTAN

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**Introduction and Objectives.** Avian influenza A (H9N2) virus has been endemic to Pakistan since 1994. Despite continued reports of outbreaks in poultry and sporadic human infection, surveillance in the country is scarce. The co-housing of different avian and other animal species are common in poultry farms and markets. Poultry workers are exposed to H9N2 through close contact with infected poultry. Here, we conducted surveillance in Pakistan to understand the prevalence and circulation of H9N2 among poultry workers, domestic animals and wild birds.

**Methods.** During January to December 2016, a total of 1,192 serum samples were collected from poultry workers (n=117), domestic animals (n=985), and migratory birds (n=38) from and in the vicinity of commercial broiler farms in Punjab Province, Pakistan. Hemagglutination inhibition (HI) assay was used to detect anti-H9N2 antibodies to a H9N2 G1 lineage virus. Microneutralization (MN) assay was also performed to confirm serum antibodies specificity against the virus strain.

**Results.** Our results show high levels of H9N2 seropositivity in poultry workers by HI (49.6%; HI titre  $\geq 1:128$ ) and MN assay (45.1%; MN titre  $> 1:20$ ). Similarly, migratory waterfowl exhibited high seropositivity (42.1%; HI titre  $\geq 1:128$ ) to H9N2 virus. Significant H9N2 seropositivity was also detected by HI assay in different mammalian species, including goat (16.8%), sheep (16.6%), donkeys (10%), cats (8.3%), dogs (4.5%), camels (4.2%), and cattle (3.8%). No seropositivity to H9N2 was detected in horses. Neutralizing antibodies against influenza H9N2 virus were confirmed by MN assay in 8 out of the 10 species tested.

**Conclusion.** Our results indicate that a large proportion of poultry workers and other mammalian species in Pakistan have been infected with H9N2 virus. It is crucial to understand the risks of H9N2 exposure among poultry workers in order to implement appropriate control and monitoring of H9N2 virus transmission in commercial poultry farms in Pakistan.

**Keywords:** Avian influenza virus, H9N2, serology, Pakistan, poultry workers

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 11212

## EVOLUTIONARY MECHANISMS CONTRIBUTING TO ELEVATED INFLUENZA B ACTIVITY IN SINGAPORE FROM 2016–2018

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**Introduction and Objectives.** Influenza A and B viruses co-circulate in humans and cause epidemics regionally and worldwide. In Singapore, influenza viruses circulate year-round with two peaks in April–June and November–January, corresponding to the winters in the Southern and Northern Hemispheres. According to statistics compiled by the Singapore Ministry of Health, influenza viruses cause ~41–52% cases of influenza-like illnesses each year. During 2016–2018, increased influenza B activity was observed in Singapore. Here, we studied the genomic evolution of influenza B viruses in Singapore and worldwide.

**Methods.** Over 1,500 influenza-positive samples were collected in Singapore from 2011–2018. Viral isolation and genome sequencing were performed using Sanger and next-generation sequencing (NGS), and genomic sequences randomly sampled and analyzed. Phylogenetic and molecular clock analysis were conducted to understand the molecular mechanisms contributing to the increased diversification of Victoria and Yamagata lineages.

**Results.** So far, 146 novel influenza B genomes have been sequenced. We show that recent Victoria viruses (2016–2018) are mostly characterized by the presence of a 2- or 3-amino acid deletion in the hemagglutinin protein. Our results also indicate that in recent years the Yamagata viruses demonstrated distinct seasonal fluctuations in genetic diversity as compared to earlier viruses. Interestingly, Yamagata viruses (2016–2018) exhibit a number of sequentially acquired amino acid mutations on the neuraminidase protein that may be associated with antigenic change and increased prevalence.

**Conclusions.** Novel genetic variants have emerged in recent Victoria and Yamagata viruses, with several significant amino acid mutations identified, especially on the NA gene. While most attention is focused on the HA, there is also a crucial need to understand the effects of NA mutations in contributing to antigenic drift.

*Keywords: Influenza B, Singapore, Phylogeny, NGS, mutations*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
Abstract No: 11267

## Phylogeny of Influenza A (H1N1pdm09 and H3N2) Viruses in Bandung District between 2008 and 2011

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**Introduction:** Influenza is a major cause of significant morbidity in Indonesia and globally. Characterizing the phylogeny of circulating viruses can provide important information to guide the development of vaccines and to monitor for the development of evolving seasonal strains. The objective of this study was to assess the epidemiology and molecular characteristics of seasonal influenza viruses circulated in Bandung, Indonesia.

**Methods:** Nasopharyngeal swabs were collected from subjects with ILI in 2 Primary Health Centers in Bandung Indonesia, during 2008-2011. Influenza was detected by standard RT-PCR. Genomic RNA segments of detected Influenza A viruses were amplified by multisegment reverse transcription PCR (M-RTPCR), using the Myseq Illumina platform. Phylogenetic analyses were performed using MEGA 6 with a Neighbor-joining algorithm and 1000 replicates.

**Results:** There were 3356 ILI patients 402 with Influenza A (12%) and 105 had Influenza B (3%). 89 sequences of influenza A H1N1pdm09 and 165 of influenza A H3N2 viruses were obtained. The phylogenetic analysis of Influenza A H1N1pdm09 revealed that subclade 6B and Influenza A H3N2 revealed subclade 3C during the study period.

**Conclusion:** The result of phylogenetic analysis improved our understanding of Influenza viruses evolution in Indonesia. Therefore, continuing the Influenza viruses surveillance activity is important.

*Keywords: Influenza A, H1N1pdm09, H3N2, phylogenetic analysis*

Topic: Virology and Pathogenesis: Influenza Evolution & Human Ecology  
 Abstract No: 11283

## Genetic Surveillance of Influenza Viruses among Selected Countries in Latin America, 2017–2018

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**Introduction and Objectives:** Since the 2009 influenza pandemic, Latin American (LA) countries have strengthened their influenza surveillance systems according to global and regional standards. Influenza genetic sequence data are important for WHO influenza vaccine consultation meetings. As part of influenza virologic surveillance strengthening, a Regional Pilot Sequencing Project, initiative of PAHO/WHO and CDC, is being developed with the main objectives of strengthen influenza genetic surveillance, generate more information for better understand patterns of viral evolution and contribute for vaccine strain selection.

**Methods:** Sequence data from selected LA countries were analyzed to map the availability of additional influenza genetic sequence data and to describe the 2017 through 2018 Southern Hemisphere influenza seasons. Influenza A/H1pdm09, A/H3, B/Victoria and B/Yamagata hemagglutinin sequences from clinical samples from 12 National Influenza Centers (NICs) in ten countries (Argentina, Brazil, Chile, Colombia, Costa Rica, Ecuador, Mexico, Paraguay, Peru and Uruguay) with collection date from epidemiologic week (EW) 18, 2017 through EW 43, 2018 were analyzed. These sequences were generated by the NICs or WHO Collaborating Center (WHO-CC) at CDC, uploaded to the Global Initiative on Sharing All Influenza Data (GISAID) platform, and used for phylogenetic reconstruction.

**Results:** Influenza hemagglutinin sequences from samples collected during the study period from participating countries (A/H1pdm09 n=326, A/H3 n=636, B n=433) were highly concordant with the genetic groups of the influenza vaccine-recommended viruses for influenza A/H1pdm09 and influenza B. For influenza A/H3, the concordance was variable.

**Conclusions:** Considering the constant evolution of influenza viruses, real-time, high-quality genetic sequence data are important to allow public health decision makers to make informed decisions about prevention and control strategies, such as the influenza vaccine composition. Countries that conduct influenza genetic sequencing for surveillance in LA should continue to work with the WHO-CCs to produce high-quality genetic sequence data and upload those sequences to open-access databases.

**Keywords:** Influenza; Sequencing, Genetic surveillance, GISAID, National Influenza Center, Latin America, Hemagglutinin

Topic: Virology and Pathogenesis: Influenza Glycobiology

Abstract No: 10131

## A NOVEL INHIBITOR OF N-LINKED GLYCOSYLATION, NGI-1, EXHIBITS ANTI-VIRAL ACTIVITY AGAINST INFLUENZA A and B

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**Introduction and objectives:** Proper N-linked glycosylation (NLG) is crucial for influenza A and B viruses (IAV and IBV) biological functions. A novel NLG inhibitor, NGI-1, reversibly blocks cellular glycosylation by targeting the oligosaccharyltransferase. This study evaluates the potential of NGI-1 for influenza-disease treatment and details virus glycosylation.

**Methods:** The ability of NGI-1 to inhibit A(H1N1) and A(H3N2) IAV and B/Victoria and B/Yamagata IBV replication was evaluated in NHBE and MDCK cells, using 0.01 or 1.0 multiplicities of infection (MOI). Trypan blue exclusion was used to determine the inhibitor's cytotoxicity. Electron microscopy of NGI-1-treated or untreated A(H3N2) IAV and mass spectrometry-based analyses of treated or untreated virus glycoproteins, hemagglutinin (HA) and neuraminidase (NA), were performed 24 hours after infection of MDCK cells with 1.0 MOI. Binding of NGI-1-treated virus was assessed by hemagglutination assays with various red blood cells (RBC), and the NA activity was measured by a standard fluorometric assay with MUNANA as the substrate. The ability of NGI-1-treated HA to generate immune responses was assessed by immunization of naïve mice with two 10 µg HA doses followed by measurement of serum antibody titers by hemagglutination inhibition (HI) and ELISA tests.

**Results:** NGI-1 was non-toxic in cells at concentrations up to 30 µM. At 10 µM, IAV and IBV titers in the NGI-1-treated cultures were 100 to 1000 times lower than those in the untreated cultures up to 72 hours after infection. Glycosylation was reduced by more than 20% in NGI-1-treated virus compared to untreated virus, leading to a two-fold increase in morphologically atypical virions, an 8- to 16-fold reduced ability to agglutinate RBC, and decreased total and protective serum antibody responses in mice.

**Conclusion:** NLG reduces influenza virus replication and stability without causing significant cytotoxicity in vitro. Small-molecule inhibition of cellular glycosylation may present a novel option in anti-influenza therapies.

*Keywords: influenza, glycosylation, inhibitor*

Topic: Virology and Pathogenesis: Influenza Glycobiology  
Abstract No: 10664

## Receptor preference of H5 avian influenza viruses to both $\alpha$ -2,3 and $\alpha$ -2,6 sialic acids

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The receptor binding properties of the hemagglutinin (HA) of influenza A virus (IAV) determine the host range of IAV and their alternation affects host accessibility causing interspecies transmission. Avian and human IAV preferentially interact with sialic acids (SA) linked to a galactose by an  $\alpha$ -2,3 and  $\alpha$ -2,6 linkage, respectively. We analyzed receptor specificity of H5 avian IAVs isolated in poultry, wild birds and mammal in 2014 and 2016 to estimate a potential of human infection. The clade 2.3.4.4 H5 from an H5N6 and H5N8 were analyzed a receptor specificity by hemagglutination assay. Turkey erythrocytes were treated with Vibrio cholera neuraminidase and resialylated with  $\alpha$ -2,3 or  $\alpha$ -2,6 sialyltransferase. For the assay, turkey, horse and sheep erythrocytes were also incubated with  $\alpha$ -2,3 neuraminidase to remove selectively SA  $\alpha$ -2,3 receptors. To determine whether neuraminidase involves in receptor binding, oseltamivir was incubated with the viruses before MDCK cell infection and virus titers were measured at 24 and 48 hrs after inoculation. Seasonal H1N1 and H3N2 and avian H9N2 IAVs were used as control. The HA titers of H5 viruses were not different among untreated, resialylated, and treated with  $\alpha$ -2,3 neuraminidase turkey erythrocytes and similar to between untreated and treated horse erythrocytes. In contrast, HA titers were not detected or significantly decreased with sheep erythrocytes treated with  $\alpha$ -2,3 neuraminidase. There was no significant difference of virus titers in MDCK cells. The H1N1 and H3N2 human IAVs were only interacted with erythrocytes retained SA  $\alpha$ -2,6 linkage. The H9N2 virus did not agglutinate with SA  $\alpha$ -2,3 linkage-removed horse and sheep erythrocytes. These results suggest the H5 IAVs bind to receptors of both SA  $\alpha$ -2,3 and  $\alpha$ -2,6 linkages and could have a potential for human infection. This study was supported by the intramural research program of the Korea Centers for Disease Control and Prevention (2017-NI43001-00).

*Keywords: Influenza virus; receptor specificity; hemagglutinin; H5 subtype*

Topic: Virology and Pathogenesis: Influenza Glycobiology  
Abstract No: 11174

## Decoding glycan substructures specific for influenza cell and host tropisms using systems biology approaches

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**Introduction and Objectives:**The initial step of the influenza A virus (IAV) infection is the binding of the hemagglutinin (HA) to the sialylated host glycans. The HA forms trimers, each of the monomers has a relatively conserved receptor binding site (RBS) that functions to engage the virus with the sialylated glycans on the host cells. The binding specificity and the binding affinity of an IAV to the glycan receptors, which can be expressed differentially on different cells and different hosts, are two of the key factors determining the virus cell and host tropisms. Typically, avian IAV prefers sialic acids (SAs) that are linked to galactose (Gal) by the  $\alpha$ 2,3-linkage (SA2,3Gal) whereas human IAV binds to terminal SAs with a  $\alpha$ 2,6-linkage (SA2,6Gal).

**Methods:**In this study, we generated a total of 200 mutants targeting the HA RBS of A/California/04/2009(H1N1) and further characterized replication efficiency of these mutants in MDCK cell and chicken embryonated egg. To identify the glycan motifs determining phenotypic diversity of these mutants, we performed virus-glycan binding analyses against 75 glycan isoformers using glycan array. The glycan motifs determining the replication efficiency in MDCK cells and eggs are learned from machine learning and further validated.

**Results and Conclusion:** Machine learning analyses showed that increasing the binding ratio of  $\alpha$ 2-3 sialic acid glycans to  $\alpha$ 2-6 sialic acid glycans can help expand the receptor spectrometry for IAV binding and that increasing of IAV binding ability to the non-sialic acid glycans can increase virus replication efficiency in both MDCK cells and eggs. In addition, a number of novel glycan substructures and mutations in HA RBS determining IAV tropisms in MDCK cell and chicken embryonated egg were also identified. In summary, the results from this study can be useful in optimization of influenza vaccine seed production and in selecting high yield influenza vaccine strains during surveillance.

*Keywords: vaccine yield, glycan array, machine learning, glycan motif, H1N1*

Topic: Virology and Pathogenesis: Influenza Glycobiology

Abstract No: 11185

## SELECTION OF A NOVEL, BUT UNFIT INFLUENZA A(H1N1)pdm09 I106M NEURAMINIDASE MUTANT AFTER PASSAGING IN A DIFLUORO SIALIC ACID INHIBITOR

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### Introduction

3-fluoro(eq)-4-guanidino difluoro sialic acid (3Feq4Gu DFSA) is a mechanism-based influenza neuraminidase (NA) inhibitor, forming a covalent link to Y406 in the NA active site. It is a potent inhibitor of influenza replication *in vitro* and *in vivo*. Based on studies of oseltamivir and zanamivir we know that the greater the similarity to the natural substrate the more difficult it is to select resistant viruses without compromising virus fitness. We predict resistance to DFSAs should be difficult to select since they are even closer to the natural substrate.

### Methods

An influenza A(H1N1)pdm09 and influenza B virus were passaged for 15 passages in 3Feq4Gu DFSA *in vitro*, to determine whether resistant variants could be selected.

### Results

Yields of both viruses decreased significantly by 12 passages, so drug concentrations were decreased from P13-P15. There was no difference in sensitivity in the MUNANA fluorescence-based assay, nor in plaque assays for the P15 stocks. No resistant influenza B plaques were isolated. Plaquing of the P15 pdm09 stock revealed isolated small diffuse plaques which after amplification had barely detectable NA or hemagglutinin (HA) activity, despite titers of  $>10^7$  PFU/ml. The NA had a novel non-active site I106M substitution, but no HA changes. The I106M viruses had similar replication kinetics in MDCK cells as wild type viruses, but their ability to bind to and infect CHO-K1 cells expressing high levels of cell-bound mucin was compromised. The I106M substitution was unstable to further passaging.

### Conclusion

Thus, resistant variants were hard to isolate and were compromised in their fitness. We propose that the novel I106M substitution may impact on movement of the loops around the NA active site. In addition, I106M NA has insufficient activity to remove carbohydrates from the virion HA and NA, thus sterically limiting HA access to chicken erythrocyte receptors resulting in poor HA binding.



Topic: Virology and Pathogenesis: Influenza Glycobiology  
Abstract No: 11269

## Proteomic characterization of influenza H7N9 virus cultivated in MDCK cell

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### Instruction

The infection of influenza virus is the major cause of severe respiratory diseases around the world, leading thousands of deaths annually. Vaccination has been considered as an effective way of preventing the virus infection, and novel platforms were developed to increase the manufacturing yield against the pandemic outbreak. However, current vaccine seed virus is generally designed for an egg-based manufacturing system, residue mutations might occur when adapting such RNA virus in different cultivation biosystem. Analyzing the protein sequence and possible post-translational modifications of antigen of virus passages become essential for understanding the structural functionality of the expressed virus particle and estimating the impact on the quality of the final vaccine product.

### Objective and Methods

In this study, the H7N9 vaccine virus, RG268, was adapted in MDCK cell-based system, and MS-based approaches was used to analysis the sequence and glycosylation of hemagglutinin of H7N9 virus passages cultivated in MDCK cells.

### Results

Proteomic results identified one-site specific mutation on the head region of hemagglutinin at the fifth passage of MDCK-derived H7N9 virus. Interestingly, such site-specific mutation generates a consensus motif of N-X-S (Asn-X-Ser, X is any amino acid except proline), and one additional N-linked biantennary glycan core was identified on HA head region of the fifth-passage H7N9 virus, which is not observed in the other earlier passages. Additionally, the virus titer increased from 3.16E+4 TCID50/ml (First passage) to 1E+8 TCID50/ml (fifth passage), which highlights this site-specific glycosylation is correlated with the virus growth in MDCK cell. By immunizing the mice with MDCK-derived H7N9 virus particles, both animal antisera are able to inhibit hemagglutination of RG268 virus with the titration value of 1339.0 (First passage) and 1612.7(Fifth-passage), respectively, suggesting the impact of additional glycan moiety is not obvious on the antigenicity of the late-passage MDCK-derived H7N9 virus.

*Keywords: Proteomics, Glycosylation, H7N9 virus, Vaccine*

Topic: Virology and Pathogenesis: Influenza Glycobiology

Abstract No: 11326

## THE ROLE IN ANTIGENICITY AND VIRAL FITNESS OF N-GLYCOSYLATIONS NEAR THE RECEPTOR BINDING SITE.

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**Introducción:** As a consequence of antigenic drift, Influenza A virus (IAV) over time have acquire several N-glycosylation motifs on the globular head of its hemagglutinin (HA). This have been associated with modulation of antigenicity, virulence and immune responses. It has been shown that N-glycosylation at residue 144 of the 2009 H1N1, near the receptor binding site (RBS) and the Sa immunodominant site can induce a polyclonal response capable of neutralizing other glycosylated H1N1 variants. **Objectives:** To study the effects of the complexity of glycosylation 144 in the modulation of the antigenicity and IAV fitness.

**Methods:** To evaluate the masking properties of glycosylations at sites 144, 142, 172, and 144-172 viral variants derived from the A/Netherlands/602/09 H1N1 we used monoclonal antibodies directed to antigenic site Sa. To evaluate the effect of the complexity of the N-glycans in antigenic masking we used virus with complex or high-mannose glycosylation produced in MDCK or kifunensine tretated MDCK cells, respectively. To study the receptor avidity we evaluate the hemagglutination activity on turkey red blood cells.

**Results:** Western blot showed a higher molecular weight of the glycosylation 144 compared to other glycosites, implying the incorporation of longer glycans. Its susceptibility to treatment with EndoH suggests that it is composed by mannose-rich or hybrid type glycans. The effects of glycan complexity in antigenic masking are discussed in each case. A decrease on hemagglutination activity of glycosylated variants was observed and this was partially mitigated when exclusively mannose-rich N-glycans were incorporated in hemagglutinin.

**Conclusions:** Our results indicates that the complexity of N-glycosylations near to RBS have an impact in antigenicity and viral infection. These results provide new insights of the biological relevance of the N-glycosylations near the RBS and their role in antigenicity and viral fitness.

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*Keywords: Influenza A H1N1, N-Glycosylations, receptor binding site, Antigenicity.*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10097

## IMMUNOGENIC RESPONSE OF A NOVEL VLP DESIGNED USING THE INFLUENZA NEURAMINIDASE (N1, N2), HEMAGGLUTININ (H1) AND MATRIX (M) PROTEINS

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**Introduction:** Influenza is a vaccine-preventable disease, however vaccine production confronts the greatest challenge due to the annual emergence of mutated or newer subtypes of influenza virus. In this context, virus-like particles (VLPs) are the next generation vaccines trending the research domain. Several research groups have highlighted the usefulness of neuraminidase (NA) as a vaccine antigen and reportedly VLPs incorporating consistent amounts of NA are designed and tested for immunogenic potential. A genome-deficient VLP vaccine offers the advantage of using multiple antigens, which can be incorporated to stimulate immune responses, equivalent to live-attenuated vaccines, in a cost-effective manner.

**Methods:** In this study, a novel influenza VLP containing hemagglutinin (HA), neuraminidase (N1 and N2), and matrix (M) proteins was developed (filed for Indian patent: 201741033978) and tested for immunogenicity in Balb/c mice. Two different concentrations of VLP (5µg or 10µg) with and without 15µg Quil-A adjuvant were injected to mice. Blood samples were screened for the presence of anti-NA and anti-HA antibodies.

**Results:** VLP at 10µg concentration administered in combination with 15µg Quil-A adjuvant was found to induce good anti-NA and anti-HA antibody responses.

**Conclusion:** Induction of anti-NA immune responses in mice strengthens the fact that VLP-based vaccines containing NA are promising influenza vaccine candidates for inducing long-term immunity. The positive results obtained in this study serve as a platform for future studies with different combinations of influenza surface proteins in different animal models.

*Keywords: Influenza; quadrivalent VLP; Quil-A adjuvant; immunogenicity; mice model*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 10176

## IMMUNOGENICITY AND PROTECTIVE EFFICACY OF UNIVERSAL LIVE ATTENUATED INFLUENZA VACCINE CANDIDATES EXPRESSING CHIMERIC HEMAGGLUTININS AND WILD-TYPE NUCLEOPROTEINS IN A FERRET MODEL

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**Introduction and objectives:** The development of universal influenza vaccines has been a priority for more than 20 years. Several approaches have been proposed that redirect the adaptive immune responses from immunodominant hypervariable regions to low-immunogenic but highly conserved regions of viral proteins. We hypothesized that sequential immunization with live attenuated influenza vaccines (LAIVs) expressing chimeric HAs (cHAs) and nucleoprotein (NP) of wild-type virus can afford heterosubtypic protection of ferrets.

**Methods:** We generated a panel of LAIVs expressing cHAs (the HA stalk domain from H1N1pdm09 virus and globular head domains from H5N1, H8N4 and H9N2 viruses). In addition, some strains contained WT NP, in order to induce CD8 T-cell immune responses more relevant to current infections. All LAIVs were engineered to contain identical N2 NA gene. Naïve ferrets were immunized with three doses of classical LAIVs containing non-chimeric HA and NP from master donor virus (MDV) [LAIVs (NP-MDV)]; cHA-based LAIVs containing NP from MDV [cHA LAIVs (NP-MDV)]; and cHA-based LAIVs containing NP from H1N1pdm09 virus [cHA LAIVs (NP-WT)]. Protection was assessed by infection of immunized ferrets with high dose of H1N1pdm09 virus.

**Results:** Challenge virus induced significant pathology in the non-immunized ferrets, including high virus titers in respiratory tissues, clinical signs of disease and histopathological changes in nasal turbinates and lung tissues. All three vaccination regimens protected animals from clinical manifestations of disease: immunized ferrets did not lose weight or show clinical symptoms, and their fever was significantly lower than in the control group. Further analysis of virological and pathological data revealed the following hierarchy in the cross-protective efficacy of the vaccines: cHA LAIVs (NP-WT) > cHA LAIVs (NP-MDV) > LAIVs (NP-MDV).

**Conclusion:** The prototype universal LAIVs that combine the two approaches of inducing anti-HA stalk antibody and more relevant CD8 T-cell immune responses are promising candidates for further clinical development.

**Keywords:** *Universal influenza vaccines, live attenuated influenza vaccine, chimeric hemagglutinin, nucleoprotein, ferrets*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10330

## SAPONIN-CONTAINING NANOPARTICLES AS AN EFFICIENT ADJUVANT/DELIVERY SYSTEMS FOR INFLUENZA VACCINE MUCOSAL IMMUNIZATION

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**Introduction and Objectives.** Mucosal immunization against influenza is attractive due the number of reasons: it safe, non-traumatic, easy for mass-immunization and can provide immune barrier in primary infection gate. Most commercial influenza vaccines today are injectable and unable to induce high levels of immune responses when delivered at mucosal sites. In order to make them more immunogenic and prepare vaccine for mucosal immunization, strong mucosal adjuvants/delivery systems are required. In the research presented novel adjuvant/delivery systems for mucosal immunization were elaborated.

**Methods.** Triterpen saponins "Glabilox" and "SO1" with low toxicity and high immunostimulatory activity were isolated from plants *G.glabra* and *S.officinalis* indigenous to Kazakhstan using HPLC fractionation. Mice were immunized intranasally various vaccine preparation in doses 3.0/5.0/10.0 µg per animal. Sera were taken 21 days after single immunization and mice challenged with influenza virus in dose 100 EID<sub>50</sub>.

**Results.** 2 kind of preparations for mucosal immunization contained "Glabilox" and "SO1" saponins were elaborated: (1) adjuvant on the base of saponin/lipid nanoparticles, (2) nanoparticles containing HA+NA influenza virus antigens, lipids and saponins. Immunostimulation activity and protection capacity of whole-virus inactivated influenza vaccine contained saponin/lipid nanoparticulate adjuvant and subunit influenza vaccine on the base of antigen/saponin/lipid nanoparticles were studied in animal vaccination/challenge experiments. It was shown that single intranasal immunization with whole-virus inactivated vaccine contained saponin/lipid adjuvant and same immunization with subunit vaccine on the base of antigen/saponin/lipid nanoparticles induced formation of high levels of IgM, IgA, IgG1, IgG2a, IgG2b antibody and stimulated production of IL-2, IL-4, IL-10, IFN-γ cytokines. Immunization/challenge experiments demonstrated 80-90% protection against influenza infection after intranasal immunization with subunit influenza vaccine and 100% protection following intranasal immunization with whole-virus inactivated influenza vaccine contained saponin/lipid nanoparticulate adjuvant.

**Conclusion.** Adjuvant/delivery systems on the base of nanoparticles containing purified triterpen saponins "Glabilox" and "SO1" are efficient for mucosal immunization against influenza.

*Keywords: Saponins, nanoparticles, influenza, mucosal immunization*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10382

## Cross-protective potential and protection-relevant immune mechanisms of whole inactivated influenza virus vaccines are determined by adjuvants and route of immunization

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### Introduction and objectives

Adjuvanted whole inactivated virus (WIV) influenza vaccines show promise as broadly protective influenza vaccine candidates. Using WIV as basis we assessed the relative efficacy of different adjuvants by carrying out a head-to-head comparison of the liposome-based adjuvants CAF01 and CAF09 and the protein-based adjuvants CTA1-DD and CTA1-3M2e-DD and evaluated whether one or more of the adjuvants could induce broadly protective immunity.

### Methods

mice were immunized intramuscularly (i.m.) or i.n. with A/Puerto Rico/8/34 (PR8) WIV with or without the different adjuvants and 2 weeks after the final immunization mice were challenged with homologous PR8, heterologous (H1N1)pdm09 or heterosubtypic X-31 (H3N2) virus to assess protection and several immune parameters.

### Results

In general, intranasal immunizations were significantly more effective than intramuscular immunizations in inducing virus-specific serum-IgG, mucosal-IgA and splenic IFN $\gamma$ -producing CD4 T cells. Intranasal immunizations with adjuvanted vaccines afforded strong cross-protection with milder clinical symptoms and better control of virus load in lungs. Mechanistic studies indicated that non-neutralizing IgG antibodies and CD4 T cells were responsible for the improved cross-protection while IgA antibodies were dispensable. The role of CD4 T cells was particularly pronounced for CTA1-3M2e-DD adjuvanted vaccine as evidenced by CD4 T cell-dependent reduction of lung virus titers and clinical symptoms.

### Conclusion

Thus, intranasally administered WIV in combination with effective mucosal adjuvants appears to be a promising broadly protective influenza vaccine candidate.

*Keywords: whole inactivated virus (WIV) influenza vaccines; liposome-based adjuvants; protein-based adjuvants; cross protection; non-neutralizing serum antibodies; CD4 T cells*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 10408

## Protection against hMPV conveyed by influenza virus vectors carrying multiple epitope antigens of hMPV in the NS protein

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**Introduction and Objectives** Human metapneumovirus (hMPV) is an important viral pathogen that causes respiratory infections in infants, elderly individuals and immune-compromised individuals, and no vaccine is currently available. This article is to develop vaccine candidates against both influenza virus and hMPV conveyed by influenza virus vectors carrying multiple epitope antigens of hMPV.

**Methods** hMPV multi-epitope gene segments were inserted into the NS gene of the influenza virus. Reverse genetic techniques were used to generate recombinant influenza viruses with the recombinant NS gene; the process was confirmed by whole genome sequencing, and the viruses were designated as rFLU/hMPV/B and rFLU/hMPV/CTL+Th. BALB/c mice were immunized intranasally with rFLU/hMPV/B and rFLU/hMPV/CTL+Th twice at two-week intervals. Virus-specific antibody titers, splenocyte cytokines and virus challenge protective effects were detected 2 weeks after the boost immunization.

**Results** We successfully constructed recombinant influenza viruses in which hMPV multi-epitope antigens were expressed. BALB/c mice were immunized intranasally with rFLU/hMPV/B and showed a viral-specific antibody response against both the influenza virus and hMPV, while mice immunized with rFLU/hMPV/CTL+Th showed an influenza virus-specific antibody response and a hMPV-specific cytotoxic lymphocyte response (significant IFN- $\gamma$  secretion). Additionally, balanced Th1/Th2 responses were elicited by rFLU/hMPV/B and rFLU/hMPV/CTL+Th, as shown by the splenocyte cytokine secretion tests. Both rFLU/hMPV/B and rFLU/hMPV/CTL+Th conveyed effective protection against subsequent influenza virus and hMPV challenges, with lower viral loads and clear attenuation of the histopathological changes associated with viral infections observed.

**Conclusions** rFLU/hMPV/B and rFLU/hMPV/CTL+Th are promising vaccine candidates. Further assessment should proceed using other animal models.

*Keywords: human metapneumovirus; influenza virus; epitope; viral vector*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10425

**INDUCTION OF NEUTRALIZING STEM ANTIBODIES BY IMMUNIZATION WITH INFLUENZA A HEMAGGLUTININS FROM THE STRAINS SENSITIVE TO NEUTRALIZING ANTIBODIES AND IDENTIFICATION OF VIRUS RESISTANCE MUTATIONS AGAINST A STEM MONOCLONAL ANTIBODY**

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**Introduction:**

The frequent emergence of influenza variants with mutations in the head region of HA requires influenza vaccines to be reformulated annually to match the dominant circulating strains. Vaccines that elicit broadly neutralizing antibodies to the conserved stem of hemagglutinin (HA) are being developed as a strategy for next-generation influenza vaccines that protect against influenza across multiple years. However, the efficient induction of neutralizing stem antibodies remains a challenge, and it is unclear whether influenza viruses can readily escape neutralization by such antibodies.

**Methods:**

As a strategy to elicit cross-neutralizing sera, we immunized mice and rabbits with HAs from influenza viruses that are sensitive to neutralizing stem antibodies. We further generated and selected mouse monoclonal antibodies for cross-neutralizing activity and the ability to confer protection against virus challenge. Viruses that escaped neutralization by a cross-neutralizing monoclonal were selected and characterized.

**Results:**

Sequential immunizations with the HA from influenza viruses that are sensitive to neutralizing stem antibodies elicited antisera with cross-neutralizing activity. A stem monoclonal antibody, 4C2, that broadly neutralizes many subtype influenza A strains from group 1 influenza viruses was isolated. The 4C2 provided protection against influenza challenge from a heterosubtypic strain. HA mutations that allow viruses to escape 4C2 neutralization were identified. Molecular modeling suggests that these mutations alter HA structure and indirectly limit antibody accessibility to the neutralizing epitope.

**Conclusion:**

These results inform vaccination approaches for eliciting cross-neutralizing antibodies and identify mutations that may allow escape from neutralization by stem antibodies.

*Keywords: influenza hemagglutinin, neutralizing stem antibody, next-generation influenza vaccine, virus escape mutations*



Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 10461

## GENERATION AND CHARACTERIZATION OF DELNS1 INFLUENZA A AND B VIRUSES: A STRATEGY FOR OPTIMIZING LIVE ATTENUATED INFLUENZA VACCINES

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### Background:

Vaccination is considered the most effective way to alleviate the disease burden and mortality associated with seasonal influenza and to curb future human pandemics. Currently available inactivated influenza vaccines are suboptimal, particularly in the most at-risk groups, and do not induce long-lasting and sufficiently broad cross protective activity to protect against antigenically drifted strains. The influenza virus NS1 protein is a key virulence element with multi-functional roles in virus replication. Deletion of is expected to create safer and more immunogenic live attenuated influenza virus (LAIV) vaccines.

### Methods & Results:

Using reverse genetic technology, we have developed two master backbones based on the 2009 H1N1 influenza A (CA4) and 2011 influenza B (HK8038) viruses which contain *NS1*-deleted (DeINS1) viral genome. We identified novel adaptive mutations which support influenza A and B DeINS1 LAIV replication in vaccine producing cells, without the need for helper virus. All the DeINS1 LAIVs characterized are highly attenuated in human cells *in vitro* and avirulent in mice but replicate well in eggs and MDCK cells. Influenza A and B DeINS1 viruses both grow better at 33°C than at 37-39°C. Vaccination with CA4-DeINS1 LAIV grants potent protection against lethal challenge with homologous virus and strong cross protection against heterosubtypic (H3N2) or antigenically distant influenza A and B mouse-adapted viruses in mice. A mechanistic study found that DeINS1 LAIVs induce considerable, broad and long-lasting antibody and CD8<sup>+</sup> and CD4<sup>+</sup> T cell immune responses. Significantly, DeINS1 LAIV can be used to enhance specific anti-influenza immunity through inclusion of an additional antigen gene in the deleted *NS1* site.

### Conclusions:

Generation of DeINS1 LAIVs with adaptative mutations promoting growth in vaccine production systems is an important strategy for making highly attenuated and immunogenic vaccines that induce broad cross protective immunity against seasonal and emerging influenza strains.

**Keywords:** influenza; vaccine; live attenuated virus; H1N1; H3N2; influenza B; CD4 T cells; CD8 T cells

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 10484

## Influenza H3N2 viruses have a low genetic barrier to resistance to broadly neutralizing hemagglutinin stem-binding antibodies

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In the past decade, the discovery and characterization of broadly neutralizing antibodies (bnAbs) that target the highly conserved stem region of influenza hemagglutinin (HA) have provided valuable insights and stimulation for development of a universal influenza vaccine. However, the genetic barrier for resistance to HA stem-binding bnAbs has not been thoroughly evaluated. Here, we perform a series of deep mutational scanning experiments to probe for resistance mutations to HA stem-binding bnAbs. Our results indicate that the genetic barrier to resistance to HA stem-binding bnAbs is generally very low for the H3 subtype, but is substantially higher for at least some H1 strains. Several resistance mutations in the H3 subtype cannot be neutralized by HA stem-binding bnAbs at the highest concentration tested, do not reduce the *in vitro* fitness and *in vivo* pathogenicity, and are often present in circulating strains as minor variants. Overall, this study reveals that different influenza viral strains and subtypes may differ substantially in their propensity to escape from stem-directed antibodies and thus underscores a potential challenge for development of a *bona fide* universal influenza vaccine.

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10508

## LIVE ATTENUATED INFLUENZA VACCINES EXPRESSING FOUR M2E TANDEM REPEATS WITHIN THE HEMAGGLUTININ MOLECULE PROTECT MICE AGAINST DIVERGENT INFLUENZA VIRUSES

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**Introduction and Objectives:** The ectodomain of the influenza membrane protein M2 (M2e) is a promising target for designing a universal influenza vaccine. However, natural M2e is poorly immunogenic. To increase the immunogenicity of the M2e various types of carriers, adjuvants and immunization strategies have been used. Despite all attempts, none of the M2e-based universal vaccines has been licensed yet. A new strategy of induction M2e-specific antibody is the expression of M2e tandem repeats within the hemagglutinin (HA) molecule of live attenuated influenza vaccine (LAIV) virus. The goal of this study is to generate prototype universal influenza vaccines based on H7, H3, H1 subtype LAIVs and characterize their protective properties in a mouse model.

**Methods:** Recombinant LAIV viruses expressing chimeric HA+4M2e proteins were generated by the means of reverse genetics. The expression of M2e epitopes by the chimeric viruses was confirmed by ELISA with M2e-specific antibody 14C2. Infectious virus titers were determined in eggs and MDCK cells incubated at different temperatures. Protective efficacy of new recombinant LAIVs against a panel of divergent influenza viruses was assessed in BALB/c mouse model, using either direct or indirect (passive immunization) strategies.

**Results:** All the LAIV-4M2e viruses efficiently replicated in eggs and MDCK cells and preserved the temperature sensitive and cold-adapted phenotypes typical for LAIV viruses. The LAIV-4M2e immunized mice developed high levels of serum anti-M2e antibodies. Both LAIV-4M2e and the LAIV vector protected mice against heterologous influenza viruses using direct immunization strategy. The passive immunization strategy revealed better protection in the LAIV-4M2e immunized group, compared to the control groups.

**Conclusion:** LAIVs expressing four M2e tandem repeats are the promising prototypes of universal influenza vaccine. Further investigation of the possible mechanisms of protection afforded by M2e-specific antibodies is needed.

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Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10509

## Improvement of influenza virus-like particle production from a new baculovirus design

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To date, vaccination remains the best strategy for preventing severe illness and death caused by influenza viruses. Currently, Egg-based production has still been the dominant method to produce seasonal influenza vaccines, but it has several drawbacks. Recently, virus-like particle (VLP) vaccines have been considered as an alternative method. In the previous study, we established an influenza H7N9 VLP expression system and optimized its downstream purification. The proteins of VLP were shown to express at a very high level under the control of very late polyhedrin and p10 promoter, however, the post-translational modification seems to worsen during the very late phases of baculovirus infection and may affect the VLP assembly. Hence, we tried to use early promoters for the protein expression of VLPs to improve its yield and quality.

The cDNA of HA, NA, and M1 derived from influenza viruses A/Taiwan/1/2013(H7N9) were cloned into the pFastBac-Dual vector to generate influenza VLP-TH by Bac-to-Bac<sup>®</sup> Baculovirus expression system. Based on the construction of VLP-TH, the promoters of HA, NA and M1 genes were exchanged to early promoters, respectively. The VLP production from these constructions was analyzed by the hemagglutination assay and dot-blot assay.

Our results showed that the constructions with the early promoter replacement of NA and HA genes had no HA titer, indicating that they were failed to produce VLPs. However, the constructions with the early promoter substitution of M1 gene, M1/HA gene, or M1/NA gene, respectively, had similar HA titer compared to VLP-TH, demonstrating that M1 expression at the early phase of baculovirus infection did not affect the VLP production. The protein composition, structure, glycosylation, and immunogenicity of new VLPs will be further investigated.

The generation of influenza VLPs in insect cells is complicated. To modulate the protein expression stage through promoter substitution could be a strategy to improve the VLP quality.

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10533

## Single-dose nanoparticle-based M2e peptide vaccine induces cross-protective immunity against influenza A viruses

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Influenza is a persistent threat to public health. Current influenza vaccines rely on the hemagglutinin protein as an antigen to induce neutralizing antibodies. However, mutant viruses acquire the ability to escape from prevailing herd immunity by antigenic drift and shift. It is necessary to yearly update the composition of seasonal influenza vaccines to match the newly circulating viruses. In this study, we aim to develop a universal influenza A vaccine to induce cross protection against divergent virus strains by using a nanocarrier for antigen delivery. The extracellular domain of influenza A ion channel membrane matrix protein 2 (M2e) is chosen as the antigen target as it is highly conserved among subtypes and considered to be a potential candidate antigen for a universal influenza A vaccine. To enhance the immunogenicity of M2e, we constructed a vaccine formulation comprising of the consensus M2e peptide and cyclic di-guanosine monophosphate (cdGMP, a STING agonist as an adjuvant) encapsulated in hollow PLGA nanoparticles (M2e-cdGMP-NPs). Contrary to the common notion that antigens need to be displayed on particle surfaces to effectively induce humoral responses, subcutaneous delivery of M2e-cdGMP-NPs induces robust anti-M2e serum IgG response in mice, with stimulation of both IgG1 and IgG2a isotypes. The antibodies induced by the M2e-cdGMP-NPs are capable of binding to the native form of M2 proteins expressed on H1N1- and H3N2-infected cells. Notably, single-dose nanoparticles-vaccinated mice showed 100% survival following a lethal challenge with A/Puerto Rico/8/1934 (H1N1) strain. Overall, M2e-cdGMP-NPs significantly enhances the immunogenicity of M2e peptide, eliciting a cross-reactive protection for better prevention of influenza virus infections. The hollow PLGA nanoparticle provides a powerful platform to improve the effectiveness of peptide vaccines.

*Keywords: Influenza A virus; universal vaccine; M2e; nanoparticles*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 10534

## A STING-activating polymeric nanoparticle enhances humoral and cellular immunity against influenza A virus

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Influenza is of public health significance, and establishing herd immunity is critical for the population. Toward a safe and effective vaccine, adjuvants are usually required to boost the immunogenicity of the protein subunit vaccines. In this study, we reported the use of nanoparticulate cdGMP (cyclic di-guanosine monophosphate), which binds to STING (stimulator of interferon genes) to activate the downstream signaling pathways and trigger a Th1 immune response, as an adjuvant for the influenza HA subunit antigen. Thin-shell hollow PLGA nanoparticles encapsulating cdGMP were prepared using a double emulsion technique. Characterizations by cryo-EM and dynamic light scattering analysis revealed that the cdGMP nanoparticles had a unimodal particle distribution with an average size of 110 nm. In mice, subcutaneous delivery of cdGMP nanoparticles with ovalbumin induced prominent T cell response than free molecules of cdGMP. Compared to free cdGMP-receiving group, mice immunized with HA protein adjuvanted with cdGMP nanoparticles showed higher level of HA-specific IgG2a, long-lasting hemagglutination-inhibiting antibodies, and increased numbers of germinal center B cells, follicular helper T cells and central memory T cells. Following live viral challenge at 3 weeks after immunization, mice receiving the cdGMP nanoparticle adjuvant had undetectable viral titers in the lung and bronchoalveolar lavage fluid. Moreover, at 5 months post immunization, the cdGMP nanoparticle adjuvant elicited a superior recall response, 100% protection against the lethal viral challenge, whereas the free cdGMP group showed 33% survival. In sum, the novel STING-activating nanoparticles in this study provide a superb adjuvancity for the existing HA protein antigen, enabling effective prophylaxis against influenza.

*Keywords: Influenza virus; STING agonist; cdGMP nanoparticles; vaccine*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10655

## Development & characterization of an aerodynamic system for pulmonary delivery of influenza vaccine

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*Influenza* means "influence", commonly referred to as flu, is an infectious disease caused by RNA viruses of family Orthomyxoviridae, that affects birds & mammals. The aim is to develop aerodynamic systems with r-H1N1Ags for safely deposition in alveoli to enhance bioavailability & control release of influenza antigen after pulmonary administration in animal model. This induces not only systemic humoral (IgG) responses, but also cell-mediated (IL-4, IFN- $\gamma$ ) and mucosal immune responses (IgA, IgG), non-invasive, propellant & needle free delivery of vaccine.

Chitosan microparticles were prepared by ionic gelation method of chitosan with tripolyphosphate (TPP). The formulations were optimized on basis of particle size, tap density & entrapment efficiency. The external morphology of the optimized formulation was studied by TEM & SEM. The zeta potential was determined along with stability studies at accelerated temperatures. The in-vivo studies involved determination of antibody titres in serum and mucosal secretions & uptake studies by fluorescence microscopy.

The results show that as the preparation was reduced to lyophilized form which increased the stability as compared to conventional liquid formulations. The fluorescence images show the uptake of microparticles by various organs and the ELISA results show comparable IgG responses along with IgA.

The Chitosan microparticles have higher positive values of zeta potential due to presence of hydroxyl group of chitosan & shows positive surface charge. In case of charged particles, as zeta potential increases, repulsion interaction will be larger leading to formation of more stable particles with more uniform size distribution. Tap Density is most necessary parameter for aerodynamic microparticles with low density were expected to avoid macrophage uptake & accumulate in deep lung epithellum, one can generate large particles of low density to both optimize aerodynamic diameter & prevent phagocytosis. Thus, Antibody production was found to be more in pulmonary as compared to other routes.

*Keywords: Influenza; Chitosan; Microparticles*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 10680

## Generation of avian influenza A DIVA vaccines with chimeric hemagglutinin recombinant viruses

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### Introduction

The H9N2 subtype is believed to spread rapidly and has become one of the most prevalent LPAI viruses in the domestic poultry industry. The number of countries and regions affected by Asian H5Nx HPAI viruses reached a maximum in 2006. Asian H5N8 HPAI viruses spread to some many countries, resulting in the emergence of the novel combination of the H5 HA and NA subtypes associated with the clade 2.3.4.4 HA. Therefore, the need for more effective and efficient vaccine development for the H9N2 and H5Nx viruses emerged.

### Materials and Methods

The chimeric H9/H5 HA virus, the HA1 region of H9N2 and the HA2 region of the H5N8 were generated. The cHA H9/H5N2 and H9N2 viruses were propagated in 10-day-old SPF embryonated eggs for vaccine preparations. Six-week-old SPF chickens were used in this study for vaccine efficacy. Three of each vaccine immunized chickens were euthanized on days 1, 3, and 5 post challenge, and multi-organ tissue samples were collected for virus titrations.

### Result

The chimeric H9/H5N2 virus showed growth properties *in vitro* and *in vivo*, and the virulence were similar to that of the LPAI H9 virus. An inactivated vaccine based on this chimeric virus induced high serum neutralizing antibodies against both H9 and H5 viruses but induced cross-reactive hemagglutination inhibition antibody only against H9 viruses. The cHA H9/H5N2 vaccination strategy provides robust protection against homologous, heterologous, and heterosubtypic viruses of both subtypes. Furthermore, each HA1- and HA2 stalk-specific antibody response was sufficient to inhibit viral replication and protect chimeric virus-immunized mice from lethal challenge with mouse-adapted H9N2, H5N2, or wild-type HPAI H5N1 virus.

### Discussion

Our results demonstrate that the novel chimeric H9/H5N2 recombinant virus is a low pathogenic virus, and this chimeric vaccine is suitable for a DIVA vaccine with broad spectrum neutralizing antibody against H5 avian influenza viruses.

*Keywords: avian influenza virus, chimeric vaccine, poultry, H9N2, H5N8, H5N1*



Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10688

## Stability and immunogenicity of influenza virus hemagglutinin monomers

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**Background:** Influenza viruses undergo continuous antigenic changes that occur mostly at the head region of hemagglutinin (HA) that shows considerable antigenic flexibility or plasticity. Antibodies against the head region are typically induced at much higher levels than those against the stem region that is highly conserved. In a recent serum antibody repertoire analysis, antibodies against the monomer interface of HA trimer were reported to represent a broad spectrum of specificities and confer protection in animal models. We showed previously that the recombinant HA ectodomain derived from a pandemic strain A/Korea/01/2009 was monomeric in solution, and a double mutant F88E/V91W of a seasonal strain A/Thailand/CU44/2006 was engineered to be a monomer.

**Methods:** Baculoviruses containing mutant genes to make monomeric HA mutants were prepared by site-directed mutagenesis. The recombinant proteins were expressed in insect cells, purified using chromatographic methods, and characterized by gel electrophoresis, differential scanning fluorimetry (DSF) and size exclusion chromatography-multi-angle laser light scattering (SEC-MALS).

**Results:** We designed HA monomers from subtypes H1, H3 and type B, by incorporating mutations to destabilize trimer conformations, and examined the stabilities and potential antigenicities of the monomeric forms. Starting with the HA trimers from seasonal strains, mutations were introduced at the intermonomer interface, corresponding to a double mutant (F88E/V91W) of H1 HA. The mutant proteins were expressed and purified using chromatographic methods. The thermal stabilities of the monomeric HA mutants were shown to be lower than those of the corresponding trimers by 10-15°C in melting temperature. Nevertheless, the immunogenicity of one of the monomeric forms was comparable to that of the trimeric form.

**Conclusion:** The monomeric mutants showed lower stability, compared to the trimeric forms. The immunogenicity of one of the monomeric forms was comparable to that of the trimeric form.

**Keywords:** Influenza virus, Hemagglutinin, Monomer, Antigenicity

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10692

## Generation of high-growth influenza virus A backbone in MDCK cells, and evaluation for the vaccine seed strain

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### Introduction

As shown during the 2009 pandemic H1N1 (A(H1N1)pdm09) outbreak, egg-based influenza vaccine production technology is insufficient to meet global demands during an influenza pandemic. Therefore, there is a need to Madin-Darby canine kidney (MDCK) adapt cell culture-derived vaccine technology using suspended cell lines for more rapid and larger-scale vaccine production.

### Methods

To generate the high-growth (HG) PR8 backbone virus, PR8 virus was serially passaged in MDCK cells and selected HP PR8 virus clones. Based on full length sequencing of HG cones, the individual characteristic mutations in PB2, PB1, PA, M, and NS were introduced into the parental PR8 internal backbone by site-directed mutagenesis system, the rescued each backbone strains was evaluated in attached and suspended MDCK cells.

### Result

Following 48 serial passages with four rounds of virus plaque purification in MDCK cells, we were able to select several MDCK-adapted plaques that could grow over 10<sup>8</sup> PFU/ml. By using a series of Rg viruses, we demonstrated the essential residues of each gene and identified a set of high-growth strains in MDCK cells (PB1 D153N, M1A137T, and NS1N176S). In order to confirm whether the high-growth property of MDCK-adapted HG virus is a general feature, we substituted this backbone into the seasonal human A/H1N1 (A/California/07/2009) and A/H3N2 (A/Perth/16/2009) strains as well as the avian HPAI H5N1(A/environment/Korea/ΔW150/2006) vaccine combination strain. Also showed significantly enhanced growth properties (more than 10<sup>7</sup> PFU/ml) in both attached- and suspended-MDCK cells compared with each representative virus and the original PR8 vaccine strain.

### Conclusion

Taken together, this study demonstrates the feasibility of a cell culture-derived approach to produce seed viruses for influenza vaccines that can be grown promptly and vigorously as a substitute for egg-based vaccines.

*Keywords: Influenza viruses, cell-derived vaccine, Madin-Darby canine kidney (MDCK) cells, bioreactors*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10703

## Antibody dependent enhancement of influenza disease promoted by increased virus fusion kinetics: Implications for Safety and Efficacy of next generation influenza vaccines and therapeutic antibodies

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**Background:** Multiple next-generation (universal) influenza vaccines and broadly neutralizing antibodies (bNAbs) in clinical development inhibit virus replication at post-entry stage or use Fc-dependent mechanisms and may mediate enhancement of viral infection or disease. Therefore, it is important to develop animal models and *in vitro* assays to evaluate both protection and potential antibody dependent enhancement (ADE) of infection and/or enhanced respiratory disease (ERD).

**Methods:** Mouse model was used to measure lung viral loads, lung pathology and lung cytokines in addition to weight loss and lethality after influenza virus challenge. Multiple doses of two HA specific MAbs were administered to mice prior to challenge with H3N2 virus. Novel *in vitro* single influenza virus particle assay was developed to track individual virions in the endocytic compartment during influenza infection to study impact of bNAbs or vaccine-induced antibodies on virus fusion kinetics.

**Results:** ADE of infection and/or ERD was manifested by increased lung pathology and changes in lung Th2/Th1 cytokines in a MAb and dose-dependent manner. One reduced lung viral loads at the high dose but increased viral loads at the lower doses, whereas another MAb did not impact the lung viral load. Both MAbs shifted the HA0 pH-induced sensitivity to trypsin cleavage resulting in increased sensitivity to higher pH. Fab induced destabilization of the HA stem domain. In a new *in vitro* assay that tracks labelled virus particles into the endocytic compartment, the two MAbs reduced the H3N2 virus residence time in the endocytic pathway, suggesting faster virus fusion kinetics.

**Conclusions:** Our findings highlight the need to carefully evaluate next generation influenza vaccines and antibody-based therapeutics for both protection and enhanced disease following influenza virus challenge. The *in vivo* model and *in vitro* assays would help pre-clinical evaluation of safety and effectiveness of next generation influenza vaccines and therapeutics.

**Keywords:** Influenza, Universal Vaccine, Antibody dependent enhancement, Stalk, Enhanced Respiratory disease

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10704

## AS03 Adjuvant Promotes H5N1 Antibody Diversity and Affinity Maturation: Improved Cross-Clade Neutralization, Higher Neuraminidase Inhibition, but Limited Cross-Subtype activity

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**Background:** Adjuvants are combined with vaccines against avian influenza with pandemic potential in order to overcome poor immunogenicity due to lack of pre-existing immunity. Full understanding of the “Adjuvant-impact” requires development of new analytical technologies.

**Methods:** We performed a comprehensive analysis of the antibody responses in a clinical trial conducted at the NIH, using the H5N1 (A/Indonesia) inactivated vaccine, administered in the presence or absence of AS03. Whole Genome Fragment Phage Display Library (GFPDL) was used to identify all the epitopes in the hemagglutinin (HA) and neuraminidase (NA) recognised by the immune sera. Surface Plasmon Resonance (SPR) was used to measure antibody binding affinity to properly folded globular head (HA1) and HA2 stem. Microneutralization assays were used to measure neutralization of vaccine matched and heterologous H5N1 viruses, and to evaluate heterosubtypic neutralization of H1N1 viruses. Neuraminidase inhibition assay (NAI) was used to evaluate the function of anti-NA antibodies.

**Results & Conclusions:** Two immunizations with AS03-adjuvanted H5N1 subunit vaccine (A/Indonesia, 3.75 µg HA/dose) induced antibody epitope spreading from HA2 to HA1 and expanded epitope diversity in neuraminidase (NA) when compared with unadjuvanted vaccine. Furthermore, a significant antibody affinity maturation to properly folded HA1 (but not HA2) domain was measured using SPR, which correlated with neutralization titers against the vaccine strain and several heterologous H5N1 strains. However, no significant increase in heterosubtypic cross-neutralization of several H1N1 seasonal strains was observed. The NA response showed increase in epitope diversity (including targets close to the enzymatic site) in the AS03-adjuvanted group that correlated with higher neuraminidase inhibition (NAI) titers following vaccination.

**Conclusions:** This study provides insight into the impact of AS03 adjuvant on antibody responses following H5N1 IIV vaccination. Better understanding of the vaccine induced immune responses will help to select adjuvants and vaccine platforms for next generation influenza vaccines and new pandemic strains.

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10706

## Immune-Focused HA1 Influenza Vaccines Provide Long Lasting Cross-Protective Immunity

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**Background.** Traditional influenza vaccine production based on inactivated viruses may not be optimal for a rapid response to influenza pandemic requiring global mass vaccination. In Influenza virus, HA1 globular domain is the target of most neutralizing/protective antibodies *in vivo*. New vaccines are required to focus the immune response to known protective targets that can be rapidly produced for more effective response than currently licensed vaccines.

**Methods.** Recombinant HA1 globular domain from multiple influenza strains including pandemic H5N1 (A/Vietnam/1203/2004 & A/Indonesia/5/05), H7N7 and H7N9 were produced in *E coli* and purified under redox refolding conditions.

**Results.** All recombinant HA1 (rHA1) domains contained functional oligomers composed of 4-6 trimers without addition of exogenous trimerization sequence. These proteins were shown to be stable for >6 months at 4<sup>0</sup>C. The purified HA1 proteins bound cell surface receptor and agglutinated RBC. The rHA1 proteins elicited high titer neutralizing antibodies against homologous and heterologous pandemic influenza viruses in rabbits and ferrets. Ferrets vaccinated with the rHA1 were fully protected from lethality and weight loss following challenge with homologous and heterologous wild type highly pathogenic H5N1, H7N7 and H7N9 viruses. Protection was associated with a significant reduction in viral loads in the nasal washes and lungs of homologous and heterologous virus challenged ferrets immunized with HA1 domain, which was not significantly reduced in ferrets vaccinated with inactivated influenza vaccine compared with unvaccinated controls. Antibody kinetic analyses demonstrated significant affinity maturation of the antibodies elicited by HA1 but not with licensed inactivated influenza vaccines. The rHA1 vaccine generated long lasting high titer neutralizing antibodies and provided protection up to 6 months following vaccination in ferrets.

**Conclusions.** Our findings suggest that “immune focused” effective HA1 based can be rapidly produced in scalable bacterial system and are ideal for rapid response to emerging pandemic threat for the global population.

*Keywords: HA1, Vaccine, Adjuvant, Universal, Protection*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10732

## Highly immunogenic influenza virus-like particles containing B-cell-activating factor (BAFF) for multi-subtype vaccine development

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Virus-like particle (VLP) technology is an attractive platform for the development of seasonal and pandemic influenza vaccines. Influenza VLPs can be obtained by the overexpression of HA, M1, NA, and/or M2 viral proteins in insect, mammalian, or plant cells. In this study, we reported to obtain highly immunogenic influenza VLPs by molecular incorporation with B-cell-activating factor (BAFF) or proliferation-inducing ligand (APRIL). Since BAFF and APRIL act as homotrimers to interact with their receptors, we engineered the VLPs by direct fusion of BAFF or APRIL to the transmembrane anchored domain of H5HA gene. Results showed that immunizations with the HA-transmembrane anchored BAFF- or APRIL-VLPs only formulated in alum but not MPL adjuvant elicited significantly higher IgG titers in sera. However, only the BAFF-VLPs formulated in alum adjuvant elicited more broadly neutralizing antibodies against the homologous and two heterologous H5N1 clade/subclade viruses and conferred protective immunity against live virus challenges. As the multi-subtype influenza vaccines containing a variety of HA subtypes can confer broader protective immunity, we also obtained multi-subtype H5H7 BAFF-VLPs and H1H5H7 BAFF-VLPs and demonstrated that these multi-subtype BAFF-VLPs were able to induce the production of neutralizing antibodies against multiple HA subtypes. Our findings provided useful information for the development of highly immunogenic, multi-subtype influenza VLP vaccines.

*Keywords: influenza VLP, BAFF, multi-subtype vaccines*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10759

## Glycan-masking hemagglutinin antigens from stable CHO cell clones for H5N1 avian influenza vaccine development

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Refocusing of B cell responses can be achieved by preserving the overall fold of the antigen structure but selectively mutating the undesired antigenic sites with additional N-linked glycosylation motifs for glycan-masking the vaccine antigen. We previously reported that glycan-masking recombinant H5 hemagglutinin (rH5HA) antigens on residues 83, 127, and 138 (g127+g138 or g83+g127+138 rH5HA) elicited broader neutralizing antibodies and protection against heterologous clades/subclades of high pathogenic avian influenza H5N1 viruses. In this study, we engineered the stably-expressing CHO cell clones for producing the glycan-masking g127+g138 and g83+g127+g138 rH5HA antigens. All of these glycan-masking rH5HA antigens produced in stable CHO cell clones were found to be mostly oligomeric structures. Only the immunization with the glycan-masking g127+g138 but not g83+g127+g138 rH5HA antigens elicited more potent neutralizing antibody titers against four out of five heterologous clades/subclades of H5N1 viral strains. The increased neutralizing antibody titers against these heterologous viral strains were correlated with the increased amounts of stem-binding antibodies, only the glycan-masking g127+g138 rH5HA antigens can translate into more protection against live viral challenges. The stable CHO cell line-produced glycan-masking g127+g138 rH5HA can be used for H5N1 subunit vaccine development.

*Keywords: glycan-masking; hemagglutinin; CHO cells; H5N1 vaccine*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10760

## Induction of heterologous protection by combined use of sequential influenza vaccination strategy in Balb/c mouse

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**Background:** Influenza remains a most common and significant public health concern worldwide. Annual vaccination is recommended as the most important step against influenza and its potentially serious complications. However, current vaccines leave the public exposed to the emerging influenza viruses from antigenic drift or shift. Therefore, it is a high priority to develop tools that can induce broader immunity to control the escaped divergent viruses.

**Method:** BALB/c mice were treated intramuscularly with 2 or 4 doses of inactivated (seasonal H1N1/A/Brisbane/59/07, pandemic H1N1/A/California/09/2009, and highly pathogenic H5N1/A/VN/1203/04; plus adjuvant) and vaccinia virus-based H5N1 live-attenuated (Wyeth/IL-15/5Flu) vaccines in different combinations (3 weeks interval). Wyeth/IL-15/5Flu is a novel pentavalent vaccine, expressing HA, NA and NP proteins from H5N1/A/Vietnam/1203/2004, M1 and M2 proteins from H5N1/A/CK/Indonesia/PA/2003 virus, and human IL-15 as a molecular adjuvant. 3 weeks post-vaccination, H9N2/Y280 was given intranasally, and weight loss were monitored daily. Lung viral titers were measured by TCID<sub>50</sub> assay at day 3. T cell recall responses from BAL at day 7 were determined by intracellular cytokine staining assay. Protective NP-specific Ab measurement in sera was conducted using ELISA at day 7.

**Results:** All studied sequential 4-dose vaccinations could induce higher degrees of heterosubtypic immune response in mice. Significantly different kinetics in regaining weight was seen ( $P < 0.01$ ). Furthermore, the lung viral load represented a faster clearance of virus after challenge ( $P < 0.01$ ). Meanwhile, recovery was partially associated with higher CD8<sup>+</sup> T cell responses in the BAL (IFN- $\gamma$  and TNF- $\alpha$ ,  $P < 0.05$ ) and higher levels of NP-specific IgG1 Ab in sera, which depended on vaccination regimens.

**Conclusion:** These vaccine methodologies may lead to the development of universal vaccine strategy for future influenza prophylactics.

*Keywords: Universal vaccine, influenza, live-attenuated, sequential vaccination*



Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10761

## A step toward a universal influenza vaccine: Pan-influenza A protection by single immunization with X-31 cold-adapted live attenuated vaccine

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**Introduction and Objectives:** A universal influenza vaccine that provides broad protection to multiple subtypes of the influenza virus has long been pursued by various approaches, prominently by hemagglutinin (HA) stalk- or M2 extracellular domain (M2e)-based vaccines. However, the breadth of protection is moderate, mostly within the same group, and the strength is weak, necessitating multiple vaccinations.

**Methods:** Using a mouse model, we tested different prime-boost combinations of cold-adapted, live attenuated influenza vaccines (CAIVs) for their ability to induce humoral and T cell responses, and protective efficacy against H1 and H5 (HA group 1) as well as H3 and H7 (HA group 2) influenza viruses. The assay involved HAI, neutralization, fusion inhibition titers of sera, lung viral titers, and the depletion of T cells and NK cells from immunized mice. The master strain of CAIV is based on the X-31ca (reassortant of A/PR8/34 and A/HK/6/68) strain.

**Results:** A single vaccination with X-31ca provides a broad and potent cross-protection covering antigenically distinct hemagglutinin (HA) group 1 and 2 influenza viruses. Notably, even in the absence of antibody-mediated neutralizing activity or hemagglutinin inhibitory activity *in vitro*, CAIVs provided a potent protection against heterologous and hetero-subtypic lethal challenges *in vivo*. *In vivo* depletion experiments demonstrated not only CD4+ and CD8+T cells, but NK cells contributed to the cross-protection, signifying the role of both cell-mediated protection, and antibody-dependent cellular cytotoxicity (ADCC).

**Conclusion:** X-31ca CAIV-based strategy can serve as a simple but powerful option for providing pan-influenza A protection, which has not been achieved yet by other vaccine strategies. We suggest the use of ancestral virus/viral antigen as an option for eliciting cross-protection from circulating viruses by minimizing strain-specific immune responses. The promising results of potency, breadth, and safety demonstrated in the mouse model support further studies in higher animal models for clinical relevance.

**Keywords:** Influenza; universal vaccine; cold-adapted live vaccine; ADCC

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 10765

## A Green Technology for Vaccines: Catechin as a Novel Inactivating Agent with Built-in Adjuvant for Influenza Vaccine

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**Introduction and Objectives:** Formalin (FA) have long been used for the preparation of inactivated vaccines or toxoids. FA extensively modifies vaccine antigens affecting immunogenicity profiles, sometimes compromising the protective efficacy or safety of the vaccines. A safe, non-toxic inactivating agent from natural resource with built-in adjuvant activity would provide a 'green technology' for inactivated viral vaccines.

**Methods:** Irreversible inactivation of influenza viruses by GT (green tea extract) or purified catechin EGCG (epigallocatechin-3-gallate) was tested in vitro, in ovo, and in vivo. Chemical modification of hemagglutinin (HA) was analyzed by mass-analysis. After immunization of mice, the immune responses were analyzed by ELISA, HAI, NT and antibody-dependent cellular cytotoxicity (ADCC). The protective efficacy was evaluated by lethal infection of immunized mice. The adjuvant effect was analyzed by isotypes switching of Abs with respect to Th1/ Th2 balance.

**Results:** Catechins resulted in complete and irreversible inactivation of all tested viruses, including influenza, dengue and RSV. In contrast to FA that reacts with lysine, a major anchor residue for epitope binding to MHC molecules, EGCG crosslinked primarily with cysteine residues, and thus preserved the major epitopes of the influenza HA. The vaccination completely protected the mice from lethal challenge. The quality of antibody responses of GT-inactivated vaccine was superior to that with FA-inactivated vaccine, in terms of antibody titer, the avidity to viral antigens and the cross-reactivity to hetero-subtypes. The immunogenicity was further increased by combining with alum. EGCG induced isotype switching, IgG1 into IgG2a, which was closely correlated with dramatic increase in ADCC activity.

**Conclusion:** As a 'green technology' for inactivation, the results may offer a vaccine platform with improved efficacy, safety, productivity, and the public acceptance. The anti-oxidant activity and built-in adjuvant activity of catechins could be translated into novel vaccine additives for a variety of inactivated or recombinant vaccines.

*Keywords: Influenza; Vaccine; Catechin; Inactivating agent; Adjuvant*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10772

## Multiplex, direct screening of CD8 T cell cytotoxicity reveals discrepancy against IFN $\gamma$ expression

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### Introduction

The effector functions of CD8 T cells can be measured by cytotoxicity assays. Due to the single-plex nature of these assays, this approach has relatively limited applications for large-scale epitope screenings. Intracellular Interferon-gamma cytokine staining (IFN $\gamma$ -ICS) assays is routinely used as a surrogate biomarker to predict the cytotoxicity effects of CD8 T cells. Here, we report the development of a highly robust, multiplex cytotoxicity assays to screen for CD8 T cell epitopes. Our work also reveals the discrepancy between CD8 cytotoxicity and ICS responses on certain epitopes.

### Methods

Mouse CD8 T cells from animal models for influenza, SARS and *Plasmodium* (malaria), and human CD8 T cells from healthy individuals and acute influenza patients were examined. Syngeneic donor cells were pulsed with peptides, differentially labelled, inoculated into recipient mice or human CD8 T cells respectively, and analyzed by flow cytometry the following day. These CD8 samples were also tested for their IFN $\gamma$  responses using corresponding peptides.

### Results

With three fluorescent dyes, we demonstrate that cytotoxic potential of up to 23 different CD8 T cell specificities can be assayed in a single reaction by flow cytometry. By incorporating irrelevant epitopes into the assay, non-specific cytotoxicity can be determined for background subtraction. Most importantly, we demonstrate for the first time that IFN $\gamma$  expression by specific CD8 T cells do not always correlate with actual cytotoxic potential. This observation occurs in all studied models, suggesting that this is a highly common phenomenon.

### Conclusion

This advanced multiplex cytotoxicity assay allows direct, simultaneous analysis of different epitope-specific CD8 T cells in a single reaction. We believe that this assay can play important roles in accurately identifying relevant epitopes to be used in potential vaccine candidates for T cell-based vaccines, such as universal influenza vaccines.

**Keywords:** Vaccine; CD8 T cells; IFN $\gamma$ ; cytotoxicity; epitope screening

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 10777

## Harnessing an RNA-mediated chaperone (Chaperna) for self-assembly of Ferritin-based Nanoparticle influenza vaccine

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**Introduction and Objectives:** As an alternative to cell culture or egg-based methods, bacterial production of recombinant HA warrants a fast delivery of vaccines in pandemic situation. However, most of vaccine antigens are produced as non-functional misfolded aggregates in bacterial hosts. The folding of monomeric antigens and their subsequent assembly into higher ordered structures are crucial for commercial production of nanoparticle (NP) vaccines in a timely and reproducible manner. Novel molecular chaperone is therefore required for folding and assembly of HA antigens into immunologically relevant conformation.

**Methods:** A novel chaperone function of RNA (chaperna) has been successfully applied for the assembly of influenza HA into ferritin NPs. HA1 domain was fused with an RNA interaction domain (RID) and ferritin. The triple fusion protein was expressed as soluble form from *E. coli*, purified, and its biophysical properties were analyzed by size-exclusion chromatography (SEC), dynamic light scattering (DLS) and transmission electron microscopy (TEM). Protective efficacy was evaluated in mouse model.

**Results:** The soluble HA1 domain was assembled predominantly into trimeric form. The ferritin-HA fusion proteins were assembled into NPs as confirmed by SEC, DLS and TEM. Mutations that affected the RNA binding to RBD resulted in aggregation of HA into amorphous structures, reducing the overall yield of NPs of a defined size. Vaccination with NP provided an efficient protection from heterologous lethal challenge. The results confirm the RNA-mediated assembly of HA into highly ordered, immunologically relevant structure.

**Conclusion:** The bacterial production of soluble HA protein is simple, efficient, amenable to easy scale-up, and warrants a speedy delivery of pandemic vaccines. The kinetic "pace-keeping" role of chaperna ensuring an 'orderly' assembly of antigen monomers holds promise for the development and delivery of NPs and virus-like particles (VLPs) as recombinant vaccines.

*Keywords: RNA, chaperone, nano particle, subunit vaccine*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
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## Development and Characterization of Oral Combination vaccine against Hepatitis B and Influenza

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Vaccination has not only become vital but a lot of revolutionary changes are being observable in the field of vaccine delivery. Vaccine antigens administered by the oral route are often degraded during gastrointestinal transit. Bile salt stabilized vesicles i.e. bilosomes are found to be effective in preventing antigen degradation and enhance mucosal penetration. The aim of the present work was to prepare a combination vaccine system against hepatitis-B (HBsAg) and influenza(r-H1N1Ags).

Oral immunization induces both mucosal and systemic immune responses, whereas mucosal responses are not generally observed following systemic immunization. Bilosomes provide needle free, painless approach for immunization, thereby increasing patient compliance and consequently increasing vaccination coverage.

Bilosomes containing HBsAg and r-H1N1Ags were prepared by a lipid cast film method. Antigen loaded bilosomes were characterized *in-vitro* for their shape, size, percent antigen entrapment and stability. Fluorescence microscopy was carried out to confirm the uptake of bilosomes. The *in-vivo* study comprised of estimation of IgG response in serum and sIgA in various body secretions using specific ELISA.

Bilosomes formed were multilamellar and were stable in gastric and intestinal fluids. Fluorescence microscopy suggested that bilosomes were taken up by the gut associated lymphoid tissues. In-vivo data demonstrates that bilosomes produced both systemic as well as mucosal antibody responses upon oral administration at higher dose levels as compared to intramuscular immunization but fail to produce any synergistic effect.

Thus, HBsAg potentiates the production anti-r-H1N1 antibody. Also measurable sIgA in mucosal secretions were observed. Thus, the bilosomes are a promising carrier for oral combination vaccines. This approach could be adapted for human use because the mucosal surfaces are the initial sites of infection and it therefore seems logical to attempt to develop vaccination strategies that evoke appropriate localized responses to counteract the early events of pathogenesis.

*Keywords: bilosomes, flu, anthrax, oral*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10798

## NS1 Deleted Influenza Virus: Potential Vaccination and Therapeutic Alternative

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**Background:** NS1 is a multifunctional protein of influenza virus. It counters the cellular host immune response against virus and also helps in viral replication. Influenza virus lacking functional NS1 gene are unable to replicate efficiently in healthy cells. But, it still can make few copies of viral antigen which could be presented to MHCs on the cell surface of infected cells. The study aim to assess the therapeutic and vaccination potential of dendritic cells primed with NS1 deleted influenza virus.

**Methodology:** Bone marrow derived dendritic cells (BMDCs) maturation was analyzed by real time PCR of maturation markers. The potential of virus primed dendritic cells to generate T cell response was assessed by co-culture of splenocytes and virus-infected lung cell. The cells for co-culture experiment were isolated from the mice received an intravenous injection of primed DC; parallel control experiment was also performed. The therapeutic ability of primed DCs was assessed by estimation of lung viral titer using western blotting and real-time PCR in the mice injected with primed DCs.

**Results:** The Real-time PCR of BMDC maturation markers such as CD80, CD86, MHC, and CCR5 show that NS1 deleted virus promotes the maturation DCs. It was observed that NS1 primed DCs can efficiently elicit T cell response in mice. The therapeutic ability of primed DCs is evident by decreased lung virus titer by western blotting and real time PCR in the mice received intravenous injection of primed DC as compared with control.

**Conclusion:** The present strategy could be an alternative vaccination and therapeutic alternative against influenza virus infection. The same strategy could also be applied to other viral infection, where therapeutic or vaccination alternative are lacking or limited

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
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## A COCKTAIL INFLUENZA VACCINE BROADLY PROTECTS MICE AND FERRETS AGAINST MULTIPLE SUBTYPES OF INFLUENZA A VIRUS CHALLENGE

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### Introduction and objectives:

Influenza viruses are a global public health concern, with a significant impact of morbidity and mortality from both annual epidemics and pandemics. Because current influenza vaccination is unlikely to protect against antigenically divergent strains or new pandemic viruses with a novel hemagglutinin (HA) subtype, there is a critical need for broadly protective vaccines that protect against all influenza A viruses, a so-called “universal” influenza vaccine.

### Methods:

Four low pathogenic avian influenza viruses (H1N9, H3N8, H5N1, and H7N3) were inactivated with Beta-propiolactone (BPL) and purified using sucrose density gradient centrifugation. Purified viral antigens were mixed at equal concentration and used for a prime-boost immunization in mice and ferrets. Animals were immunized twice either intranasally or intramuscularly (6ug/mouse, 400ug/ferret) and challenged with various homo- and heterosubtypic viruses (H1N1, H2N2, H2N7, H3N2, H6N1, H7N1, and H10N7). Clinical signs, mortality, viral replication, and lung pathology were compared between the vaccinated and control groups to assess protection.

### Results:

The cocktail vaccination provided a broad range of protection against a wide variety of viral infections both in mice and ferrets. Vaccinated animals showed significant protection against every homo- and heterosubtypic viral challenge. Especially in mice, where all the viral challenges were lethal, vaccinated mice showed greater than 95% aggregate survival combining all lethal infections. Both in mice and ferrets, viral replication in lungs (mice, ferret) and upper respiratory tract (ferret) was significantly decreased in vaccinated animals in all challenge experiments. Importantly, a striking decrease in lung pathology and viral antigen was observed in vaccinated animals.

### Conclusion:

These studies demonstrated the broadly protective efficacy of the cocktail influenza vaccine and suggest a promising and practical strategy for developing broadly protective “universal” influenza vaccines.

*Keywords: universal influenza vaccine; hetero-subtypic challenge; mouse; ferret*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 10934

## OVX836, A novel universal influenza A vaccine candidate, provides CD8-mediated protective efficacy in mice

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### Introduction

Cellular immunity to the conserved influenza nucleoprotein (NP) is correlated with protection against influenza disease, providing a strong rationale to develop NP-based vaccines. OVX836 is an unadjuvanted recombinant vaccine obtained by fusing the NP sequence of A/WSN/1933 (H1N1) to Oligodom®, OSIVAX' proprietary multimerization platform. OVX836 protects mice from lethal challenges with multiple A-strains and induces high level of NP-specific CD8+T cell responses in spleen and lungs.

### Method

Adaptive transfers were performed to evaluate the role of CD8+T cells in OVX836 protection. Donor mice were immunized twice three weeks apart with OVX836 or PBS. Serum and lungs were collected 7 days after the second injection:  $5 \times 10^5$  purified CD8+T cells or 300µl serum were transferred from PBS or OVX836 vaccinated mice to recipient mice, by i.v. or i.p. injection respectively. Recipient mice were infected intra-nasally with a lethal dose of H1N1 A/WSN/33 virus 24h after the transfer. Mice were observed for illness and weight loss, and sacrificed if weight loss reached 20%.

### Result

After viral challenge, the mice who received CD8+T cell from mice immunized with OVX836 had a much higher survival rate than the mice who received CD8+T cell from PBS mice (67% vs 17%;  $p=0.04$ ). The survival rate was similar to the mice directly immunized with OVX836 (67% vs 83%;  $p=0.3991$ ). Mice who received serum from either OVX836 or PBS donor mice were not protected.

### Conclusion

OVX836 vaccination protects mice through a CD8+T cell mediated mechanism of action. These results confirm that NP-specific CD8+T cells play a critical role in mediating viral clearance following acute respiratory infections in mice, and support further development of OVX836.

*Keywords: Universal-Flu-Vaccine; CD8; NucleoProtein; Protection; Mice*



Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 10936

## OVX836, A novel universal influenza A vaccine candidate, protects ferrets against viral challenge

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### Introduction

Cellular immunity to the well-conserved influenza nucleoprotein (NP) is correlated with protection against influenza disease, providing a strong rationale to develop NP based vaccines. OVX836 is an unadjuvanted recombinant vaccine candidate generated by the fusion of the A/WSN/1933(H1N1) NP to Oligodom®, Osivax's proprietary multimerization platform. We have previously shown that OVX836 induces NP-specific CD8+ T-cell responses and protects mice from multiple influenza A lethal challenges.

### Method

Naïve ferrets were vaccinated three times three weeks apart with OVX836 or PBS. Animals were euthanized 10 days after to evaluate the NP-specific cellular responses in spleen and lung by ELISPOT.

In a second experiment, naive ferrets were vaccinated with OVX836, TIV, a combination of OVX836+TIV, or PBS, and then challenged with an H1N1pdm09 influenza virus (included in the TIV). Animals were monitored daily for body weight, disease symptoms and viral shedding.

### Results

OVX836 vaccination induced NP-specific IFN $\gamma$  CD4+ and CD8+ T-cells in the spleen and lungs of ferrets. Additionally, animals vaccinated with OVX836, TIV or the combination OVX836+TIV were similarly protected against viral challenge in terms of body weight change, nasal secretions and viral shedding, when compared to the PBS group (+ 6-7% weight at Day 13 in vaccinated animals vs PBS,  $p < 0.05$ ). Importantly, although OVX836 vaccination did not induce HAI titers (as expected), it did not impact the HAI response to the TIV when both products were administered in combination.

### Conclusion

After the challenge, OVX836 performed as well as the TIV used in homologous conditions, although the NP antigen used in OVX836 was from H1N1 1933 and the challenge strain was H1N1 2009. The protection is assumed to be mediated by the NP specific cellular response. These results demonstrate the ability of OVX836 to cross protect ferrets against a different strain and support further development of this vaccine candidate.

*Keywords: Universal-Flu-Vaccine; CD8; NucleoProtein; Protection; Ferrets*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10981

## OPTIMIZED NS1-TRUNCATED LIVE VACCINES PROVIDE ROBUST PROTECTION FROM HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS INFECTION

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### Introduction and Objectives

Several strains of H5 and H7 highly pathogenic avian influenza (HPAI) viruses can transmit from infected poultry to humans resulting in severe morbidity and high mortality rates. It is of great importance to control HPAI viruses in poultry to prevent the emergence of novel strains and reduce the risk of avian-to-human transmission. Using the genetic backbones of several naturally-selected influenza virus mutants that encode C-terminally truncated NS1 proteins, we have constructed live-attenuated influenza vaccine (LAIV) candidates that are optimized for mammalian and avian hosts. In this study, two LAIV candidates bearing either H5 or H7 hemagglutinins were tested in chickens as single vaccines or prime-boost vaccination regimens where one-day-old chickens were primed with LAIV and boosted with inactivated vaccine prepared with same LAIV strain 3 weeks later.

### Methods

H7-vaccinated birds were challenged with a heterologous H7 (North American-lineage) or heterosubtypic H5 (Asian-lineage) HPAI viruses. H5-vaccinated birds were challenged with the heterologous Asian lineage H5 virus.

### Results

Based on hemagglutinin-inhibition cross-reactivity titers, substantial antigenic differences exist between H7 vaccine and H7 HPAI virus (11-fold), H5 vaccine and H5 HPAI virus (128-fold), and H7 vaccine and H5 HPAI virus (>8000-fold). Despite these antigenic differences, in all heterologous vaccination-challenge groups and independently of the vaccination regimen used, there was complete protection against clinical disease and death, and only a few or no birds shed virus in the trachea and cloaca at 4 days post-infection. Further, the immunity elicited by the H7 prime-boost vaccination regimen was partially cross-protective against heterosubtypic H5 virus challenge in terms of delayed clinical signs and mean death time.

### Conclusion

Collectively, these data demonstrate that our optimized NS1-truncated LAIV candidates can broadly protect against antigenically distant heterologous HPAI viruses. These vaccines will be used to develop universal bivalent vaccination regimens against all H5 and H7 HPAI viruses.

*Keywords: Highly pathogenic avian influenza virus; live-attenuated influenza vaccine; universal influenza vaccine*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 10984

## UNIVERSAL MONOCLONAL ANTIBODY BASED INFLUENZA HEMAGGLUTININ QUANTITATIVE ENZYME-LINKED IMMUNOSORBENT ASSAY.

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### Introduction

World Health Organization guidelines ask manufacturers to determine the potency at the time of release and throughout the shelf-life to assure optimal potency of the influenza vaccine. In this regard, a Single Radial Immunodiffusion (SRID) assay has been used as a standard method for influenza vaccine potency assay via quantitating hemagglutinin (HA) in the vaccine. However, SRID requires seasonal reference reagents that should be updated annually. To overcome the limitation, there have been extensive efforts to develop alternative potency assays.

### Method

The consensus HA (cHA) stalk for group 1 influenza A virus (IAV), group 2 IAV and influenza B virus (IBV) were designed based on the conserved domains that were analyzed via bioinformatical approach. The cHA stalks were expressed from *E.coli* by fusing with RNA interaction domain (RID) of lysyl tRNA synthetase of mouse and were purified by one step Ni<sup>+</sup> affinity chromatography. Group specific universal monoclonal antibodies were generated through hybridoma technology and validated by ELISA. Lastly, HA quantitative ELISA using universal monoclonal antibody were established and compared with SRID.

### Result

The consensus HA stalk for each group were produced as a soluble form and well purified. Group specific universal monoclonal antibody that can bind to various strains and drift strains within same groups were generated. '1G5' and '2C12' are antibodies for group 1 IAV, '4F11' are antibody for group 2 IAV and '10F8' are antibody for IBV. The antibodies showed universal binding to HA from various strains without cross reactivity to HAs from the other groups. Furthermore, the results of quantitative ELISA using universal antibodies showed highly comparable with those of SRID.

### Conclusion

In this study, we introduced the concept of new hemagglutinin quantitative ELISA using group specific universal monoclonal antibody that can bind to HA antigens from various subtypes and drift strains.

*Keywords: Influenza vaccine, Potency assay, Universal antibody, ELISA*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11007

## **Broadly neutralizing influenza A (H3N2) monoclonal antibodies against hemagglutinin receptor binding site and vestigial esterase domain and induction of competitive antibody responses in a phase 2 trial in older adults**

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### Introduction

Currently licensed influenza vaccines often fail to fully protect due to immune responses that do not adequately address seasonal antigenic drift variants, and mismatches between vaccine and circulating strains due to egg-adaptive mutations. To address these limitations we have developed a quadrivalent hemagglutinin (HA) based protein – detergent nanoparticle vaccine (qNIV) with a saponin based Matrix-M™ adjuvant that has completed phase 1 and 2 trials in older adults demonstrating broadly cross-reactive antibody responses.

### Methods

Two IgG1 neutralizing mouse monoclonal antibodies were produced against the A/Hong Kong/4801/2014 (H3N2) HA, sequenced, and used for 2D nanoimaging. Binding specificity was evaluated by FACs with wild-type and mutant HA, affinity was determined by SPR to wild type and HAs with drifted and egg-associated mutations, and antibody competitive binning (Octet) with known head and stem HA monoclonal antibodies to was used to generate epitope heat maps and evaluate antibody responses in sera from immunized older adults.

### Results

Two broadly neutralizing H3N2 HA head-based monoclonal antibodies were identified that mapped to conserved regions at the HA receptor binding domain (RBD) and vestigial esterase (VE) subdomain. Furthermore, antibody competition studies with sera from qNIV vaccinated older adults in a phase 2 trial demonstrate qNIV responses against RBD and VE conserved HA domains and, like two commercial vaccine in the study, lower competitive antibody levels specific for the HA stem.

### Conclusion

The recombinant qNIV vaccine is genetically stable, not subject to substrate-induced mutations and when presented as a HA – detergent nanoparticle presents novel epitopes with the potential to induce broadly protective immunity and improved vaccine efficacy.

*Keywords: Influenza vaccine epitopes neutralizing*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11019

## SYSTEMATIC DEVELOPMENT OF NEXT-GENERATION NS1-TRUNCATED LIVE ATTENUATED INFLUENZA VACCINES BY TARGETING VIRAL SUBPOPULATIONS WITH ENHANCED INTERFERON-INDUCING CAPACITY

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### Introduction and Objectives

The continued worldwide outbreaks of influenza A viruses with new antigenic makeups has necessitated the development of more broadly reactive vaccines. Among the currently available vaccines, intranasally administered live-attenuated influenza vaccines (LAIVs) can provoke efficient mucosal and cellular immunity in addition to systemic antibody responses. However, the effectiveness of the currently licensed LAIVs has been fluctuating in recent years. In a series of studies, we have clearly shown that a strong positive correlation exists between the *in vivo* efficacy of NS1-truncated LAIVs and the magnitude of type I interferon induced by these vaccines in species-specific cell culture systems. In addition, NS1-truncated LAIV candidates were also shown to maintain large subpopulations of defective-interfering particles *in vitro*. The objective of the present study was to systematically produce next-generation LAIV backbones.

### Methods

The viral population of our current vaccines was deconstructed through plaque purification and new vaccine candidates were selected based on their interferon-inducing capacity as an *in vitro* phenotypic marker.

### Results

Of the 100 new avian-specific candidates generated through this process, a few emerged as super high inducers of interferon. Some of these next-generation LAIV candidates have acquired additional large deletions in the NS1 protein and produce high levels of defective viral genes. Further improvement of our mammalian-specific LAIV is underway using a similar approach but at a single-cell resolution by the employment of single cell sorting techniques on interferon reporter A549 human epithelial cells. In addition, data obtained through deep sequencing will reveal the profiles of single nucleotide polymorphisms and deletion junctions of defective viral genes of the best vaccine candidates.

### Conclusions

Novel LAIV candidates were revealed through systematic deconstruction of the original NS1-truncated LAIV particle population and analysis of genomic and phenotypic markers of vaccine efficacy. The vaccine phenotypes will be confirmed in animals.

*Keywords: influenza virus subpopulations; live-attenuated influenza vaccine; defective influenza virus genomes*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11058

## A single shot of an adenoviral vectored influenza vaccine provides protection against heterosubtypic lethal influenza virus challenge in mice

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**Introduction and Objectives:** Licensed influenza A virus (IAV) vaccines do not confer heterosubtypic protection and manufacture can be negatively impacted by production in eggs. A universal influenza virus vaccine that provides heterosubtypic protection and is egg-independent, would be highly desirable. Non-replicating adenoviral (Ad) vectors represent a promising vaccine platform due to their safety and immunogenicity in humans and capacity for rapid, egg-independent production.

**Methods:** We engineered Ad5 expressing influenza hemagglutinin H1 (Ad5\_H1). A single shot of Ad5\_H1 was administered intramuscularly (*im*) to mice at doses of 10<sup>6</sup>-10<sup>8</sup> infectious units. Positive controls included a single shot of matched, inactivated influenza vaccine (IIV) administered *im* (1.5µg). Antibodies (Abs) to full length H1 and hemagglutinin stalk-only were measured by ELISA on day 28 (D28). Flow cytometry with intracellular cytokine staining (ICS) was performed on D14 and D28 to measure stalk-specific T-cells. Mice were challenged at D30 with homologous (i.e. H1N1) or heterosubtypic (i.e. H5N1) IAV at 5 mLD50.

**Results:** At all doses, Ad5\_H1 resulted in H1 endpoint titers greater than 75-fold higher than H1-IIV (geometric mean >574333 versus 7480). High titers of anti-stalk Abs were detected following 10<sup>8</sup> and 10<sup>7</sup> Ad5\_H1 (>23000), but were negligible following vaccination with H1- or H5-IIV. H1 stalk-specific CD8<sup>+</sup> and H5 cross-reactive CD4<sup>+</sup> T-cells were detected by ICS in Ad5\_H1-vaccinated mice. Upon homologous H1N1 challenge, all doses of Ad5\_H1 provided 100% protection against weight loss, comparable to H1-IIV. Upon challenge with heterosubtypic H5N1, 100% survival was observed in mice receiving 10<sup>8</sup> Ad5\_H1 and in positive controls (i.e. H5-IIV). Mice vaccinated with H1-IIV were not protected.

**Conclusion:** Ad5\_H1 immunization can provide 100% protection in mice against homologous (10<sup>6</sup>-10<sup>8</sup>) and heterosubtypic challenge (10<sup>8</sup>). Considering the low sequence identity between the H1/H5 hemagglutinin head, it is likely that stalk Abs confer survival. Stalk-specific T-cells may also contribute to increased heterosubtypic protection.

*Keywords: vaccine universal influenza*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 11126

## ENHANCED CROSS PROTECTIVE EFFICACY OF RECOMBINANT LIVE INFLUENZA H3N2 VIRUS EXPRESSING CONSERVED M2 EXTRACELLULAR DOMAIN IN A CHIMERIC HEMAGGLUTININ CONJUGATE

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### Introduction

Influenza virus is highly contagious and can cause life-threatening illness leading to death of animals and humans. However, hemagglutinin (HA)-based current vaccines are ineffective in providing cross protection against antigenically distinct influenza viruses due to the hypervariability of HA. Instead, the conserved extracellular domain (M2e) of M2 ion channel of influenza has emerged as a candidate of universal vaccine. Although its ability to confer a broad cross protection has been confirmed in animal studies, M2e immunity alone vaccine is less efficacious due to the protection via non-neutralizing immune mechanism.

### Objectives

To improve the efficacy of HA-based current influenza vaccine, we generated reassorted H3N2 influenza virus vaccines (HA and NA from A/Switzerland/2013/62) expressing chimeric H3(cH3) HA-4xM2e conjugate proteins in the N-terminus. Its backbone is from A/Puerto Rico/8/34 H1N1 virus.

### Methods

Recombinant influenza seasonal virus vaccines were generated by reverse genetics. Balb/c mice were intranasally administered with wild type HA or cH3 HA-4xM2e virus vaccines. Immunized mice including naive mice were challenged with A/Vietnam/1203/2004 H5N1 reassortant virus.

### Results & Conclusion

The reassortants H3N2 cH3 HA-4xM2e viruses displayed highly attenuated phenotypes in mice with restricted growth in the upper respiratory tract(nose) but not in the lower respiratory track lung tissues of the mice. Moreover, cH3 HA-4xM2e virus did not cause weight loss in mice while the control wild type A/PR8 virus induced severe weight loss even with lower doses. Recombinant H3N2 virus carrying cH3 HA-4xM2e led cross protective M2e specific IgG antibody responses as well as HA immunity. Mice that intranasally inoculated with cH3 HA-4xM2e virus showed enhanced cross protection against antigenically distinct viruses after prime or prime-boost doses. The findings in this study support a novel approach to improve the efficacy of current influenza vaccine platforms by recombinant influenza virus vaccines inducing immunity to both HA and cross protective M2e antigens.

*Keywords: Influenza, universal vaccine, M2e*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11129

## PRACTICAL IMPLEMENTATION OF CELL ISOLATED CANDIDATE VACCINE VIRUSES FOR LARGE SCALE, CELL-BASED INFLUENZA VACCINE MANUFACTURE

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### Introduction

Cell culture based influenza vaccines have the potential for many benefits compared to vaccines manufactured using traditional egg based technology. One specific advantage of interest is that influenza viruses isolated on Madin-Darby Canine Kidney (MDCK) cells are not subject to the adaptation pressure of egg isolation that results in reduced candidate vaccine virus (CVV) recovery rates and unwanted egg adaptations that may affect their antigenic properties, particularly in H3N2 viruses.

### Methods

To realize the potential coverage benefit and enable a high volume cell culture based vaccine supply, it is necessary to ensure a steady source of high yielding cell isolated CVV strains for manufacturing. Unlike for the egg based vaccines, the cell equivalent of a well-established infrastructure for supplying high growth reassortants does not yet exist to ensure a steady supply of highly productive cell isolated CVV strains for production of vaccine. Instead, a large number of wild type cell isolated CVV strains supplied by two WHO Collaborating Centers for Influenza must be screened to assess their performance in the cell culture based manufacturing process. A highly automated screening process has been developed for this purpose which utilizes the ambr® 15 microscale bioreactor system with an array of scaled down bioreactors that mimic the environmental conditions of the full scale manufacturing process, a one-step ultracentrifugation based sample purification process, and a robust high throughput HPLC method for rapid assessment of HA yield.

### Results

HA yield data are organized and summarized using a graphical leaderboard system which is used as a tool to determine which CVV strains are suitable for manufacture in the cell culture process.

### Conclusion

These performance data can then be shared with the WHO Collaborating Centers isolating CVV strains on MDCK cells to prioritize antigenic characterization and further virus isolations.

*Keywords: MDCK*



Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11134

## Lung-resident memory CD8 T cells require the help of non-resident CD8 T cells to provide effective anti-influenza immunity

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### Introduction:

Tissue-resident memory CD8 T cells (TRMs) provide rapid, specific immunity against subsequent pathogen challenge, including influenza. However, it is unclear whether and how influenza-specific lung TRMs are directly involved in controlling the spread of influenza infection. Here, we use 1) human lung *ex vivo* influenza infection, and 2) mouse treated with a vaccinia-based universal influenza vaccine as models to study TRMs.

### Methods:

Human lung samples were analyzed for lung TRM frequency by flow cytometry and assayed for viral titer after challenged with pandemic H1N1 virus *ex vivo*. Mice were vaccinated, followed by lethal influenza challenge. Lung influenza-specific TRM levels and cytotoxicity were quantified by flow cytometry and multiplex *in vivo* cytotoxicity assay. For adoptive transfer experiments, sorted CD8 TRMs from vaccinated mice were intravenously transferred into recipients that were then challenged with a lethal dose of influenza virus.

### Results:

TRM numbers in human lungs are correlated with decrease in lung viral titer. Intranasal, but not subcutaneous, vaccination in our mouse model confers efficient protection against lethal PR8 (H1N1) and mouse-adapted HK68 (H3N2) challenge. This is associated with induction of influenza-specific CD8 TRMs in mouse lungs. Lung CD8 TRMs display poor cytotoxic potential, though during the course of challenge this increases markedly due to recruitment of effector influenza-specific CD8 T cells. Adoptive transfer of sorted lung CD8 TRMs into subcutaneously vaccinated mice, but not naïve mice, leads to partial protection when challenged, which correlates with increases in influenza-specific CD8 T cells in the lungs of these mice.

### Conclusion:

In both humans and mice, lung influenza-specific CD8 TRMs reduces lung viral titer during influenza infection. These cells can be established through intranasal vaccination and requires the participation of influenza-specific CD8 T cells from lymphoid organs to confer anti-influenza immunity.

*Keywords: Universal vaccine, Tissue-resident memory T cells, CD8+*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11146

## INVESTIGATION OF T CELL IMMUNE PRESSURE ON THE INFLUENZA GENOME WITHIN A UNIVERSAL VACCINATION MODEL

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### Introduction:

Many potential candidates for a universal influenza vaccine are currently in development. Many of these utilise T cell recognition of conserved viral epitopes to confer heterosubtypic immunity. Influenza has already been shown to be capable of anti-viral resistance and immune escape from vaccine mediated T cell immunity may also occur. Should increased immune pressure elicited by T cells trigger immune evasion, it could result in the loss of immunodominant epitope recognition.

### Method:

We aim to investigate the role of universal vaccine induced T cells on mutations arising during the course of an influenza infection in a mouse model. Mice are vaccinated using Wyeth/IL15/5Flu, a live vaccinia backbone encoding IL-15 as an adjuvant and H5N1-derived proteins, HA, NA, NP and Matrix. Lungs were homogenized day 7 post H1N1 challenge and submitted for next generation sequencing by Illumina MiSeq. T cell responses for newly identified variants and viral loads were determined in parallel.

### Result:

In preliminary results, we have seen that vaccination can increase the rate of SNPs across the influenza genome but these mutations are occurring outside of known T cell epitope regions. Examining nonsynonymous mutations occurring at >10% by *in vitro* peptide stimulations have shown that these regions are not uncharacterised epitope regions. High incidence of variants may correlate with increased viral loads in regions outside of vaccine-targeted proteins. Experiments are ongoing to confirm variants and identify conditions of escape.

### Conclusion:

Results suggest that T cell directed immune pressure can induce a higher frequency of mutations across the influenza genome. An overall increased occurrence of SNPs could possibly lead to mutations within epitope regions and co-mutations to bypass fitness restrictions, leading to a loss of immune recognition. These findings should be taken into consideration to help inform future vaccine strategy and design.

**Keywords:** *T cell; Universal Vaccines; NGS; Escape mutant; Immunology*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 11168

## Augmented germinal center formation underpins enhanced immunogenicity of self-assembling protein nanoparticle vaccines for influenza

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**Introduction and Objectives:** Current seasonal influenza vaccines provide protection that is highly strain-specific, resulting in costly global surveillance efforts and annual reformulation. Protein-based, self-assembling nanoparticles have tremendous potential as novel vaccine platforms – demonstrating superior immunity compared to soluble protein vaccines in a number of contexts including influenza. However, immune mechanisms that underpin this greater immunogenicity remain poorly defined.

**Methods:** Here we undertook an in-depth characterization of the immunogenicity of a prototypic protein-based nanoparticle vaccine, based on a spherical ferritin core with eight trimeric influenza haemagglutinin (HA) spikes on the surface.

**Results:** Vaccination of mice with HA-ferritin nanoparticles elicited 10-fold higher serum antibody titers and greater protection against experimental influenza challenge compared to vaccination with soluble HA protein. Confocal microscopy of draining lymph nodes following HA-ferritin vaccination revealed markedly augmented germinal center reactions compared to soluble HA. Quantification by flow cytometry confirmed a 2-3-fold increase in both bulk and HA-specific germinal center B cells compared to soluble HA vaccination. Using an activation-induced marker assay to measure T follicular helper responses, we found no evidence that the ferritin carrier supported HA-specific B cells via linked recognition.

**Conclusion:** Our findings suggest the display of antigens in highly ordered and repetitive arrays directly drives the germinal center formation, in a mechanism that is likely B cell intrinsic. Better understanding the basis of nanoparticle vaccine immunogenicity will facilitate the rational design of nanoparticle vaccines for broad and durable protection against diverse pathogens.

**Keywords:** *nanoparticle; nanotechnology; vaccine*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11204

## EXPOLRING DIFFERENT HETEROLOGOUS PRIME-BOOST VACCINATION REGIMENS WITH H1N1 INFLUENZA VIRUSES OF SWINE

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In previous pig vaccination studies, we used antigenically distinct whole inactivated H1N1 influenza virus strains for primary and booster vaccinations. This strategy could at best induce seroprotective hemagglutination inhibition (HI) antibody titers ( $\geq 40$ ) against 50% of a panel of 24 antigenically diverse H1N1 viruses. Here we explored a different heterologous prime-boost vaccination strategy by using different virus strains as well as different vaccine platforms and immunization routes.

Pigs were vaccinated intranasally with a live attenuated Suid herpesvirus 1 vector expressing the hemagglutinin of the 2009 pandemic H1N1 (vector-pdm09) virus and/or intramuscularly with whole inactivated vaccine (WIV). WIV vaccines were based on three H1N1 strains belonging to distinct H1 lineages: pdm09, European avian-like and European human-like swine influenza virus. Three control groups received a mock vaccination, vector-pdm09 only or two administrations of WIV-pdm09. Two heterologous prime-boost groups received two different WIV. Three heterologous prime-boost groups were primed with vector-pdm09 and boosted with either WIV-pdm09, or one of both antigenically distinct WIV. There was a 4-week interval between primary and booster vaccination. Sera collected one month after the last vaccination were examined in HI against the above-mentioned virus panel. At this timepoint pigs were challenged intranasally with one of two H1N2 swine influenza viruses and euthanized 3 days later for virus titrations of respiratory tract samples. The HA1 of the challenge viruses showed 10-32% amino acid heterogeneity with those of the vaccine strains.

The breadth of the serum HI antibody response was only slightly higher after priming with vector-pdm09 and boosting with heterologous WIV (seroprotective HI titers against 29-38% of H1N1 viruses) than after prime-boost vaccination with two heterologous WIV (21-33%) or different types of pdm09 vaccine (29%). Virus titrations are pending.

Profound comparisons of these heterologous prime-boost regimens require further studies of mucosal antibody responses and evaluations of virus titers post-challenge.

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11225

## AVIAN H5 INFLUENZA A VIRUSES: HOW TO COPE WITH ANTIGENIC DIVERSITY FOR VACCINE DESIGN?

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In 1997, highly pathogenic avian influenza (HPAI) viruses of the A/goose/Guangdong/1/96 (H5N1) lineage emerged. Since, they have continued to cause outbreaks in poultry globally, with occasional human infections, raising concerns about a new pandemic. Due to continued circulation for 20 years, the hemagglutinins (HAs) of HPAI H5 influenza viruses evolved into distinct genetic and antigenic clades, posing a serious challenge to pandemic preparedness and vaccine design. Here, we describe the quantification and visualization of the global antigenic diversity of H5 HA in an antigenic map and the rational design of immunogenic, broadly reactive H5 vaccine candidates.

Recombinant viruses carrying synthetic HA genes of globally representative H5 viruses were generated. A subset of these viruses was used to raise post-infection antisera in ferrets. Results from hemagglutination inhibition (HI) assays were used to compute an antigenic map using multidimensional scaling algorithms to visualize the antigenic relatedness between viruses and antisera. The antigenic map was used to rationally design vaccine candidates aimed to provide protection against a broad range of H5 HPAI viruses. Ferrets were vaccinated with these vaccines and the height and breadth of the immune responses were assessed in HI assays.

The antigenic map contained 108 H5 viruses and 24 post-infection sera, spanning the global evolution and antigenic diversity of HPAI H5 viruses from 1997 to 2016. Antigenic diversity increased rapidly in the first 10 years of circulation, but more recent lineages continued to cross-react with older ones. Post-vaccination sera raised against rationally designed vaccine candidates reacted with a HI titer >40 – a correlate of protection – with up to 65% of H5 strains tested.

The designed vaccine candidates induced antibodies that cross-reacted with most H5 HPAI viruses, including strains explicitly selected to be outliers. Further evaluation of these vaccines upon challenge with a HPAI H5 virus in ferrets is warranted.

*Keywords: H5N1; avian influenza; antigenic diversity; vaccine*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 11255

## Evaluation of chimeric virus-like particle vaccines displaying dual inter- and intra- clade HA protein of Highly pathogenic H5 subtype Avian Influenza Virus.

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**Introduction and Objectives:** Since the first broke out in China in 1996 (Gs/GD lineage), HPAIV has evolved into 10 genetically distinct virus clades. In 2003 and 2004, outbreak of clade 1 H5N1 viruses caused worldwide damage in domestic poultry. From 2008, clade 2.3.2.1 H5N1 virus showed wide dispersion and outbreaks. Through reassortment between H5N1 virus and local low pathogenic Influenza virus (LPAIV), novel H5N6 and H5N8 of clade 2.3.4.4 emerged and spread from East Asia to North America, West Asia and Europe and caused recent outbreaks. As these H5 subtypes are genetically and antigenically distinct, they are difficult to eradicate.

In this study, we developed two chimeric VLPs, one expressing HA proteins from clade 1 and 2.3.2.1 HPAIV H5 viruses and another expressing HA proteins from clade 2.3.2.1 and 2.3.4.4 HPAIV H5 viruses. Immunogenicity against inter-clade and intra-clade H5 subtype HPAIV was observed.

**Methods:** HA genes of 3 viruses A/Vietnam/1194/2004 (H5N1, clade 1) virus, A/mandarin duck/K10-483/2010 (H5N1, clade 2.3.2.1) and A/Mandarin Duck/K16-187/2016 (H5N6, clade 2.3.4.4) were synthesized or extracted from virus propagated in chicken embryo. Recombinant baculovirus (rBV) encoding clade 1 HA (rBV\_clade1), clade 2.3.2.1 HA (rBV\_clade 2.3.2.1), clade 2.3.4.4 HA (rBV\_clade 2.3.4.4) and two HAs at one rBV (rBV\_clade 1 & 2.3.2.1, rBV\_clade 2.3.2.1 & 2.3.4.4) were generated using Bac-to-Bac baculovirus expression system. After vaccination on SPF chickens, immunogenicity between other clades of HAs were observed by HI assay.

**Results:** Through vaccination with chimeric VLP vaccines, we observed broad antibody responses against various clades and subclades of H5 viruses.

**Conclusion:** In this study, chimeric VLP showed broad spectrum of immunization against clades of H5 HPAIV. Along with this feature, safety of VLP technology and DIVA compatibility through NP ELISA render VLP as a promising vaccine strategy in control of AIV.

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11266

## **A heat-killed lactic-acid bacteriasupplemented in the inactivated HPAI vaccine provides early onset of protection against lethal HPAI challenge**

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Highly Pathogenic Avian Influenza (HPAI) is one of most devastating disease of poultry industry resulting in significant economic losses worldwide. Vaccination against HPAI is one of few methods available for controlling HPAI. Most of HPAI vaccine used in current poultry industry are inactivated, oil-adjuvanted types. The used inactivated vaccines are occasionally unable to provide early onset of protection. Therefore, novel measures must be considered in order to induce an early protection against the HPAI, such as through the addition of immunostimulatory compounds like Lactic-acid Bacteria (LAB). Here, we observed the adjuvanticity of heat-killed LAB on the effectiveness of inactivated HPAI vaccine.

In the present study, *Lactobacillus plantarum*, which is known to enhance the systemic immune response by activating various immune cells, was employed as an immunostimulatory supplement of inactivated HPAI vaccine. At first, Reverse genetics-derived H5N9HPAI vaccine strains was propagated and inactivated. Low and high doses of inactivated antigens or each doses of antigens supplemented with LAB were used to formulate water-in-oil emulsion inactivated vaccines. To analyze the induction of an early onset of immunity through these vaccines, chickens were immunized with each vaccine formulation one week before lethal challenge.

In high dose-vaccinated groups, complete protection against early HPAI challenge was conferred regardless of the addition of the LAB. In these groups, no clinical signs were observed, and no virus RNA was detected by real-time RT-PCR. In low dose-vaccinated groups, we found that LAB-Addition group substantially strengthened the antibody response and showed protection against early challenge. All of the chickens in the control group and 5 of 8 chickens in the LAB-Non addition group died within 3days after early challenge. This data demonstrates that LAB in the inactivated vaccines significantly improved the induction of early protection against HPAI and can be used as a novel immunostimulatory supplement for the HPAI vaccine.

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11271

## Evaluation and screening of 4 adjuvant candidates for enhancing immunogenicity of microneedle vaccination against influenza virus in mice.

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### Introduction

A microneedle is an array of micron-sized needles which penetrates and delivers therapeutic materials into the underlying dermis, immune cell- rich regions. However, when considering improved vaccine efficacy in microneedle vaccination, it is important to find a proper adjuvant can be used in transdermal delivery.

### Objectives

To enhance the immunogenicity of the microneedle vaccine, 4 promising adjuvant candidates were applied in this study: PLGA(lactic-co-glycolic acid)-encapsulated resiquimod(R848), sulfated 1,3-beta-glucan(1,3BG), LPS from E.coli and Desaminotyrosine(DAT).

### Methods

Each adjuvant was coated on microneedle with inactivated swine-origin influenza A/H1N1 virus. Six-week-old BALB/c mice were vaccinated by microneedle with each adjuvant (n=6). The serum was collected at 2 and 4 weeks post vaccination. Hemagglutination inhibition (HAI) titers were assessed and IgG, IgG1, IgG2a level in serum were determined using ELISA(enzyme-linked immunosorbent assay). After 5 weeks post-vaccination, the mice were anesthetized and challenged intranasally with the same virus strain and their survival rate and weight were recorded for 10 days.

### Results and Conclusion

The mean serum IgG levels in the R848, LPS and DAT were higher than 1,3BG group. In addition, the IgG1 and IgG2a level were higher in sera from the mice injected adjuvant except for 1,3BG. For HAI test, the highest HAI titers were observed in the LPS group followed by R848. For 1,3BG and DAT group, however, HI titer did not change following immunization. The survival rate was 100%(6/6) in the R848, LPS, and DAT group while the challenge was lethal(0/6) in the 1,3BG and control group. Mice delivered vaccine with R848 had lost about 14.8% of their initial weight by day 7, but gradually regained their initial weight at day 10(97.6%) and similar patterns were detected in LPS and DAT groups. By day 5, the mice in both the 1,3BG and control groups had lost over 25% of their body weight.



Topic: Virology and Pathogenesis: NextGen/Universal Vaccines

Abstract No: 11282

## Correctly folded, but not functional, influenza virus neuraminidase is required to produce protective antibodies in mice

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**Introduction.** The influenza virus neuraminidase (NA) plays an integral role in influenza virus replication cycle by facilitating multi-cycle infection through the release of virions from infected cells. NA-specific antibodies can impede the virus replication cycle by binding to the NA and blocking enzymatic activity. Because of the ability of antibodies to block these fundamental role it is believed that strong anti-NA immunity could provide significant protection from influenza. NA included in current influenza virus vaccines is not abundantly immunogenic, potentially due to a compromised structural integrity cause by the production process and vaccine formulation.

**Methods.** To determine how certain stresses that might occur during vaccine production could influence the antigenicity of NA we performed a series of *in vitro* experiments where we treated NA with formalin, EDTA or heat treatment. We measured NA enzymatic activity and structural integrity, followed by vaccination studies in mice.

**Results.** We found that increasing concentrations of formalin or EDTA and increasing temperature abolished the enzymatic activity of both A/Philippines/2/1982 (H3N2) purified virus and recombinant NA protein. Although enzymatic activity was diminished, formalin and EDTA treatment did not affect conformational epitopes found on the NA, whereas heat destroyed conformational epitopes. Following these studies, we vaccinated mice with recombinant N2 treated with formalin, EDTA or 100°C in a prime-boost regimen three weeks apart. Four weeks post boost, we challenged mice with a lethal dose of A/Philippines/2/1982 (H3N2) and monitored weight loss and survival. We found that vaccination with inactive NA (EDTA and formalin) induces robust antibody responses and that are able to protect mice from lethal influenza virus challenge. In contrast, destroying conformational epitopes following heat treatment, results in no antibody production and significant weight loss during infection.

**Conclusions.** Together, our data suggests that enzymatically active NA is not required to induce antibody responses.

*Keywords: NA, enzymatic activity, vaccination*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11330

## Evaluation of the safety and efficacy of a chimeric influenza virus HA antigen as the candidate vaccine in swine model systems

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Influenza A viruses (IAV) infect a variety of avian and mammalian hosts, including humans and pigs. An effective vaccine that can induce broadly protective immunity against heterologous IAV strains is needed. Recently, we demonstrated that broad immunity within the IAV subtype (H1N1) could be achieved using a molecular breeding (DNA shuffling) strategy to create chimeric hemagglutinin (HA) as candidate vaccine antigen [McCormick et al., 2015; PLoS One, 10(6):e0127649]. A panel of chimeric HAs was constructed using parental HAs from the 2009 pandemic virus and swine influenza viruses that had a history of zoonotic transmission to humans. These HAs represented four distinct phylogenetic clades. In this study, one of the chimeric HA constructs, HA-129, was selected to be expressed in the context of a whole virus backbone of A/swine/Texas/4199-2/98-H3N2 as a recombinant virus, designated as TX98-129. Immune response induced by this recombinant virus was initially evaluated in a nursery pig model. The results showed that TX98-129 induced broad immunity against genetically diversified influenza viruses, and antibody levels were correlated with protection against infection. The safety and efficacy of the TX98-129 candidate vaccine was further evaluated in a pregnant sow-fetus model system. The result consistently showed that the vaccine induced an immune response against the TX98-129 virus and the parental viruses. After challenge with a virulent IAV, a significant increase in antibody titers was observed in vaccinated sows at 5 and 22 days post challenge (dpc), and challenge virus was detected in only one vaccinated sow with low titer (1:2) at 5 dpc. No challenge virus was detected in all the fetuses from IAV-challenged sows, but lung lesions were observed in those fetuses from non-vaccinated sows at 5 dpc. This study provides comparative swine model systems to study the effect of influenza vaccine in host (maternal) immunity and fetal development.

*Keywords: Influenza; Vaccine; Hemagglutinin*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11339

## Intranasal administration of multi-antigen IDLV protect from homologous and heterosubtypic influenza challenge

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**Background:** One effective strategy for establishing protective responses against influenza would entail induction immune responses at the respiratory mucosal site of viral entry; however, the design of a mucosal vaccine remains a challenge.

We have developed integrase-defective lentiviral vectors (IDLV) as a gene-based vaccine platform to express influenza antigens. This approach offers the safety advantage that transgenes are expressed within the context of a non-integrating, non-replicating virus. Expressing antigens in the context of IDLV also improves immunogenicity compared to killed vaccine. We previously showed that two immunizations with IDLV expressing the highly conserved nucleoprotein (NP) provided cross-protection against influenza challenge when administered by the intranasal (i.n.) route. To broaden the immune responses elicited by IDLV, we constructed a multi-antigen vaccine expressing HA and NA on the surface and NP from the transgene, and we tested its protective efficacy after i.n. administration.

**Methods:** We developed 1. IDLV expressing NP and pseudotyped with HA and NA from influenza A/California/07/2009 (H1N1) (multi-antigen IDLV-NP H1N1) 2. IDLV expressing GFP and pseudotyped with the same HA and NA (IDLV-GFP H1N1); 3. non-pseudotyped IDLV-NP. IDLV were administered to 6-8 week old Balb/c mice. *In vivo* antibody production was assessed by ELISA, and interferon- $\gamma$  production from isolated mouse lymphocytes was measured by ELISPOT. Mice were lethally challenged using mouse-adapted A/California/07/2009 (H1N1) or recombinant IAV VNH5N1-PR8/CDC-RG and monitored for weight loss and survival.

**Results:** We administered IDLV-NP H1N1, IDLV-GFP H1N1, and IDLV-NP to groups of 10 mice, and found that IDLV elicit specific antibody and cell-mediated immunity to the antigens expressed (H1, N1 or NP). A single dose of multi-antigen IDLV-NP H1N1, but not IDLV-NP was able to fully protect from homologous and heterologous challenge.

**Conclusion:** IDLV represent a novel platform for simultaneous delivery of multiple protective antigens by the i.n. route and induction of cross protective immunity.

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11779

## Autoreactive potential of universal influenza vaccines

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**Introduction:** A universal influenza vaccine could save millions of lives in the event of a deadly pandemic. It is not clear why antibodies specific for conserved regions of influenza viruses are so rare. One possibility is that these antibodies have a higher potential to cross-react to self-proteins, and therefore B cells that generate these antibodies are deleted through tolerance mechanisms. In support of this, infections and vaccinations with the 2009 H1N1 pandemic strain induced more antibodies that were cross-reactive against multiple influenza strains than were induced by previous seasonal strains. However, they were also associated with a higher risk of autoimmune disorders, including narcolepsy and Guillain-Barré syndrome. Therefore, we examined whether cross-reactive influenza antibodies had a higher potential to be autoreactive than antibodies specific for one subtype of influenza.

**Methods:** We previously demonstrated that H3N2-vaccinated mice treated with a low dose of rapamycin had more cross-reactive influenza antibodies and were better protected against subsequent lethal infections of multiple subtypes. Thus, we utilized rapamycin to increase the frequency of influenza cross-reactive antibodies, and tested whether these antibodies were more reactive to self-proteins than strain-specific antibodies. In addition, we tested broadly neutralizing antibodies isolated from humans.

**Results:** We found that mice with increased levels of cross-reactive influenza antibodies also had more antibodies specific for self-antigens. Although the increase in autoreactive antibodies was transient, it correlated with increased susceptibility to disease in mouse models of multiple sclerosis and Guillain-Barre Syndrome. We also found that some antibodies generated in humans in response to influenza infection showed reactivity to antigens targeted in Guillain-Barre Syndrome.

**Conclusions:** Together, our results suggest that influenza cross-reactive antibodies have the potential to be autoreactive. These data have important implications for developing universal influenza vaccines designed to generate durable influenza cross-reactive antibodies.

*Keywords: universal vaccine, autoimmunity, cross-reactive antibodies*

Topic: Virology and Pathogenesis: NextGen/Universal Vaccines  
Abstract No: 11817

## Targeting the “universal” Influenza A vaccine candidate M2e to Clec9A-expressing Dendritic Cells

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### INTRODUCTION

Over the past century there have been at least four devastating pandemics caused by Influenza A that took the lives of millions, and the threat of the next great pandemic remains a top global health concern today. Currently available vaccines need to be annually reviewed and updated to match circulating strains, and are accompanied by long and complicated production cycles with limited production capacity. In response to these limitations, increasing efforts have been devoted to develop “universal” flu vaccine candidates that would provide broad, cross-clade protection against all influenza A strains. Such candidate is the highly conserved non-glycosylated 24-amino acid ectodomain of M2 protein (M2e). However, its low immunogenicity has slowed down its clinical development and novel vaccine approaches are needed to improve the protective potential of M2e.

### METHOD

Our strategy is to target M2e to a specific sub-population of dendritic cells (CD8<sup>+</sup> DCs) as a way to boost the immunogenicity of M2e. This is achieved by engineering a chimeric anti-Clec9A monoclonal antibody fused at its heavy chains with six copies of M2e antigen. In this presentation, we will show the immunogenicity and protective efficacy upon a single dose administration of the Clec9A-M2e construct combined with different adjuvants. Furthermore, a dose response study and the long-term anti-M2e antibody response will be featured.

### CONCLUSION

Our data support that the Clec9A targeting strategy represents a promising approach to boost the immunogenicity of M2e with the additional benefit of dose and antigen sparing. Since the equivalent DC sub-population exists in humans (CD141<sup>+</sup> DCs), translation to human should be possible.

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 10320

## Monitoring of influenza A virus circulating in swine population in the northern and western regions of Kazakhstan in 2018

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Influenza virus causes an acute, highly contagious disease due to its constant variability, prevalence, and availability of natural reservoirs. Swine can act as a “melting pot” or an intermediate host in which reassortment takes place between avian, porcine, and human genes. Reassortment contributes to the emergence of new antigenic variants of influenza virus. Monitoring of influenza A virus circulation in the swine population is necessary for timely anti-epidemic and anti-epizootic measures to prevent epidemics, pandemics, and epizootics.

183 nasopharyngeal swabs were collected from swine during the 2018 spring season at livestock farms and private farmsteads located in the Kostanay and Aktobe regions.

The primary screening of biological samples in RT-PCR using AmpliSens reagents (Moscow, Russian Federation) demonstrated the presence of influenza A virus genetic material in five nasopharyngeal swabs (2.73% of the total number of examined samples). Subtyping showed the presence of influenza A/H1N1 virus RNA in three samples (1.64%), influenza A/H3N2 virus RNA in two samples (1.09%).

Therefore, the RT-PCR results obtained with the samples collected in spring 2018 from the northern and western regions of the Republic of Kazakhstan indicate the co-circulation of influenza A/H1N1 and A/H3N2 viruses in the swine population.

*Keywords: Influenza, virus, swine population*

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 10344

## Highly pathogenic avian influenza virus in Russia, 2016-2018.

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**Introduction and Objectives.** For the last years there has been challenging situation regarding avian influenza in Russia. This was due to the spread of highly pathogenic H5Nx avian influenza viruses of clade 2.3.4.4, which caused multiple outbreaks among wild birds and poultry in European part of Russia. The investigation and comparison of genetic and biological properties of newly and previously isolated highly pathogenic avian influenza viruses are presented.

**Materials and methods:** Sample collection and analysis were conducted according to WHO and OIE manuals. Whole genome sequencing was performed on an Illumina MiSeq using MiSeq reagent kit v3. Sequence alignment and phylogenetic analysis were performed using MEGA 6 software. All applicable international, national and institutional guidelines for the care and use of animals were followed.

**Results and conclusion.** All of the studied strains showed high rate of virulence for 9-day old embryonated chicken eggs. The infectious titers (EID<sub>50</sub>) of viruses in allantoic cavity were equal to 7,6 – 9.0 log EID<sub>50</sub>/ml. The infectivity rate of newly isolated viruses of H5N8, H5N5 and H5N2 subtypes was higher than infectivity rate of strains circulated in Russia before 2016. The infectious dose (ID<sub>50</sub>) index was equal to 1,6 – 2,8 logEID<sub>50</sub> and the lethal dose (LD<sub>50</sub>) index was equal to 1,8 – 4,4 logEID<sub>50</sub> after intranasal infection of Balb/c mice. The analysis of nucleotide sequences of the isolated strains genomes revealed several mutations potentially related to antigenic properties, virulence and changes in host specificity of viruses. The continuous emergence and broad distribution of novel H5 viruses including A(H5N8) and A(H5N2) viruses with highly pathogenic genotype and phenotype to poultry and mammals, which poses serious threat to agricultural animals and public health, highlights crucial importance of continuous surveillance, thorough investigation and risk assessment of H5Nx viruses.

**Keywords:** HPAI, avian influenza, H5Nx, surveillance, Russia.

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 10417

## PATHOGENICITY OF HPAI H5N8 VIRUS, FIRST ISOLATED IN THE RUSSIAN FEDERATION, FOR CHICKEN.

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**Introduction.** The natural reservoir of Influenza A viruses are waterfowl, which have no clinical signs of infection in contrast to lethal outcome in poultry. HPAI H5 continues to cause major economic problems in affected countries, especially in South-East Asia and China. Clade 2.3.4.4 A(H5N8) viruses are currently an agricultural and public health concern. They were identified in China in 2010, and then spread via wild birds along main migratory flyways. Health risks posed by novel HPAI A(H5N8) virus to poultry, animals and humans remain uncertain, so the primary objective is to investigate A(H5N8) susceptibility of chickens as widespread representatives affected by HPAI epizootics.

**Objectives.** To study the pathogenicity of new virus *A/great crested grebe/Uvs-Nuur Lake/341/2016(H5N8)* in chicken.

**Methods.** In study we used *A/great crested grebe/Uvs-Nuur Lake/341/2016(H5N8)* strain and 6-week-old SPF Rode Island chickens. Allantoic fluid from infected eggs was diluted in PBS to a final titer of  $10^6$  EID<sub>50</sub>/ml. Six chickens were separately oronasally inoculated with virus. On day 3 post-infection, samples of brain, lungs, spleen, kidneys, liver and small intestine were taken for RT-PCR and titration in MDCK. Viral growth in culture was measured by cytopathic effect and calculated. Tissue samples of the following visceral organs of birds were taken 3 dpi and processed using HE staining for histopathology.

**Results and Conclusion.** Histopathological examination confirmed the gross pathology findings, low-to-moderate levels of virus were detected mainly in respiratory and digestive tracts. Experimental infection with H5N8 showed the following characteristic pathologies in chickens: interstitial pneumonia, congestive phenomena in liver and kidneys, significant destruction in small intestine, heart fibrosis and activated immunogenesis in spleen. Multiple organ failure was accompanied by high virus titers in all examined organs.

Our study confirms high pathogenicity of new influenza A virus *A/great crested grebe/Uvs-Nuur Lake/341/2016(H5N8)*. The emergence of new reassortant viruses and their circulation in bird populations is a major concern as virus can spread via migratory wild birds.

The reported study was funded by RFBR, project №18-54-70006

**Keywords:** H5N8, Russia, pathogenicity, chicken



Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 10480

## UKRAINE IS THE UNIQUE REGION FOR SURVEILLANCE AND RESEARCH OF ECOLOGY OF EMERGENCE H5, H7, H9 SUBTYPES OF AVIAN INFLUENZA IN EURASIA

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**Introduction.** Wild birds are the primary natural reservoirs of avian influenza viruses (AIV). Emergence of new strains with new properties, particularly H5, H7, H9 subtypes that are pathogenic in new hosts, requires constant analysis.

**Methods.** Surveillance for AIV was conducted in Ukraine from 2006 to 2019 in regions aligning with intercontinental flyways. In sum, 21,511 samples were collected from 105 species of wild birds (2006-2016), and 8,463 samples were collected from 40 species (2017-2019). Fecal samples were analyzed by RT-PCR, and selected AIV were amplified for serological subtyping and genome sequencing. A limited collection of avian sera was analyzed by hemagglutination inhibition (HI).

**Results.** In 2006-2016 the AIV infection prevalence among wild birds was 0.45% (PCR), and 0.88% in Anatidae. In 2017-2019, AIV prevalence was 2.23% (1.94% in Anatidae), with most AIV (69%) were found in mallard (*Anas platyrhynchos*), other *Anas* spp. (19%), white-fronted goose (8%), and whooper swan (4%). In total, 23 antigenic combinations, with 15 avian HA subtypes of AIV were isolated. Most of these isolates were low pathogenic AIV although a few were highly pathogenic AIV. In total, 14 HPAIV H5 viruses (8 H5N1, 6 H5N8), 3 H5N2 LPAIV, 9 H7 LPAIV (3 H7N3, 1 H7N6, 3 H7N7, 2 H7N2), 3 H9N2 LPAIV, and 3 H9 LPAIV. The closest related sequences of H5N1 (clade 2.2) and H5N8 (clade 2.3.4.4) HPAIV were in Russia and Europe; while other subtypes were found in the Eurasian LPAIV pool. Wild birds were seropositive to H5 (2.3-4%), H7 (2.3%), H9 (5.5-15.4%).

**Conclusions.** Our results demonstrate the great genetic diversity of AIV including H5, H7, and H9 subtypes in wild birds in Ukraine, highlighting the importance of this region for the ecology of emergent pathogen. Identification of local reassortant strains suggests an exchange of genes and continual introduction of new variants in Eurasia.

**Keywords:** avian influenza viruse; wild birds; Ukraine; ecology of emergent pathogen; surveillance

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 10525

## Isolation and Characterisation of Equine Influenza Virus (H3N8) from an Equine Influenza Outbreak in Malaysia in 2015

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### Introduction and Objectives

Equine influenza is a major cause of respiratory infections in horses and can spread rapidly despite the availability of commercial vaccines.

### Methods

Molecular characterization of Equine Influenza Virus (EIV) isolated from the Malaysian outbreak in 2015 was carried out by Sanger sequencing of the HA and NA gene segments.

### Results

The nucleotide and amino acid sequences of H3 were compared with representative Florida clade 1 and clade 2 strains using phylogenetic analysis. The Florida clade 1 viruses identified in the Malaysian EIV outbreak revealed numerous amino acid substitutions as compared to the current World Organisation for Animal Health (OIE)-vaccine strain recommendations and representative strains of circulating Florida clade 1 and clade 2 sub-lineages. Differences in HA included amino acids located within antigenic sites which could lead to reduced immune recognition of the outbreak strain and alter the effectiveness of vaccination against the outbreak strain.

### Conclusion

Detailed surveillance and genetic information sharing could allow antigenic and genetic drift of equine influenza viruses to be monitored more effectively on a global basis and aid in refinement of vaccine strain selection for EIV.

*Keywords: Equine Influenza; H3N8*

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 10526

## Isolation and Characterisation of Avian Influenza Virus (H9N5) from a migratory Common Redshank in Singapore in 2015

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### Introduction and Objectives

Migratory birds play significant roles in biodiversity in all ecosystems and migratory birds are known to be natural reservoirs or hosts for Avian Influenza Virus (AIV). As part of the national surveillance activities, swab samples were obtained from wild birds at nature reserves and parks.

### Methods

Routine PCR testing for AIV using the influenza virus matrix gene detected a H9N5 AIV from 2 Common Redshank birds. The PCR results was confirmed by serology techniques such as Hemagglutination Inhibition (HI) and Neuraminidase Inhibition (NI). Subsequently, virus isolation using chicken embryonated eggs was carried out and further characterisation was performed using Sanger sequencing and Next-Generation Sequencing (NGS).

### Results

NGS facilitated in-depth understanding of the H9N5 whole virus genome, allowing us to classify the H9N5 virus under the H9.3.3.3 lineage and ascertain genome mutations that can facilitate adaptation to other hosts.

### Conclusion

The study emphasises the importance of continued surveillance using the One Health approach.

*Keywords: Avian Influenza; H9N5*

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 10527

## Molecular Characterisation of Canine Influenza Virus (H3N2) from a Canine Influenza Outbreak in Singapore in 2018

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### Introduction and Objectives

Influenza A viruses occasionally spill over from their reservoirs in waterfowls and infect several mammalian hosts, including human, swine, horse, and recently, cats and dogs. Influenza virus in dogs, commonly known as dog flu, causes respiratory disease in dogs. There are two major Canine influenza virus (CIV) subtypes, CIV H3N2 of avian origin emerged in Asia in the mid-2000s and CIV H3N8 of the equine origin that epidemic in dog populations in North America. In late April to May 2018, there was an outbreak of Canine influenza in several dog shelters in Singapore and hundreds of dogs are infected. The virus shedding stopped within 14 days and most dogs are recovered within weeks.

### Methods

Molecular characterization of the CIV strains isolated from this outbreak was subsequently carried out by Next-Generation sequencing of the HA and NA genes as well as internal gene segments.

### Results and Conclusion

As a result, avian-origin CIV H3N2 was identified responsible for the Singapore CIV outbreak. Detailed sequence analysis and phylogenetic studies will be presented during the conference.

*Keywords: Canine Influenza; H3N2*

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 10779

## PREVALENCE OF INFLUENZA A/H3 VIRUS IN WILD BIRD POPULATIONS IN DIFFERENT REGIONS OF KAZAKHSTAN IN 2004-2017

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**Introduction.** Influenza A virus is characterized by a unique genetic variation and its main ecological niche is the wild birds of the aquatic and near-water complexes. Influenza A/H3 viruses cause annual outbreaks of morbidity in the population and are able to infect other species of terrestrial and aquatic mammals, as well as birds.

**Method.** RT-PCR screening of swab samples and successive passages in embryonated chicken eggs of positives ones. Determination of antigenic formula of influenza A viruses was carried out by means of BLAST analysis of nucleotide sequences of HA and NA genes of isolates in GenBank.

**Result.** Virological study of 8,279 biological samples from 5,994 birds belonging to 155 species of 37 families from 17 orders, carried out in Kazakhstan during 2004-2017, resulted in isolation of 288 hemagglutinating agents, 193 of them were positive for the M gene of influenza A virus in PCR. From this amount 29 isolates were identified as influenza virus A/H3. Representatives of H3N6 subtype were isolated from four bird species of (mallard, pintail, common tern, teal) of the *Anatidae* and *Laridae* families in 2004, from graylag goose (*Anatidae* family) in 2006, from black-headed gull, slender-billed gull (*Laridae* family) in Central Kazakhstan in 2015. Influenza A (H3N8) virus was isolated from a teal, common gull, red-crested pochard, Eurasian wigeon, grebe, graylag goose in 2006, from a teal in 2008, also from a teal and common pochard (*Anatidae*, *Laridae* and *Podicipedidae* families) in Central and South Kazakhstan in 2010. Ecological and virological studies in other regions of Kazakhstan did not reveal prevalence of influenza A/H3 virus in avifauna.

**Conclusion.** The influenza A/H3 virus circulates among representatives of the avifauna of the *Anseriformes*, *Charadriiformes* and *Podicipediformes* orders on the territory of the RK.

*Keywords: A/H3, isolate, influenza A virus, wild bird*

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses

Abstract No: 10899

## IMPROVING AVIAN PARAMYXOVIRUS VECTOR VACCINE AGAINST HPAI

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**Introduction:** Avian paramyxovirus serotype 10 (APMV-10) is suitable as a virus vector of the emergency vaccine against highly pathogenic avian influenza (HPAI) because most chickens don't have anti-APMV-10 antibody which could interfere with the vaccine efficacy. Though some APMVs can serologically cross-reactive with each other, APMV-10 can evade the immunity against APMV-1 (Newcastle disease virus), which commercial chickens acquire by the routine vaccination. We have constructed the recombinant APMV-10 vector vaccine (rAPMV-10/HA) that have an HPAIV HA expression cassette in 5' untranslated region (UTR) of P gene. However, the vaccine efficacy of rAPMV-10/HA were not satisfactory in chickens. In present study, we improved rAPMV-10/HA vaccine by enhancing the expression of exogenous HA protein.

**Materials and Methods:** The HA gene cassette in rAPMV-10/HA was modified to be flanked by 5' and 3' UTRs of each APMV-10 gene (rAP10-UTRs) to improve protein expressions. As a control, rAP10-nonUTR that didn't contain any UTRs was generated. The effects of UTR in mRNA transcription, protein expression of the HA and vaccine efficacy for chickens were examined.

**Results:** Addition of UTRs increased the proportion of HA protein mRNA among the vector-derived mRNAs (1.55- to 1.84 fold) and the expression of HA protein (255- to 396-fold) in cells infected with rAP10-UTRs compared to that with rAP10-nonUTR. All chickens vaccinated oculonasally with rAP10-UTRs were survived after HPAIV challenge at 2-week post vaccination. Challenging virus were detected in neither throat nor cloaca swab of chickens when vaccinated with rAP10-NP-UTR, F-UTR or HN-UTR. In contrast, with rAP10-nonUTR, three out of ten chickens challenged with HPAIV were died and five of them shed the virus in cloaca or throat swabs after challenge.

**Conclusion:** The UTRs of APMV-10 could enhance the expression of the inserted HA protein, resulting in the improvement of vaccine efficacy of rAPMV-10/HA vector vaccine.

*Keywords: Avian paramyxovirus (APMV); Highly pathogenic avian influenza (HPAI); vaccine vector; emergency vaccination; Untranslated region (UTR)*

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 11143

## CHICKEN AND DUCK ENDOTHELIAL CELLS DISPLAY A MARKEDLY DIFFERENT INNATE IMMUNE RESPONSE TO VIRAL CHALLENGE

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**INTRODUCTION:** Highly pathogenic avian influenza viruses (HPAIVs) represent an ongoing threat to the poultry industry, impacting animal health and causing major economic losses worldwide. Chickens (*Gallus gallus*) are highly susceptible to HPAI whilst ducks (*Anas platyrhynchos*) are typically resistant. In chickens, HPAIVs primarily infect the endothelium leading to cell death, oedema, haemorrhaging, microthrombosis, disseminated intravascular coagulation and disruption of the innate immune response. Together, these features help account for the rapid and high mortality rates of HPAI in gallinaceous species. In contrast, HPAIVs rarely infect the duck endothelium and this is likely to account for the reduced disease severity seen in these birds. Here, we seek to characterize species dependent differences in the innate immune response of avian endothelial cells in order to understand their differential susceptibility to HPAI.

**METHODS:** Primary chicken and duck endothelial cells were cultured from the aorta and bone marrow of embryonated eggs. The identity and purity of these cell cultures was confirmed by RT-PCR, uptake of Ac-LDL and tube formation assays. Cells were subsequently challenged with poly (I:C) (a TLR3 agonist and mimic of viral infection) or HPAIV (A/chicken/Vietnam/0008/2004 (H5N1) and the transcriptome was characterized.

**RESULTS:** Chicken endothelial cells from both the bone marrow and aorta expressed significantly more pro-inflammatory cytokines than duck endothelial cells after challenge with poly (I:C). In contrast, no difference was observed in IFN $\alpha$  expression. To further understand these species dependent differences, RNA-Seq is currently being performed on duck and chicken endothelial cells infected with A/Vietnam/04(H5N1).

**CONCLUSIONS:** Here, we provide the first evidence that chicken and duck endothelial cells have a differential innate immune response to viral challenge. These data represent an important first step towards understanding species dependent differences in the pathogenesis of HPAIVs.

*Keywords: Innate Immune response; Avian; HPAIV*

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 11224

## EVALUATION OF LACTOBACILLUS ADDITION IN HPAI VACCINE AS AN ADJUVANT IN DUCKS.

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### Introduction and Objectives :

High Pathogenic Avian Influenza(HPAI) cause a big problem in the poultry industry worldwide. HPAI is highly contagious and leads to low egg production and weight loss and mortality rate is maximum 10% in ducks. Generally, stamping out policy is adopted in Korea when HPAI occurs in poultry farm. However, when the disease spreads rapidly, it is difficult to control the outbreak with stamping out policy, and therefore urgent vaccination needs to be prepared. Since the live vaccine virus is likely to be recombined with the field virus, only inactivated vaccines can be used, but inactivated vaccine has the disadvantage of late antibody formation. For rapid response with high antibody titer, we evaluated safety and efficacy of lactobacillus addition as adjuvant.

### Methods :

We used 5 groups of 1-day-old duck (n=10) and vaccine is made by clade 2.3.4.4 H5N9 that reverse genetically made. We used inactivate lactobacillus. The groups were divided by antigen dose(high or low) and whether lactobacillus was added or not. Also we tested same vaccine in 2-week-old ducks (n=10). The vaccine was subcutaneously inoculated into the duck neck. For 2 weeks after the vaccination, we checked body weight and serum antibody HI titer.

### Results and conclusion

There was little difference in body weight between vaccinated ducks. Also, antibody titer was formed faster and higher with the addition of lactic acid bacteria as adjuvant. Additionally, when the same dose of antigen is injected, antibody response of 2-week-old duck was faster and stronger than that of 1-day-old duck. Lactobacillus adjuvant in vaccines is safe enough to avoid weight loss. Also, lactobacillus adjuvant in vaccine showed better antibody response in ducks. Lactobacillus can be one of the good candidates for inactivated vaccine adjuvant in ducks.

*Keywords: HPAI; duck; adjuvant; lactobacillus; vaccine*



Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 11272

## ISOLATION AND CHARACTERIZATION OF LOW-PATHOGENIC H7N5 AVIAN INFLUENZA VIRUS FROM WILD DUCK IN SOUTH KOREA

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### Introduction and Objectives

Wild aquatic birds are considered the natural reservoir of avian influenza viruses (AIV). National surveillance of avian influenza virus (AIV) in South Korea has been annually conducted since 2008. We report on the result of isolation and genetic characterization of H7N5 AIV in South Korea, 2018.

### Methods

Wild bird surveillance was performed every winter season for detecting AIV. The virus isolation was performed by egg inoculation. The isolate was identified with sequencing with Miseq(Illumina), and assembled with CLC genomics workbench(Qiagen). Phylogenetic analyses are conducted with reference sequences of Global Initiative on Sharing All Influenza Data (GISAID).

### Results

On December 2018, AIV was isolated from wild bird feces in Chungnam province of South Korea. This isolate was identified as low pathogenic H7N5 AIV by nucleotide sequencing, Host species of the fecal sample was identified as wild duck by DNA barcoding assay. This isolate was designated as A/wild duck/Korea/H337/2018(H7N5). Previously, it has been few reports on detection of H7N5 AIV, nor had been this virus during the national surveillance between 2008 and 2017 in South Korea. Phylogenetic analysis including nucleotide sequences of H7N5 AIVs revealed that all genes of this virus clustered into Eurasian lineage. Highest nucleotide identity of HA and NA genes of this isolate were 97.75% with HA gene of A/mallard duck/Georgia/10/2016(H7N7) and 98.94% with NA gene of A/duck/Fukui/181015/2015(H12N5), respectively.

### Conclusion

These results suggest that this H7N5 AIV might have emerged through reassortment between AIVs of Eurasian lineage. Ongoing AIV surveillance efforts will provide understanding of genetic diversity of AIV.

**Keywords:** avian influenza, H7N5, wild bird, Phylogenetic analysis

Topic: Virology and Pathogenesis: Non-Human Influenza Viruses  
Abstract No: 11324

## Human seasonal pdmH1N1 infection in pigs

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**Introduction:** Since the introduction and spread of the 2009pandemicH1N1 (pdmH1N1), this virus has become part of the human seasonal virus trends, demonstrating evolution and repeated cross-hemispheric spread. The pdmH1N1 virus dominated the 2013-14 and 2015-16 north American human seasons and in western Canada the same trend was observed in pigs, accompanied with reports of flu activity in people on the same farms.

**Methods:** Nasal swab and oral fluid samples were collected from pigs on farms with previous influenza activity and farms undergoing acute respiratory outbreaks. Both suckling pigs (14 to 24 days of age) and nursery pigs (6 and 8 weeks of age) were sampled on the same farm, when available. Nasal swabs were tested in pools of 3 and oral fluids individually using a commercial PCR kit. One positive (Ct<38) pool per age group was subtyped and virus isolation was attempted on at least one pool Ct<30. Hemagglutinin gene sequencing was completed on isolates (1/subtype/submission).

**Discussion:** The 2018-19 December/January peak of pdmH1N1 in north American humans was accompanied by a similar peak of detection in pigs. The pig viruses were genetically similar to those found in North American humans who had no pig exposure during the same time period. As in previous years, the human peak of pdmH1N1 was followed by a surge in H3 viruses. This trend was also found in pigs with the pig H3N2 viruses. One farm had a worker's child confirmed to have pdmH1N1 at the start of the farm's epizootic. Although this virus has evolved significantly (both genetically and antigenically) over the last decade, it continues to readily infect both humans and pigs, causes a significant respiratory disease burden for both species. The findings here underscore the importance of public education on vaccines and making updated vaccines available for pigs.

**Keywords:** *human-to-pig transmission, swine influenza, surveillance*

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 10264

## COMPUTATIONALLY DESIGNED MINI-PROTEINS BROADLY BIND AND NEUTRALIZE DIVERSE INFLUENZA A STRAINS AND AFFORD POTENT AND DURABLE PROTECTION IN MICE AND FERRETS

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**Introduction:** Seasonal influenza costs the world's economy billions of dollars annually and there exists a real risk of a global pandemic that could lead to millions of deaths. Current influenza vaccines have insufficient population penetration, poor coverage of the diverse range of viral strains, and fail to elicit immunity in some patients. Monoclonal antibodies targeting the conserved hemagglutinin stem region of influenza A have shown significant promise in the preventing infection and disease in animal models but mono-clonals require intravenous injections, are expensive to manufacture, and exhibit reduced potency with repeated use.

**Methods:** Computationally designed hyperstable small proteins (minibinders) that are 50-fold smaller than monoclonal antibodies were designed that bind the HA stem region with greater affinity than broadly neutralizing monoclonal antibodies (Mab) and can be synthesized for more cost-effective manufacture. A minibinder that binds all Group 1 influenza A strains was compared to a potent broadly neutralizing antibody (FI6) for breadth of neutralization in vitro and for in vivo protection from influenza challenge in mice and ferrets.

**Results:** In vitro neutralization studies showed that the minibinder more potently neutralized diverse Group 1 influenza viral strains (H1, H2 H6, H5, H9) than FI6. Furthermore, inhaled delivery of the minibinder in mice afforded more potent and durable protection from influenza infection and disease when compared to the FI6 MAb. In a preclinical study, the minibinder was safe, non-immunogenic and protected ferrets from viremia and clinical signs of disease. Minibinders that bind Group 2 influenza strains have been designed and biopotency studies are in progress to investigate efficacy against representative Group 2 influenza A strains (H3, H7).

**Conclusions:** These results have significant implications for the use of inhaled computational designed antivirals against influenza and other respiratory diseases.

**Keywords:** influenza A, antiviral, computational design, do novo protein, mice, ferrets

Topic: Virology and Pathogenesis: Viral Replication

Abstract No: 10351

## STRUCTURE-ASSISTED DESIGN OF ANTIVIRALS AGAINST INFLUENZA VIRUS HEMAGGLUTININ

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**Introduction and Objectives** Influenza virus remains a constant threat to global health. Therefore, there is an urgent need to design more effective vaccines and therapeutics to protect against the multiple strains and types of influenza virus.

**Methods** Crystal structures of human broadly neutralizing antibodies (bnAbs) that target the conserved functional regions of head and stem of the hemagglutinin glycoprotein have inspired and assisted in design and subsequent structural and functional characterization of a variety of HA inhibitors.

**Results** The bnAb -HA crystal structures revealed common motifs for HA recognition despite different antibody origins and germlines. Multidomain antibodies, small proteins, peptides, and small molecules have now been designed based on these antibody -HA interactions and shown to inhibit HA receptor binding and fusion. My lab has structurally characterized the interaction and mechanism of these inhibitors in complex with influenza HAs. These inhibitors can block HA receptor binding, conformational changes associated with membrane fusion in the HA stem, or both in the case of multidomain llama antibodies. Data supporting the results and conclusions can be found in recent papers (2017-2019) with our collaborators in *Science* (3), *Nature*, *Nature Biotechnology*, *PNAS* (2) and *Bioorg Med Chem*. Our various collaborators include but are not limited to Janssen (Netherlands, Belgium and USA), University of Pennsylvania, Hong Kong University, David Baker's lab, Univ. Washington, and Dennis Wolan's lab, Scripps.

**Conclusion** Structural and functional characterization of human bnAbs against the HA has provided exciting new opportunities for design of novel therapeutics that can afford protection against entry of influenza virus into host cells.

*Keywords: hemagglutinin, small molecules, antivirals, structure-assisted design, structural biology*

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 10497

## Bispecific Fcγ Receptor engaging single domain antibodies protect against influenza A

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### Introduction and objectives:

Influenza-specific antibodies that primarily act through FcγReceptor-dependent mechanisms have entered early stage clinical trials. Our objective was to develop a simpler and more stable biological that could protect in a similar way.

### Methods:

We generated bi-specific single domain antibody (VHH) constructs that are directed against the conserved, membrane exposed part of matrix protein 2 (M2) on the one hand, and against a Fcγ Receptor family member on the other hand. These bi-specific VHHs, isolated from an immunized llama, were produced in *Pichia pastoris* and used in a mouse model of influenza as potential therapeutics.

### Results and Conclusions:

We isolated a VHH that binds the M2 ectodomain (M2e) with submicromolar affinity. Co-crystal structure analysis revealed that the complementarity determining regions 2 and 3 of this single domain antibody embrace M2e. Next the M2e-specific VHH was genetically fused to a VHH that specifically binds to mouse Fcγ Receptor I or IV, or human Fcγ Receptor IIIa. *In vitro*, these Fcγ Receptor engaging single domain antibody constructs selectively activated mouse Fcγ Receptor I, IV and human Fcγ Receptor IIIa, respectively, and did so only in the context of influenza A virus-infected or M2-expressing target cells. In addition, the M2e/Fcγ Receptor I and IV bispecific construct induced phagocytosis by macrophages of influenza A virus infected cells in a phagocyte-target cell co-culture system. Importantly, we demonstrate that the M2e/Fcγ Receptor IV bispecific VHHs could protect mice against challenge with influenza A virus and this protection was associated with a reduction in lung virus load. Finally, intranasal delivery of *in vitro* transcribed mRNA encoding these novel bispecific Fcγ Receptor engaging VHHs also protected against influenza A. In conclusion, the selective engagement of one activating FcγReceptor by a bi-specific VHH-based biological directed against M2 can protect against influenza A virus challenge.

**Keywords:** Single domain antibody, Fcγ Receptor, therapeutic, mRNA

Topic: Virology and Pathogenesis: Viral Replication

Abstract No: 10639

## UNLOCKING THE ANTIVIRAL TARGET POTENTIAL OF INFLUENZA A VIRUS NS1 PROTEIN

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**Introduction:** According to WHO Influenza Strategy 2019-2030 plan is essential to develop alternative influenza treatments. Influenza NS1 protein is among the most promising druggable targets, based on its structure, global function in replication and pathogenesis. So far no anti-NS1 therapeutics are available. Our project aims to identify antiviral target hotspots within NS1 and future prioritize new compounds for the design of influenza A virus (IAV) NS1 inhibitors.

**Methods:** We have comprehensively characterized the sequence-to-structure NS1 features in IAV by conservation, druggability and docking studies using bioinformatics tools. Eleven mutations (located in structurally/functionally important highly conserved druggable sites) were induced by mutagenesis to NS1 clones. A(H1N1)pdm09 reassortants bearing wild-type and mutated NS1 proteins were generated by reverse genetics. Growth kinetics was evaluated (*in-vitro*) from 12-60h post-infection by Hemagglutination titer, TCID<sub>50</sub> and virus particle number.

**Results:** Based on 28392 NS1 sequences (1918-2018) we have found that 87.34% of human IAV(H1AV) NS1 protein is conserved. The docking experiments suggested that the NS1-effector domain(ED) might represent the most favorable target domain. We have found three major conserved druggable pockets within the NS1-ED. Additionally to hotspots described in literature, we have identified 17 new potential hotspots residues within predicted binding pockets: W102, M104, Q109-K110, P114, D120-Q121, I128, A155, V157, G168-H169, K175, V180, G184, F201-A202. The experimental assays regarding the phenotypic outcome of NS1 mutations are in the final stage of completeness and will be presented in the communication. Outstanding hotspots are going to be explored by virtual screening for identification and *in-vitro* evaluation of top chemical inhibitors.

**Conclusions:** The disclosed hotspots are mostly surface exposed and represent attractive targets for pharmacological modulation. We anticipate to enlighten the potential of the analyzed mutations in reducing viral load. This project will establish/validate the NS1 protein as an anti-H1AV target and lays the basis for structure-based design of anti-influenza drug candidates (effective against all H1AV) targeting the NS1 protein.

**Keywords:** Anti-influenza strategy; druggable pockets; conservation; druggability; non-structural protein 1

Topic: Virology and Pathogenesis: Viral Replication

Abstract No: 10640

## PB1-HA FUNCTIONAL COMPATIBILITY CONTRIBUTES TO GENOME SEGREGATION PATTERNS AND TO THE OVERALL VIRAL FITNESS IN A(H1N1)PDM09 VACCINE SEEDS

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**Introduction:** We have previously demonstrated the functional compatibility between PB1 and the antigenic proteins neuraminidase(NA) and hemagglutinin(HA) as a molecular determinant of viral fitness and adaptation. We have also found that PB1 from swine-origin influenza A virus(IAV) retains interspecies transmission traces and genomic markers related to viral adaptation. Hence, we aim to evaluate the phenotype of reverting mutations naturally acquired by H1N1pdm09-IAV, identified as putatively enhancing PB1-HA functional compatibility and IAV adaptation.

**Methods:** Mutations K386R, V517I and I298L were induced by mutagenesis to PB1 clones. A/Portugal/82/2009(PT82)-H1N1pdm09 reassortants bearing wild-type and (single or triple) mutated PB1 genes were generated by reverse genetics. From 12-60h post-infection, growth kinetics and antigen yield were evaluated *in-vitro* (Hemagglutination titer[HT], TCID50, virus particle number and NA activity). Competitive transfection assays were performed: HA,NA and PB1 variants were added to induce competition. The incorporation frequencies and gene segregation pattern were determined.

**Results:** Inducing the triple mutation K386R-V517I-I298L resulted in significant increase in virus particles production(12-36hpi); increase in HT(24hpi); and NA activity(24-60hpi). The role that competitive fitness played on viral progeny selection (co-segregation of PB1 preferably with either one/both antigenic proteins); and the role that individual residues played in establishing PB1 interactions will be discussed in the communication.

**Conclusion:** Inducing these PB1 variants changed growth kinetics dynamics. These results raise the question of how a growth impaired mutant dominated the competition, which may be explained by competitive fitness determinants other than higher replicative capacity; or PB1-HA interactions at vRNA level. Clarifying the mechanisms driving PB1 co-segregation with antigenic proteins will allow adapting the rationale to vaccine seeds production for optimal gene constellations. These findings are profitable towards an overall more cost-effective and timely delivery vaccine manufacture. Could also contribute to risk-assessment of IAV increasing awareness to the importance of gene constellation as part of virology surveillance.

**Keywords:** *Gene constellation; Influenza overall fitness; Influenza vaccine seeds; PB1-HA interaction; Selective gene incorporation*

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 10710

## BIOLOGICAL SIGNIFICANCE OF NEURAMINIDASE OF EGG-ADAPTED INFLUENZA A(H3N2) VIRUS WITHOUT AMINO ACID SUBSTITUTIONS IN THE ANTIGENIC SITES OF ITS HEMAGGLUTININ

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### Introduction

The antigenic alteration due to egg-adaptation has been a major concern for A(H3N2) vaccine virus selection and its effectiveness. Recently, we have successfully isolated cell-derived and egg-propagated A/Saitama/103/2014 virus (H3N2) (Saitama) virus, which did not have amino acid (aa) substitutions in hemagglutinin (HA) antigenic sites and retained the antigenicity similar to the original cell-propagated virus. Interestingly, several aa substitutions in neuraminidase (NA) of this Saitama virus were introduced. After cloning by limiting dilution, viruses possessing either 2 or 7 aa substitutions in NA were obtained. Because Saitama virus acquired those NA aa substitutions after egg passages, it was speculated that NA might play an important role for the Saitama virus to grow in eggs. In this study, we focused on NA from egg-adapted Saitama virus and attempted to characterize their virological features.

### Method

To investigate the receptor binding activity of Saitama virus NAs, hemadsorption assay was performed by expressing NA from clinical isolate, cell-passaged, or egg-passaged Saitama virus on COS-7 cells. We also generated recombinant viruses by reverse genetics possessing NAs from Saitama virus and the HA with reduced binding activity to sialic acid receptors to examine whether Saitama virus can grow in eggs without HA binding activity.

### Result

Cells expressing NA of egg-adapted Saitama virus adsorbed chicken red blood cells much better than cells expressing NA derived from clinical isolate or cell-passaged Saitama viruses. Furthermore, viruses possessing NA of egg-adapted Saitama virus grew well in eggs without HA binding activity.

### Conclusion

Our results suggest that aa substitutions in NA confer receptor binding activity for Saitama virus to efficiently grow in eggs. This NA function may become a resolution to prevent egg-adapted antigenic change in HA of H3N2 vaccine strains.

*Keywords: H3N2, NA receptor binding, egg adaptation*



Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 10758

## TREATMENT OF HIGHLY PATHOGENIC AVIAN INFLUENZA A/H5N1 VIRUS INFECTION WITH MESENCHYMAL STROMAL CELL-DERIVED EXOSOMES

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**Introduction:** Antivirals like oseltamivir have been used to treat influenza virus infection, yet unable to reduce the severe mortality rate of highly pathogenic avian influenza (HPAI) H5N1. We have previously used mesenchymal stromal cells (MSCs) to reduce lung edema and improve survival of HPAI H5N1 infected mice in vivo. The therapeutic effect of MSCs attributes to the extracellular vesicles secreted by MSCs containing growth factors and anti-inflammatory proteins. Exosomes are tiny extracellular vesicles containing mRNA, lipids and proteins which are released by host cells into the lung microenvironment upon stimulation. These exosomes require less storage facility and are more appealing therapy than MSCs therapy. We therefore propose to investigate the therapeutic effects of exosomes released by MSCs (MSC-exosomes) on lung edema of H5N1-infected primary human alveolar epithelial cells (AECs) in vitro through the modulation of host innate immune response by increasing alternatively activated M2 macrophages.

**Method:** Exosomes extracted from umbilical cord-derived MSCs were co-cultured with human peripheral blood-derived macrophages to study the immunomodulatory responses and alveolar fluid clearance (AFC) of the H5N1-infected AECs using in vitro lung injury model. These exosome-educated macrophages will be also analyzed for the phenotypic changes of inflammatory M1 and anti-inflammatory M2 macrophages.

**Result:** Both MSC-exosome and exosome-educated macrophages restored the impaired AFC and reduced the pro-inflammatory cytokine production of the H5N1-infected AECs. Exosome-educated macrophages were found to have activated into anti-inflammatory M2 phenotype with more increased anti-inflammatory cytokine expression when compared to macrophages without MSC-exosomes co-culture.

**Conclusion:** These results suggested that MSC-exosomes can increase edema clearance after H5N1 virus infection by inducing phenotypic changes of host macrophages to promote anti-inflammatory effect. In conclusion, MSC-exosomes can be a novel alternative treatment for human infection of HPAI H5N1 virus.

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 10774

## Genome packaging of Influenza A viruses is regulated by an interplay of vRNA packaging sequences and NP acetylation.

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### Introduction:

The genome of Influenza A virus (IAV) is allocated to eight distinct gene segments, each present in form of a viral ribonucleoprotein (vRNP) complex. All vRNPs are equally organized: the respective viral RNA (vRNA) is lengthwise encapsidated by multiple copies of the viral nucleoprotein (NP) and terminally bound by the viral polymerase complex. Though beneficial for rapid adaptation, segmented genomes require highly sophisticated packaging mechanism. In this context, it is known that highly conserved vRNA packaging sequences mediate genome packaging. Recent studies indicate that NP likewise contributes to this process. Considering that packaging sequences form specific secondary RNA structures during the packaging process we speculated that NP might shape these structures accordingly. Intriguingly, we could previously show that upon mimicking NP hyper-acetylation vRNA binding is affected.

### Objectives:

Here we sought to identify interdependence between vRNA packaging sequences and NP acetylation. Considering that single vRNA packaging mutants but also NP acetylation mutants do not display a detectable packaging defect alone, we speculated that a combination of both should result in a packaging defect if IAV genome packaging is regulated by NP acetylation in a spatio-temporal manner.

### Methods:

We generated recombinant A/H7N7 (SC35M) viruses comprising mutations in the vRNA packaging sequences of individual gene segments (PB1 and PA) together with arginine (R) or glutamine (Q) substitutions in NP at position 229, mimicking either constant non-acetylation (NP<sub>K229R</sub>) or acetylation (NP<sub>K229Q</sub>), respectively. We then analyzed their viral replication properties by performing growth kinetics and their viral genome packaging by qPCR.

### Results/Conclusion:

We herein demonstrate that genome packaging is indeed affected by mutations mimicking either non-acetylation or constant acetylation at NP<sub>K229</sub>. Based on these results we speculate that vRNA packaging sequences adopt a certain secondary structure during viral particle release, which is dictated by the vRNA sequence but regulated by NP modifications, including acetylation.

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 10780

## Enhanced reactivity of a neutralizing antibody against influenza B virus redesigned using rational sequence- and structure-based approach

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### Introduction and Objectives:

Increasing numbers of broadly neutralizing antibodies (bnAbs) targeting influenza virus hemagglutinin have been identified in recent times; these hold promise for the development of novel anti-influenza drugs and a universal influenza vaccine. However, the generation of bnAbs remains challenging, because the epitopes they target are generally non-immunodominant and may not be accessible by conventional antibody screening methods. Thus, it is necessary to develop new convenient and rapid approaches to identify anti-influenza bnAbs.

### Methods:

An influenza B antibody targeting hemagglutinin that demonstrated high reactivity to Yamagata lineage viruses and little reactivity to Victoria lineage viruses was selected and engineered by combining structure-based and sequence-based rational design.

### Results:

The redesigned antibody, 11B9m5, exhibits higher potency and breadth of neutralization *in vitro* when compared to wild type 11B9. The 11B9m5 antibody also cross-protects against lethal infection with both Yamagata and Victoria lineage influenza B viruses in mice. Data showed that a structure-guided optimization of the HCDR3 region of 11B9 allows the loop to adopt a more lock-and-key-like binding mode, while sequence-based rational design of the sequences of the other five 11B9 CDRs contributes to high-affinity binding, presumably, by facilitating interaction formation, stability of interaction surroundings and availability of low-energy binding sites.

### Conclusion:

Combining structure-based and sequence-based rational design presents a promising approach for optimizing the potency and breadth of reactivity of influenza specific antibodies, and accelerating the development of therapeutic antibodies against influenza.

*Keywords: Influenza antibody; potency and breadth enhancement; computational docking; empirical optimization*

Topic: Virology and Pathogenesis: Viral Replication

Abstract No: 10792

## DNA-LINKED INHIBITOR ANTIBODY ASSAY (DIANA) AS A NEW METHOD FOR SCREENING INFLUENZA NEURAMINIDASE AND POLYMERASE INHIBITORS

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### Introduction and Objectives

Influenza neuraminidase is responsible for the escape of new viral particles from the infected cell surface. Several neuraminidase inhibitors are used clinically to treat patients or stockpiled for emergencies. However, the increasing development of viral resistance against approved inhibitors has underscored the need for the development of new antivirals effective against resistant influenza strains. A facile, sensitive, and inexpensive screening method would help achieve this goal.

### Methods

Recently, we described a multiwell plate-based DNA-linked inhibitor antibody assay (DIANA). This highly sensitive method can quantify femtomolar concentrations of enzymes. DIANA also has been applied to high-throughput enzyme inhibitor screening, allowing the evaluation of inhibition constants from a single inhibitor concentration.

### Results

Here, we report the design, synthesis, and structural characterization of a tamiphosphor derivative linked to a reporter DNA oligonucleotide for the development of a DIANA-type assay to screen potential influenza neuraminidase inhibitors. The neuraminidase is first captured by an immobilized antibody, and the test compound competes for binding to the enzyme with the oligo-linked detection probe, which is then quantified by qPCR.

### Conclusion

We validated this novel assay by comparing it with the standard fluorometric assay and demonstrated its usefulness for sensitive neuraminidase detection as well as high-throughput screening of potential new neuraminidase inhibitors. In addition, we have recently developed DIANA for high-throughput screening of influenza polymerase inhibitors targeting cap-binding and inter-subunits protein-protein interaction.

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 10804

## IDENTIFICATION OF A TYPE-SPECIFIC PROMOTER ELEMENT THAT DIFFERENTIATES BETWEEN INFLUENZA A AND B VIRUSES

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**Introduction and Objectives:** Genetic reassortment of influenza virus plays a key role in the virus evolution and the emergence of pandemic strains. Type A and type B influenza viruses (FluA and FluB) are two major human pathogens that share common structural and functional features. The reassortment occurs extensively within either FluA or FluB but never between them. Here, we studied whether the FluA and FluB type-specific promoter element in contributing the differentiation between FluA and FluB viruses.

**Methods:** We first bioinformatically compared the promoter sequences of all available influenza A and B viruses (FluA and FluB) vRNAs in the NCBI influenza virus database and confirmed the presence of the type-specific promoter elements. We then performed mutagenesis studies of the type-specific promoter elements in the context of FluA and FluB RNP reconstitution and virus infection systems *in vivo*. We also conducted *in vitro* transcription assays to examine the effects of the promoter element mutants on promoter activities.

**Results and Conclusion:** Our results identified, for the first time, that a type-specific promoter element - the nucleotide at position 5 in the 3' end of the vRNA, plays key roles in modulating polymerase activities in a type-specific manner. Interestingly, swapping the promoter element between FluA and FluB recombinant viruses showed different consequences. The replacement of the FluA-specific U5 with the FluB-specific C5 in A/WSN virus could be immediately reverted to U5 after 2-3 passages. In contrast, the replacement of the FluB-specific C5 with FluA-specific U5 in B/Yamagata virus could be maintained but with significantly reduced replication efficiency. Therefore, our finding indicates that promoter variation between FluA and FluB contributes to their RNP incompatibilities, which shed new light on understanding the mechanisms of intertypic exclusion of reassortments between FluA and FluB.

**Keywords:** influenza A and B viruses, type-specific promoter element, virus polymerase

Topic: Virology and Pathogenesis: Viral Replication

Abstract No: 10896

## Generation of a purely clonal DI virus

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### 【Introduction and Objectives】

A defective interfering (DI) influenza virus carries a large deletion in a viral gene segment and shows potential for antiviral therapy by interfering with the replication of infectious virus. 244DI virus is one of the most-characterized DI influenza viruses, which has a 1946 nt deletion in the coding region of the PB2 vRNA. To obtain the cloned 244DI virus without contamination of infectious helper viruses, we tried to establish a system for propagation of only the cloned 244DI virus.

### 【Materials】

To prevent expression of undesired proteins, all start codons in the 3'-terminal 244 nt of the PB2 244DI vRNA reading frame were mutated. 244DI(UUG) virus, which expresses this mutated PB2 244DI vRNA, was generated by reverse genetics. 244DI(UUG) virus was propagated in AX4/PB2 cells, a cell line stably expressing PB2. We investigated this virus' titer, growth and ability to interfere with infectious virus replication *in vitro*.

### 【Results】

The 244DI(UUG) virus grew efficiently in AX4/PB2 cells, but not in MDCK cells. 244DI(UUG) virus contained PB2 244DI(UUG) vRNA, but not wild-type (WT) PB2 vRNA, indicating that the cloned 244DI(UUG) virus was successfully obtained. When WT virus was mixed with 244DI(UUG) virus and together propagated in MDCK cells, the infectious virus titer became lower dependent on the amount of 244DI(UUG) virus in the mixture. RT-PCR analysis showed that amount of 244DI(UUG) vRNA incorporated in virus particles is related to the suppression of infectious virus titer, suggesting that the cloned 244DI(UUG) virus interferes with infectious virus replication.

### 【Conclusion】

We generated purely clonal DI virus without the need for infectious helper viruses for DI virus replication, and confirmed its ability to interfere with infectious virus replication. Our approach will contribute to the understanding of DI virus interference mechanisms, and can be used for the development of DI virus-based antivirals.

*Keywords: influenza, defective interfering*

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 10903

## Combination therapy with nitazoxanide and oseltamivir reduces the impact of influenza virus infection in vitro and in vivo.

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### Introduction and objectives

Nitazoxanide (NTZ) is a first generation thiolzolid used against parasitic infections that has been repurposed to treat influenza and is thought to exert its anti-influenza activity through the inhibition of haemagglutinin maturation. Second generation thiolzolides such as RM-5061 were developed to improve systemic absorption. We investigated whether the combination of oseltamivir (OST) and NTZ or RM-5061 would be more effective than monotherapy.

### Methods

To establish whether tizoxanide (TIZ) and oseltamivir-carboxylate (OST-C), the active metabolites of NTZ and OST, had a synergistic, additive or antagonistic interaction, a 24-hour yield reduction assay was conducted in MDCK cells. For in vivo experiments, mice and ferrets were treated twice daily for 5 days with either OST, RM-5061, OST+RM-5061 or placebo (mice) or NTZ, OST, OST+NTZ or placebo (ferrets), starting 2 hours prior to infection with either 10<sup>4.5</sup> PFU of HKx31 (H3N2) (mice) or via an A(H1N1)pdm09 infected donor ferret. Animals were monitored daily for any signs of illness and viral burden was examined at various time points post-infection.

### Results

In vitro experiments revealed that TIZ and OST-C had a largely additive interaction, with some synergy at certain concentrations. In mice, the combination of OST+RM-5061 significantly reduced viral burden 5 days post-infection compared to placebo-treated animals, but there was no difference from treatment with OST alone. Lower levels of CCL5 were found in OST+RM-5061 treated mice compared to monotherapy. In ferrets, NTZ+OST treatment reduced infection rates by 75%, compared to placebo, and by 44% compared to OST monotherapy. Furthermore, NTZ+OST combination therapy reduced the progression of the virus from the upper to the lower respiratory tract, whereas OST or NTZ monotherapy were unable to prevent this.

### Conclusions

Combination therapy was superior to monotherapy in vitro and in both animal models. The benefit of NTZ+OST in ferrets was greater than RM-5061+OST in mice.

*Keywords: Nitazoxanide; influenza; influenza antivirals; ferrets; mice*

Topic: Virology and Pathogenesis: Viral Replication

Abstract No: 10973

## PRECLINICAL EFFICACY, PHARMACOKINETICS, AND SAFETY OF CB-012, A NOVEL ANTIVIRAL Fc-CONJUGATE AGAINST INFLUENZA

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### Introduction

CB-012 is a novel antiviral Fc-conjugate (AVC) comprising a potent small-molecule antiviral and the Fc domain of human IgG1. CB-012 is metabolically stable, long-acting, and demonstrates robust efficacy in lethal mouse models of influenza. Studies were conducted to confirm stability and assess pharmacokinetics, safety/tolerability, and efficacy in a prevention model.

### Methods

CB-012 stability was assessed after 0-24 h incubations in mouse/human plasma and liver microsomes at 37°C using MALDI-TOF mass spectrometry. Single-dose pharmacokinetics were studied in mouse (1-50 mg/kg), rat (5 mg/kg), and monkey (5-20 mg/kg). Plasma concentrations were measured using anti-human IgG-Fc ELISA. Two-week safety/toxicology was evaluated in rats (5-50 mg/kg) and monkeys (5-20 mg/kg) using IV slow bolus on days 1 and 8 with necropsy on day 15; clinical signs, chemistries, hematology, cytokines, and histopathology were evaluated. Preventative efficacy was studied in a lethal influenza mouse model using a single dose of CB-012 1.25-50 mg/kg 28 days prior to intranasal challenge with an LD<sub>90</sub>(~75 PFU/mouse) of A/Texas/36/91 (H1N1).

### Results

CB-012 was stable after incubations in mouse/human plasma and liver microsomes. In mouse, rat, and monkey, CB-012 t<sub>1/2</sub> was 7-10 days. Dose-proportional increases in exposure were observed in each species, notably from 1-50 mg/kg in mouse. Plasma concentration after IV administration in rat/monkey evaluated for a month post-dose remained linear with no evidence of anti-drug antibodies. High bioavailability (>60%) was observed after subcutaneous administration. A single IV dose of 2.5 mg/kg administered 28 days prior to infection provided 100% protection from death (Figure 1E). In 2-week rat toxicology studies, there was no effect on bodyweight, clinical chemistry, hematology, coagulation, cytokines, or urinalysis. Histological findings related to CB-012 were absent at all doses tested.

### Conclusion

Exceptional stability, safety, and extended half-life underscore the potential of CB-012, a long-acting, novel antiviral for prevention and treatment of influenza.

*Keywords: pharmacokinetics; prevention; treatment; H1N1; AVC*



Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 10974

## NOVEL ANTIVIRAL Fc-CONJUGATE CB-012 DEMONSTRATES POTENT ACTIVITY IN CYTOPATHIC EFFECT (CPE) AND VIRAL GROWTH INHIBITION ASSAYS AGAINST INFLUENZA A AND B STRAINS

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### Introduction

A series of long-acting antiviral Fc-conjugates (AVCs) against the influenza virus has been generated. Herein we characterize the *in vitro* activity of the AVC CB-012 in CPE and viral growth inhibition assays against influenza A and B strains.

### Methods

CPE assays were performed in MDCK cells challenged with A/California/09 (H1N1) and B/Brisbane viruses using ten, two-fold serial dilutions of CB-012 (160–0.3125nM) and oseltamivir (9.6µM–18.75nM). To determine the 50% effective concentration (EC<sub>50</sub>), MDCK cells were infected (MOI=0.001; 1-h incubation) and stained with crystal violet at 3- or 5-d post-infection (influenza A and B, respectively). Viral growth inhibition assays were performed in A549 cells using A/WSN/33 (H1N1), A/Wyoming/3/03 (H3N2), A/California/04/09 (H1N1), A/Vietnam/1203/04 (H5N1) HALo, and B/Lee/40 Victoria. Cells were pre-treated with study molecules at 1µM, 100nM, or 10nM for 2h and infected with indicated strains (MOI=0.01) for 1-h incubation, after which cells were washed and study molecules were reapplied at the same concentrations. Production of viral particles in the supernatant was determined via plaque assay. The selectivity index was determined in parallel using MDCK and A549 cells.

### Results

In the CPE assay, CB-012 EC<sub>50</sub> values were 4 and 52nM against A/California/09 and B/Brisbane compared with 390 and 1,065nM, respectively, for oseltamivir. CB-012 also performed well in the viral growth inhibition assay with 10nM reducing the production of viral particles more potently than 1µM oseltamivir against all strains by 72h, with the exception of A/Vietnam/1203/04 which required 100nM of CB-012 to outperform oseltamivir. CB-012 and oseltamivir had no impact on the viability of MDCK or A549 cells across all concentrations evaluated.

### Conclusion

Both CPE and viral growth assays demonstrated that CB-012 has more potent activity than oseltamivir against a variety of influenza A and B strains, supporting further development of this novel AVC for the prevention and treatment of influenza.

*Keywords: AVC; prevention; treatment; oseltamivir; H3N2*

Topic: Virology and Pathogenesis: Viral Replication  
 Abstract No: 10979

## EFFICACY OF CB-012, A NOVEL ANTIVIRAL Fc-CONJUGATE, IN LETHAL MOUSE MODELS OF OSELTAMIVIR-SENSITIVE AND -RESISTANT INFLUENZA A H1N1 AND H3N2 ISOLATES.

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### Introduction

Cidara Therapeutics is developing a new generation of antivirals that couple a neutralizing small molecule to the effector domain (Fc) of a human antibody (IgG1). These long-acting, antiviral Fc-conjugates (AVCs) directly attack the virus while simultaneously engaging the immune system. CB-012 is an AVC against influenza that demonstrates robust, broad-spectrum activity in lethal mouse influenza models.

### Methods

Efficacy studies were conducted in BALB/c mice challenged intranasally with virus. CB-012 was administered as a single dose intravenously at various concentrations and dose times. Oseltamivir was dosed orally at 20 mg/kg, bid, x5 days. Body weights (BW) and health scores were monitored daily, with 20% BW loss recorded as mortality.

### Results

Against H1N1, a single 0.4 mg/kg dose of CB-012 delivered 4 hours prior to infection was fully protective and statistically significant versus the Fc control (p=0.0135) (**Figure 1C**). Importantly, mice treated with CB-012 showed only transient BW loss (<1%) while oseltamivir-treated mice (200 mg/kg cumulative dose, starting at T+8hours) suffered ~10% BW loss before recovery. Against H3N2, a 0.4 mg/kg dose of CB-012 was fully protective, demonstrating the potency of CB-012 against two important seasonal influenza types. Against an H1N1 isolate harboring the oseltamivir<sup>R</sup> mutation (H275Y), CB-012 was fully protective at 2 mg/kg (p=0.003) whereas 200 mg/kg oseltamivir (cumulative dose) was not.

In a final study, administration of CB-012 was delayed up to 72 hours. A single dose of CB-012 10 mg/kg conferred an 80% survival rate, which was significant versus control (p=0.03). In contrast, only 20% of oseltamivir-treated mice survived when treatment was delayed.

### Conclusion

The novel AVC CB-012 demonstrated robust efficacy in multiple, lethal influenza challenge models of H1N1 and H3N2, oseltamivir resistance, and delayed treatment (up to 72 hours). These results support further development of CB-012 as a novel antiviral for the prevention and treatment of influenza.

*Keywords: AVC; H1N1; H3N2; immunotherapy; efficacy*

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 11055

## A RESTRICTION DIGESTION AND LIGATION INDEPENDENT TECHNIQUE FOR CLONING INFLUENZA GENE SEGMENTS INTO PHW2000

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### Introduction

Reverse genetics enables to make tailor-made influenza viruses of a desired genotype or phenotype. Very often, difficulties are encountered while cloning influenza gene segments into standard reverse genetics vector (pHW2000, pHH21), either due to the presence of internal *BsmBI* or *BsaI* restriction sites in the gene segments or due to the instability of the polymerase genes and thus, virus rescue de novo becomes more challenging. Here we report easy and efficient restriction digestion and ligation independent cloning method to clone all influenza gene segments into pHW2000.

### Concept

The method involves two sequential PCR cycles. The first PCR generates the desired amplicon (megaprimer) having termini complementary to the pHW2000 vector/template plasmid. The second PCR involving the megaprimer and the template plasmid is followed by *DpnI* digestion and transformation.

### Methods

The whole procedure involves the following steps:

1. Primer designing and synthesis
2. RNA extraction of the target virus and reverse transcription (cDNA synthesis).
3. PCR I – amplification of cDNA with designed primers, followed by gel extraction of the desired amplicons (Megaprimer).
4. PCR II – involving Megaprimer and the bait plasmid (empty pHW2000 or cloned pHW2000)
5. *DpnI* digestion and transformation
6. Screening of positive colonies

### Results

We efficiently cloned PB1 and PB2 of A/chicken/Bangladesh/23527/2014 (H9N2) and the PB2 and PB1 of A/Chicken/Vietnam/H7F-14-BN4-315/2014 (H9N2) using ligation and restriction digestion independent technique. Recombinant viruses could be rescued using the cloned PB1 and PB2. We efficiently cloned all the gene segments of A/Chicken/Vietnam/H7F-14-BN4-315/2014 (H9N2) into pHW2000 vector

### Conclusion

This technique can contribute significantly towards the rapid generation of influenza viruses by reverse genetics.

*Keywords: Ligation Independent Cloning; Influenza; Reverse Genetics*

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 11094

## **Fc-MEDIATED EFFECTOR FUNCTION CONTRIBUTES TO POTENCY OF NOVEL ANTIVIRAL Fc-CONJUGATE CB-012**

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### **Introduction**

Cidara's novel antiviral Fc-conjugates (AVCs) comprise potent, surface-acting, small-molecule antiviral agents conjugated to the Fc domain of human IgG1. AVCs demonstrate dual mechanism of action - direct antiviral activity and immune-mediated viral clearance.

CB-012 was identified from a series of long-acting AVCs against influenza that has been generated and demonstrates potent, broad-spectrum activity and efficacy in multiple influenza infection models. Herein, we evaluated the contribution of immune-mediated effector functions to CB-012 activity.

### **Methods**

Fcy receptor interaction was determined by binding of AVCs to Fcy receptor immobilized on ELISA plates. BALB/c mice were challenged intranasally with 3E2 PFU of mouse-adapted influenza A/Puerto Rico/8/1934 (H1N1) and treated 2 h post-challenge intravenously with a single dose of AVC, ranging from 0.1 – 1 mg/kg. Body weight loss of >20% was recorded as mortality.

### **Results**

The contribution mediated by engagement of immune cells (such as NK cells) via the Fc to the activity of CB-012 was demonstrated by the relative efficacy of CB-012 to CB-012a, an immune-silent analog with a mutant Fc that we showed to result in loss of Fcy receptor binding. In a lethal mouse model of influenza, 100% survival was achieved with CB-012a only at 1 mg/kg, whereas 100% survival was achieved with CB-012 at 0.3 mg/kg (Figure). The ability of CB-012 to bind to Fcy receptors significantly enhanced the viral clearance by >3-fold. The superior activity of CB-012 is mediated by engaging immune effector cells.

### **Conclusion**

These results demonstrate that immune engagement significantly enhances the robust antiviral activity of CB-012 and support further development of AVCs for the prevention and treatment of influenza.

*Keywords: Immunity; In Vivo; Novel; Antiviral; Broad-Spectrum*

Topic: Virology and Pathogenesis: Viral Replication

Abstract No: 11154

## Inhibitory Effect of Baloxavir acid on Replication of Avian and Swine Influenza A Viruses harboring distinct variants of PA gene *in vitro*

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### Introduction and Objectives

Influenza pandemics can occur when avian and swine-origin viruses gain the ability for human-to-human transmission. Baloxavir acid (BXA) potentially inhibits the cap-dependent endonuclease within the PA of influenza A and B viruses. Susceptibility data of a wide range of human viruses to BXA and co-crystal structures of BXA binding to PA endonuclease domain support broad spectrum activity of this class of compounds; however, information on the inhibitory activity of BXA against avian and swine viruses is limited. Here, we analyzed the diversity of PA genes of influenza A viruses (IAVs) by using a phylogenetic method. Moreover, we evaluated the antiviral activities of BXA against several avian and swine IAVs belong to each resulting phylogenetic cluster.

### Methods

For phylogenetic analysis, the genetic distances between the full-length PA gene nucleotide sequences of IAVs obtained from databases (NCBI and GISAID) were calculated, and then hierarchical clustering was conducted, followed by plotting of sequences in two dimensions by classical multidimensional scaling methods. The virus yield reduction assay was performed, and the 90% effective concentration (EC<sub>90</sub>) was calculated to evaluate the susceptibility of the tested virus to BXA.

### Results

In phylogenetic analyses, PA genes of IAVs were divided into 16 clusters. Avian and swine IAVs formed 12 of these clusters, and half of clusters were constituted with viruses from both hosts. BXA showed broad-spectrum activity against each of the viruses tested (the EC<sub>90</sub> values: 0.6-3.6 nmol/L) regardless of the host, isolated years, regions and subtypes, including viruses harboring amino acid polymorphisms at positions 20, 24, 37 and 38 in the PA, implicated in BXA binding to PA endonuclease domain.

### Conclusion

Results of phylogenetic analyses support broad-spectrum activity of BXA against a diverse representation of circulating avian and swine IAVs, suggesting that BXA must be useful against future pandemic virus strains.

*Keywords: baloxavir, influenza A virus, PA gene, phylogenetic analysis, genetic diversity*

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 11164

## A NOVEL CONCEPT FOR THE DESIGN OF ANTIVIRAL DRUGS FOR INFLUENZA

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**Introduction and Objectives:** Currently registered antiviral drugs for influenza are designed to bind to and block the function of viral proteins. Their modes of action target steps of the replication cycle to reduce the overall viral load; they are not designed to prevent infection. Compounds that could theoretically prevent cells from infection by blocking the haemagglutinin (HA) receptor-binding pocket have proven elusive. Here we introduce drug candidates, designed to inhibit viral infection by creating an environment that is incompatible with viral entry.

**Methods:** Several compounds have been designed by Aus Bio Ltd that induce the low pH-mediated conformational change in HA that normally occurs after internalisation of the virus to allow endosome-escape of the viral genome. This change is irreversible and if it occurs prior to contact with the cell, the virus is not capable of binding to the receptor and entering. The Aus Bio compounds use a modified form of sialic acid to anchor the compounds to the viral neuraminidase and have an effector domain that provides a negatively charged environment to trigger a premature conformational change in the neighbouring HA.

**Results:** These compounds have greatly enhanced *in vitro* potency compared to zanamivir, and unlike neuraminidase inhibitor drugs, show marked inhibition of viral entry and of hemagglutination. Results with two lead candidates that show remarkable *in vivo* prophylactic longevity and also therapeutic effectiveness in the mouse model after a single dosing will be presented as well as preliminary data showing protection in the ferret model. Results confirming the mode of action of the compounds will also be shown.

**Conclusion:** Compounds of this nature present an exciting new strategy to inhibit infection by influenza virus and increase our toolbox of options for influenza control.

*Keywords: viral inhibitors; low pH conformation; blocking entry*

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 11202

## RESISTANCE STUDIES ON A NOVEL INFLUENZA ANTIVIRAL WITH A DUAL MODE OF ACTION

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### Introduction

Here we present data on first in kind antivirals against influenza, products of AusBio Ltd, which have a modular structure and a dual mode of action. These compounds possess an effector domain that creates an acidic microenvironment mimicking the low pH found inside the endosome. This domain induces the irreversible pH dependant conformational change in the haemagglutinin and reduces attachment of the virus. The acidic effector domain has been linked to a zanamivir-like anchoring domain, to facilitate attachment to virions via the neuraminidase (NA). This anchor not only provides proximity for the effector domain, but also suppresses the enzymatic activity of the NA and prevents spread of progeny virions, like a traditional NA inhibitor.

### Methods

While NAI escape mutants are rare in the wild, the development of antiviral resistance is a significant concern for any new drug and is an important consideration for any further clinical development. Due to the structural similarities of the anchor domain to zanamivir we tested these compounds against common NAI resistant strains. Compounds were evaluated in enzyme inhibition and cell culture-based assays. We also passaged viruses *in vitro* in inhibitors to determine if we could select for resistance to these compounds.

### Results

We found that sensitivity to the AusBio compounds was unaffected by the H274Y substitution conferring oseltamivir resistance. Resistance to the E119G mutant was ten-fold less than to zanamivir. Mutants with decreased sensitivity were selected, but they were unfit.

### Conclusions

Amantadine is no longer recommended for the treatment of influenza due to high levels of resistance, and escape mutants occurred in up to 23% of Baloxavir Marboxil treated patients. However, we do not expect resistance to be a significant factor in the clinical development of the AusBio antivirals, since any novel mutants must overcome the dual mode of action of these compounds.

*Keywords: Compromised fitness, dual acting inhibitor*

Topic: Virology and Pathogenesis: Viral Replication

Abstract No: 11223

## Competitive Fitness of the Influenza Viruses with PA/I38T Substitution in Primary Human Airway Epithelium Cells

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### Introduction and Objectives

Baloxavir marboxil (BXM) is a novel cap-dependent endonuclease (CEN) inhibitor. In the treatment-emergent variant monitoring of the BXM clinical studies, treatment-emergent mutations were identified in the PA region, which were defined as AA changes after a dose of BXM. Sanger sequencing confirmed that all variants with reduced Baloxavir acid susceptibility harbored a single isoleucine to threonine substitution at position 38 in the PA protein (PA/I38T). To evaluate the impact of the single PA/I38T substitution on the viral fitness, we performed a viral competitive fitness assay in Madin-Darby canine kidney (MDCK) cells and primary human airway epithelium cells (MucilAir).

### Methods

Viral competitive fitness assays were performed with reverse genetics-derived influenza viruses or isolates derived from patients in BXM clinical study (CAPSTONE-1 study) in MDCK cells or MucilAir. The ratio of wildtype:mutant viruses was analyzed by Sanger sequencing or next-generation-sequencing.

### Results

In a viral competitive fitness assays with reverse genetics-derived viruses, H1N1, H3N2 and type B viruses with PA/I38T substitution became undetectable after 3 passages. In viral competitive fitness assays with viruses derived from patients in BXM clinical study in MucilAir, the ratio of viruses with PA/I38T substitution were also significantly diminished.

### Conclusion

The results of the viral competitive fitness assays suggest that the replicative capacity of viruses with PA/I38T substitution was impaired compared to the wild-type in MDCK and primary human airway epithelium cells.

*Keywords: Baloxavir, influenza virus, PA/I38T, competitive fitness, MucilAir*



Topic: Virology and Pathogenesis: Viral Replication

Abstract No: 11301

## Low polymerase activity attributed to PA drives the acquisition of the PB2 E627K mutation of H7N9 avian influenza virus in mammals

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**Introduction and objectives:** Avian influenza viruses (AIVs) must acquire mammalian-adaptive mutations before they can efficiently replicate in and transmit among humans. The PB2 E627K mutation is known to play a prominent role in the mammalian adaptation of AIVs. The H7N9 AIVs emerged in 2013 in China easily acquired the PB2 E627K mutation upon replication in humans. However, the underlying mechanism for its emergence has not been resolved.

**Methods:** Here, we generated a series of reassortant or mutant viruses in the backbone of an H7N9 AIV, harboring genes or mutations from an H9N2 AIV, and monitored the residue phenotype of PB2 627 during viral passages in mice.

**Results and conclusion:** We show that the low polymerase activity attributed to the viral PA protein is the intrinsic driving force behind the emergence of PB2 E627K during H7N9 AIV replication in mice. Four residues in the N-terminal of PA are critical in mediating the PB2 E627K acquisition. Notably, due to the identity of viral PA protein, the polymerase activity and growth of H7N9 AIV are highly sensitive to changes in expression levels of human ANP32A protein. Furthermore, the impaired viral polymerase activity of H7N9 AIV caused by the depletion of ANP32A led to reduced virus replication in *Anp32a*<sup>-/-</sup> mice, abolishing the acquisition of PB2 E627K mutation and instead driving the virus to acquire the alternative PB2 D701N mutation. Taken together, our findings show that the emergence of the PB2 E627K mutation of H7N9 AIV is driven by the intrinsic low polymerase activity conferred by the viral PA protein, which also involves the engagement of mammalian ANP32A.

**Keywords:** H7N9; Avian influenza virus; PB2 E627K mutation; viral PA protein; ANP32A

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 11320

## Compatibility among viral ribonucleoprotein (vRNP) genes of Influenza A viruses are limited, but not random.

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**Introduction and Objectives:** The composition of viral ribonucleoprotein (vRNP) complex affects influenza A virus (IAV) reassortment biases and viral replication efficiency but the impacts on host tropisms remain to be fully understood. A systematic analysis of the compatibility among vRNP complexes of contemporary IAVs is still lacking. This study aims to assess the genetic compatibility of contemporary IAVs and further develop and validate a computational model to predict genetic compatibility given vRNP sequences alone.

**Methods:** Through genotypic analyses, a set of vRNP were selected to cover representative genetic lineages of each vRNP gene from contemporary epidemic/enzootic human (H1N1, H3N2), avian (H4N6, H5Nx, H7N9, H7N2, H9N2, and H10N8), swine (H4N6 and H6N6) and canine (H3N2 and H3N8) IAVs. The replication efficiency of ~4,000 combinations of vRNPs are quantified using plasmid-based, minigenome assay, and machine learning is applied to these data to identify key residues for determining compatibility among vRNP.

**Results:** Among these testing combinations, only ~12% showed increased replication efficiency; whereas, the majority of the combinations resulted in decreased replication efficiency. A computational model is then developed to predict replication efficiency of a vRNP given their genotype (i.e., protein sequence). Whereas, the genetic origin of each vRNP segment affects the compatibility of these vRNP genes, mutations acquired during host adaptation can significantly alter the compatibility among vRNP genes. For example, vRNP from avian H4N6 IAV is compatible with those from avian origin canine H3N2 IAV rather than equine origin canine H3N8 IAV. The vRNP from two different human seasonal H3N2 IAVs have different compatibility with those from another IAVs.

**Conclusions:** In summary, genetic compatibility among vRNP segments seems to be limited but not random, and the mutations in vRNP genes acquired during host adaptation can alter compatibility of vRNP genes. The computational model derived from this study can facilitate risk assessment of IAVs.

*Keywords: influenza; ribonucleoprotein; vRNP; reassortment; compatibility;*

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 11338

## THE BROAD ANTIVIRAL POTENTIAL OF MEK INHIBITOR PD0184264 IN PRECLINICAL STUDIES AGAINST INFLUENZA VIRUSES COMPARE TO STANDARD OF CARE

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### Introduction

Influenza viruses (IV) infection is a public health concern worldwide. Currently, all available vaccines as well as antiviral drugs that target the virus itself are prone to resistance. It is proven that influenza viruses able to modulate and control cellular pathways involved in the viral life cycle like Raf/MEK/ERK signal pathway which the nuclear export of vRNPs is strongly dependent on the virus-induced activation.

### Objectives

Along this line, we demonstrated the antiviral potential of MEK inhibitor PD0184264 (ATR002), the active metabolite of CI-1040 against influenza viruses over *in vitro* and *in vivo* levels.

### Results and Conclusion

We were able to show that IC<sub>50</sub> value against different IV strains is about 0.6841 µM. Moreover, the current data revealed that ATR002 is also effective against Tamiflu resistant strains. Using a mouse model, we demonstrate that ATR002 can reduce IV lung titers *in vivo* and is also effective even after 72 h post infection when Tamiflu has no effect. Furthermore, superior antiviral activity has also been found upon a comparison between the MEK inhibitor and the newly licensed antiviral drug (Baloxavir). In conclusion, the available data showed that the MEK inhibitor (ATR002) offers an interesting perspective for anti-IV approach in comparison with the available antiviral drugs e.g. Tamiflu. Currently, this MEK inhibitor is directed to further investigations in the direction of clinical trials due to its potential.

Topic: Virology and Pathogenesis: Viral Replication  
Abstract No: 11774

## FISH AND SINGLE-MOLECULE LOCALIZATION MICROSCOPY AS A TOOL TO STUDY INFLUENZA VIRUS TRANSCRIPTION AND REPLICATION

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The genome of influenza comprises eight RNA segments. Each viral segment forms a ribonucleoprotein complex (vRNP) with the viral RNA polymerase (RNAP) and nucleoprotein (NP). After being released from virus particles in the cytoplasm, negative-sense vRNPs are transported into the nucleus, where they perform both transcription and a two-step replication process. During replication, negative-sense vRNPs are copied into structurally analogous positive-sense cRNPs that serve as templates for newly synthesized vRNPs.

Studies combining data from X-ray crystallography and cryo-electron microscopy have revealed important insights into the structure of RNPs and their function in transcription and replication. However, structural studies of transcribing and replicating RNPs are unable to reliably identify and localize key features of an RNP, such as the position of the polymerase or of a specific sequence position of the viral genome. As a result, many open questions on the mechanism of transcription and replication remain.

Here, we are reporting the use of fluorescence-in-situ hybridization (FISH) to obtain high-resolution information about the location of specific RNA sequences in vRNPs, and elucidate the molecular mechanisms of Influenza transcription and replication. We demonstrate that individual FISH probes bind to single RNPs with a high efficiency and specificity, and we use it to detect individual virus particles and isolated RNPs by the co-localization of only two spectrally distinct FISH probes. Moreover, the high efficiency of the hybridization enabled us to visualize the outline of RNPs by combining FISH with stochastic optical reconstruction microscopy (STORM).

By single-molecule localization (SMLM) of FISH probes on the outline of transcribing and replicating RNPs, we are currently visualizing the relative positions of specific target sequences on a RNP segment and test for conformational changes during these processes. We are also planning to specifically label RNAP and localize it by SMLM on the fluorescent RNAP outline. Our methods are general and should be useful to understand other processes of the viral life cycle.

*Keywords: single molecule; super resolution; replication; FISH; RNP*

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission  
Abstract No: 10424

## A STABILIZED HA PROTEIN INCREASES A/H1N1(2009) PATHOGENESIS IN MICE BY DAMPENING TYPE I INTERFERON RESPONSES IN DENDRITIC CELLS

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### INTRODUCTION AND OBJECTIVES:

HA stability has been linked to influenza A virus transmissibility in humans and ferrets. To study the impact of HA stability on pathogenicity, we infected DBA/2 mice with A/H1N1/2009 WT (pH 5.5), Y17H (pH 6.0), and R106K (pH 5.3).

### METHODS AND RESULTS:

Growth curves were performed using MDCK, A549, LA-4, mNEC, mTEC, and Raw264.7 cells. Mouse lung pH was measured with a micro-pH sensor. Environmental stability was measured at pH 6.4 and 7.0. MID50, MLD50, viral titers in lungs, weight loss, and survival were measured in DBA/2 mice. Histopathology, cellular infiltration, cytokine, and chemokine expression were measured in DBA/2 mice. Differential RNA transcription was measured by Affymetrix. STAT1 phosphorylation was measured by Western blot. Bone-marrow derived macrophages and dendritic cells were used to compare cytokine/chemokine induction and the kinetics of viral infection.

### RESULTS:

The MID50 of R106K was 7-times smaller than WT, but the mutant yielded similar lung titers, weight loss, and mortality. The destabilized Y17H had MID50 and MLD50 values 10- and 34-fold higher than WT, respectively. Lung pH in DBA/2 mice was 7.0-7.5. At pH 7.0, Y17H and WT had similar environmental stabilities. Y17H had growth curves similar to WT (MDCK and LA-4), higher than WT (Raw264.7 and A549), and lower than WT (mNEC and mTEC). Y17H lung titers, infiltration, and host responses could be normalized to WT with a 500-fold higher dose. In vivo and in vitro (DC) experiments showed the destabilized Y17H had increased early infection and enhanced type I interferon responses, which led to attenuation.

### CONCLUSIONS:

HA destabilization that enhances early infection in DCs and stimulates greater interferon responses causes attenuation in mice.

*Keywords: H1N1; virulence; HA stability; mouse model*

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission  
Abstract No: 10472

## Mutation NP-Q357K in Eurasian H1N1 Swine Influenza Viruses Determines the Virulence Phenotype in Mice

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### Introduction and Objectives

Two main genotypes EA H1N1 viruses, JS1-like and HuN-like, have been recognized to be infected humans in China. Our study finds that the JS1-like virus is avirulent in mice and the HuN-like virus is virulent.

### Methods

Contributions of each gene segments or candidate substitutions to the pathogenesis of EA H1N1 viruses was studied by using reverse genetic methods in mice models.

### Results and Conclusion

The NP gene determines the virulence of the EA H1N1 viruses in mice. A single substitution, Q357K, in the NP protein of the EA H1N1 viruses alters the virulence phenotype. The NP-Q357K substitution was supposed to be readily occurred when avian influenza viruses circulate in pigs, and may facilitate the mutants to circulate in humans. Our study demonstrates that the substitution NP-Q357K plays a key role in the virulence phenotype of EA H1N1 SIVs, and provides important information for evaluating the pandemic risk of field influenza strains.

*Keywords: Eurasian avian-like H1N1 swine influenza viruses; NP gene; NP-Q357K; Pathogenicity; Pandemic potential*

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission  
Abstract No: 10483

## Nanopore sequencing of novel avian influenza viruses in wild birds and poultry in Ukraine: localized reassortment with HPAIV strains from migratory birds

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**Introduction & Objectives:** Avian influenza viruses can cause zoonotic disease, and highly pathogenic avian influenza viruses (HPAIV) can cause mortality in wild birds and poultry. In 2014, HPAIV of subtype H5N8 (goose/Guangdong lineage clade 2.3.4.4) spread across Eurasia and into North America. Interestingly, this HPAIV subtype reassorted with local low pathogenic (LPAIV) strains in local settings in wild birds and poultry.

**Methods:** In order to better understand the AIV genotypes circulating in Eurasia, we adopted nanopore (MinION) sequencing technology for whole genome sequencing of viruses from wild birds and domestic poultry, with a focus on samples collected in migratory wetlands and nearby poultry in Ukraine. A total of 13 strains were directly sequenced from clinical tissue samples, whereas 16 were amplified *in ovo* before RNA extraction. After multi-segment RT-PCR to amplify the genome, cDNA were sequenced on a MinION Mk1B device using a ligation sequencing protocol.

**Results & Conclusions:** By using an iterative reference-based approach for genome assembly, 10 AIV were identified as HPAI H5N8 (clade 2.3.4.4B), 2 were locally reassorted H5N5; one LPAIV H5 and three H7 reassortants were also sequenced. Reassortment occurred in wild mute swans, wild mallard ducks, and domestic chickens. Long read nanopore sequencing also mapped full-length defective interfering (D.I.) RNA, an immunosimulatory influenza replication product, in clinical samples of HPAIV infection of wild mute swans. Additionally, a 18-amino acid NA stalk deletion was discovered in H5N8 from chickens, a mutation that increases adaptability and virulence of H5 viruses. Rapid nanopore sequencing of avian influenza illustrates the diversity of pathogenic and reassortant subtypes in the poorly understood ecology of wetlands in Ukraine.

**Keywords:** H5N8; HPAI; defective interfering RNA; nanopore sequencing; MinION

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission  
 Abstract No: 10663

## Multiple amino acid substitutions of hemmagglutinin are determinants that enhanced virulence in H3N2 mouse-adapted virus

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Limited availability of seasonal influenza virus H3N2 with sufficient virulence in mice hampered further investigation of corresponding virus pathology in vivo. To circumvent the limitation, seasonal H3N2 influenza virus (A/Switzerland/9715293/2013, SW) were serially passaged in mice and enhanced virulence strains were obtained. As a result of genetic analysis of the mouse-adapted H3N2 virus (MA-SW), amino acid substitutions were verified in five (PB2, PA, HA, NP and NA) major genome segments compared to the wild type virus. Pathogenicity experiments were conducted to find out the viral characteristics in mammals against HA and PB2 mutations associated with receptor binding and virulence, respectively. Using reverse genetic system, reassorted viruses containing the HA and PB2 of MA-SW with wild type SW virus as a backbone were produced respectively. To evaluate pathogenicity, the mouse 50% lethal dose (MLD<sub>50</sub>) were determined in Balb/c mice intranasally inoculated with serial dilution of the virus by monitoring clinical signs and loss of body weight for 14 days. Six mice of each group were inoculated intranasally with 10<sup>5</sup> TCID<sub>50</sub> of virus and organs of three mice were collected at 3 and 6 days post-infection. The MLD<sub>50</sub> for the HA mutant virus was calculated to be 10<sup>5.1</sup> TCID<sub>50</sub>/ml, and 10<sup>2.7</sup> to 10<sup>4.3</sup> TCID<sub>50</sub>/ml titer of the virus was detected in respiratory organs. In contrast, the group infected with PB2 mutant virus had no specific clinical symptoms and showed a viral titer of 10<sup>0.8</sup> to 10<sup>1.5</sup> TCID<sub>50</sub>/ml in respiratory organs, which is lower than that of HA mutant group. These results suggest that assorted HA mutation were significantly involved in functional alteration which might enhance virulence of the virus in mice. This study was supported by the intramural research program of the Korea Centers for Disease Control and Prevention (2017-NI43001-00).

*Keywords: Influenza virus, Mouse adaptation, Pathogenesis, Mutation*



Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission  
Abstract No: 10842

### Clade 2.3.4.4 H5 highly pathogenic avian influenza viruses showed various pathogenicity in domestic ducks

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[Introduction and Objectives] Since 2013, highly pathogenic avian influenza (HPAI) outbreaks caused by clade 2.3.4.4 (C2.3.4.4) H5 viruses have reported worldwide. We have isolated many C2.3.4.4 HPAI viruses (HPAIVs) from domestic ducks in wet markets in Vietnam. In Japan, similar HPAIVs were firstly detected in duck farms in 2016-17 winter. In this study, such C2.3.4.4 HPAIVs were examined for the pathogenicity in ducks to assess whether their phenotypes accelerate viral dissemination.

[Methods] Thirteen C1, 2.3.2.1, 2.3.4 or 2.3.4.4 HPAIVs were used.  $10^6$  EID<sub>50</sub>/100 uL of each virus was intranasally inoculated into 1-week-old domestic ducks. The birds were observed and checked for their viral titers in oral/cloacal swabs and tissues.

[Results] All the ducks inoculated with C1, 2.3.2.1 or 2.3.4 H5N1 HPAIVs died at 2-3 d.p.i. The ducks with Vietnamese C2.3.4.4 H5N1/N6 HPAIVs isolated in 2013-14 died at 5-8 d.p.i. In these birds, the viral titers in the swabs near the time of death were lower than those in acute phase. Vietnamese H5N6 HPAIVs in 2015 took only 4 days to kill the ducks. Japanese C2.3.4.4 H5N8 HPAIVs in 2014 and one H5N6 in 2017 caused inapparent infection, while the other H5N6 in 2016 showed high mortality. Throughout the study, viruses were recovered from the systemic tissues of the dead birds. As the birds took longer duration to death, their titers were relatively lower.

[Conclusion] The diversity of the pathogenesis was observed among C2.3.4.4 HPAIVs. The results inferred that the pathogenicity of the epidemic HPAIVs in domestic ducks in East Asian countries was temporarily decreased according to the shifts of circulating viruses, C2.3.2.1 to 2.3.4.4. Afterwards, such C2.3.4.4 HPAIVs may have acquired higher pathogenicity by perpetuating in poultry populations. It is suggested that the viral replicability in duck species is one of the key roles for the dissemination of epidemic strains.

*Keywords: duck;pathogenicity;H5*

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission  
Abstract No: 10857

## BALOXAVIR REDUCES RESPIRATORY DROPLET TRANSMISSION OF INFLUENZA A VIRUS IN FERRETS

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### INTRODUCTION

Baloxavir acid (BXA), the active form of baloxavir marboxil (Xofluza™), is a novel inhibitor of influenza cap-dependent endonuclease. BXA treatment results in a fast reduction in influenza titer, suggesting a beneficial effect on reducing transmission of the virus in a population. We investigated the effect of BXA treatment on influenza A virus transmission between ferrets.

### METHODS

Twelve donor ferrets were inoculated intranasally with pandemic H1N1 (2009) virus. On day 1 post infection, BXA was administered to 4 donor ferrets via subcutaneous injection. Oseltamivir phosphate (OS) was orally administered to a comparator group, and a third group was untreated. Each donor was co-housed with one naïve direct contact sentinel ferret, and one respiratory droplet exposed sentinel ferret was housed in the adjacent cage from day 1 to day 4 post infection. The virus titre and amount of viral RNA in the nasal washes, weight loss, and body temperature change were assessed daily during infection.

### RESULTS

BXA treated donors shed lower infectious virus in nasal washes, with lower area under curve compared to OS and untreated donors ( $P = 0.012$ ). BXA treated donors transmitted to 1/4 respiratory droplet sentinels, as compared with 3/4 for OS and 4/4 for untreated. BXA treatment did not prevent direct contact transmission, however the mean time to first positive nasal wash in the BXA and OS sentinel animals was delayed by 4 days compared to the untreated control group. BXA and OS treated donors showed less weight loss and reduced fever compared to untreated donors.

### CONCLUSIONS

A single administration of BXA was superior to both OS and no treatment in reducing viral load and respiratory droplet transmission of influenza virus.

*Keywords: Transmission; Resistance; Antiviral;*

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission  
Abstract No: 10905

## BALOXAVIR REDUCES TRANSMISSION OF INFLUENZA VIRUS BY DIRECT CONTACT IN FERRETS

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### Introduction and Objectives:

Using a ferret model of influenza virus infection and transmission, we investigated the effect of baloxavir acid (BXA), the active form of baloxavir marboxil, on viral shedding and transmission of virus to naïve co-housed ferrets.

### Method:

'Donor' ferrets infected with influenza A(H1N1)pdm09 virus were treated with BXA, oseltamivir or placebo before co-housing at a 1:1 ratio with naïve 'recipient' ferrets (n=4 pairs/group). The effects of altering the time of antiviral treatment and co-housing were evaluated in different experiments. Nasal washes were collected daily for virological analysis, where infectious virus in recipient ferrets was considered the primary indicator of successful viral transmission.

### Results:

Following treatment of donor ferrets at 24 hours post-infection, only BXA-treated animals cleared the virus within 72-96 hours post-infection. BXA treatment reduced transmission such that only one naïve co-housed ferret became infected, compared to oseltamivir- or placebo-treatment where all four recipient ferrets became infected. Even when treatment was delayed until 48 hours post-infection, BXA treatment of donor ferrets resulted in the clearance of virus within 72-96 hours post-infection, while all oseltamivir- and placebo-treated ferrets continued to shed virus. BXA treatment at this delayed time-point resulted in two naïve co-housed ferrets becoming infected, compared to all four recipient ferrets exposed to oseltamivir- or placebo-treated donors. Co-housing ferrets immediately following treatment of the donor ferret, or delaying by 24 hours had no impact on transmission frequency. Viruses with reduced BXA-susceptibility did not emerge, although donor ferrets were not sampled beyond 2-3 days post-treatment. Similar experiments with influenza A(H3N2) and B/Yamagata viruses are ongoing.

### Conclusion:

Baloxavir treatment at 24 or 48 hours post-infection significantly reduced the duration of viral shedding in treated ferrets and reduced transmission of virus to co-housed ferrets by 75% and 50% respectively, compared to both oseltamivir and placebo.

*Keywords: baloxavir, antiviral, cap-dependent endonuclease inhibitor, influenza, transmission, ferret*

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission  
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## The marmoset as an animal model of influenza

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**Introduction and Objectives:** To control infectious diseases in humans, it is important to understand the pathogenicity of the infecting organism(s). Although nonhuman primates, such as cynomolgus and rhesus macaques, have been used as influenza virus infection models, their size can limit their use in confined animal facilities. In this study, we investigated the susceptibility of marmosets to influenza viruses to assess the possibility of using these animals as a nonhuman primate model for influenza research.

**Methods:** Marmosets were inoculated with an influenza A (H1N1)pdm09 virus to compare two inoculation routes: the conventional route, via a combination of the intratracheal, intranasal, ocular, and oral routes; and the tracheal spray route. We also inoculated some marmosets with an influenza A(H5N1) highly pathogenic avian influenza (HPAI) virus via the tracheal spray route.

**Results:** In marmosets inoculated via the tracheal spray route, we found inflammation throughout the lungs and trachea. In contrast, in marmosets inoculated via the conventional route, the inflammation was confined to roughly the center of the lung. These data suggest that the tracheal spray route may be more suitable than the conventional route to inoculate marmosets with influenza viruses. We also found that some marmosets inoculated with HPAI H5N1 virus via the tracheal spray route showed weight loss, decreased body temperature, and loss of appetite and activity; H5N1 virus replication in their respiratory organs was confirmed.

**Conclusion:** Our findings indicate that marmosets have potential as an animal model for infection with seasonal or HPAI viruses.

*Keywords: animal model; marmoset; non-human primate; A(H5N1)*

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## MODEL FOR COMPARISING THE TRANSMISSIBILITY OF INFLUENZA USING DATA FROM FERRET EXPERIMENTS

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### Introduction

We present a mathematical model to designed to quantitatively investigate the difference in influenza transmissibility between different transmission mechanisms and strains using data from transmission chain experiments in ferrets.

### Method

We fit a model to the viral shedding data of an infected donor individual. We then assume that the hazard of transmission from an infected individual to a naïve individual is a function of the donor viral shedding, with different hazards for direct contact and respiratory droplet transmission. Each hazard has a corresponding parameter; we can compare these two parameter values to investigate if the data indicate differences in transmissibility between the two transmission routes.

### Results

We have an expression for the likelihood of the parameters giving rise to the data in our model framework. We have tested the model by recovering known parameter estimates from simulated data. Initial analyses of experimental data suggest strain-specific differences in transmission mechanisms.

### Conclusion

Mathematical models can help extract additional information from high-value laboratory transmission models.

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## THE MECHANISMS OF HEMAGGLUTININ ADAPTATION IN THE EMERGENCE OF THE 1968 H3N2 PANDEMIC VIRUS

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### Introduction

The 1968 H3N2 pandemic was the most recent pandemic resulting from adaptation of an avian influenza virus to humans. The H3 hemagglutinin (HA) of the emerged virus differs from the putative avian precursor (AP) by seven substitutions. Q226L and G228S are known for switching the receptor preference from avian-like to human-like binding. The role of the remaining five substitutions, R62I, D81K, N92K, A144G, N193S, is unclear.

### Methods

We rescued the pandemic virus A/Aichi/2/1968 (Aichi68), and a precursor with seven segments of Aichi68 and the AP HA, and also two putative intermediates S2 (Q226L and G228S) and S5 (5 compensatory mutations). Each virus was assayed for replication in primary cultures of well differentiated human airway epithelium (HAE), HA acid stability, and transmission in the ferret model.

### Results

In HAE culture, the virus with AP HA had reduced growth at 33°C compared to Aichi68. The putative intermediate viruses S2 and S5 grew to significantly lower titres at early time points (8 and 24 h.p.i.), compared to Aichi68 and AP viruses suggesting a fitness trough for evolution from AP to Aichi68. The HAs of Aichi68, AP and S5 had similar acid stability, while S2 was more sensitive to low-pH treatment, indicating that Q226L and G228S substitutions concomitantly decrease acid stability, but that compensatory mutations can alleviate this deficit. The order dependency of HA substitutions, their role in within host fitness vs transmissibility and the stringency of the transmission bottleneck will be elucidated by deep sequencing viruses from a chain of transmission in ferrets.

### Conclusion

Multiple compensatory HA mutations were required to adapt the AP virus for human-to-human transmissibility. This included changes in both receptor specificity as well as acid stability. Each change alone incurred a fitness cost to virus. The circumstances by which all necessary changes are achieved remain unclear.

*Keywords: Hemagglutinin; Transmission; H3N2*

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## PATHOGENICITY OF CLADE 2.3.4.4B H5N6 HIGHLY PATHOGENIC AVIAN INFLUENZA IN SOUTH KOREA

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### Introduction and Objectives

South Korea suffered from highly pathogenic avian influenza outbreak in November 2017 due to a novel H5N6 virus, classified into clade 2.3.4.4B. In this study, we evaluated the pathogenicity and transmissibility of the H5N6 virus in SPF chickens and ducks.

### Methods

A/duck/Korea/HD1/2017(H5N6) (HD1) was propagated in SPF chicken eggs. The intravenous pathogenicity index (IVPI) was decided based on the OIE regulation. To evaluate the pathogenicity, groups of five-week-old SPF chickens and two-week-old ducks were intranasally inoculated with serially diluted HD1. After eight hours, naïve birds were co-housed with the group inoculated at the titer of  $10^6$  EID<sub>50</sub>/100 $\mu$ l. While the birds were observed for two weeks, the tissue and swab samples were taken for DF-1 cell culture. Blood samples were taken at 14 dpi for HI test.

### Results

The IVPI and mean lethal dose (LD<sub>50</sub>) of HD1 were measured 2.98 and  $10^{3.4}$  EID<sub>50</sub>, respectively. When challenged at the titer of  $10^6$  EID<sub>50</sub>, the mean death times (MDTs) of inoculated and contact SPF chickens were 2.2 and 7 days, respectively. A higher titer of HD1 was recovered from oropharyngeal swabs than cloacal ones. In the experiment conducted at ducks, no deaths of clinical signs were observed. However, HD1 efficiently transmitted from infected to naïve ducks by direct contact, leading to sero-conversion of the contact ones. Virus shedding was much higher and prolonged in ducks than in SPF chickens. A significantly lower titer of HD1 was detected in certain tissues of ducks, whilst it persisted in all tissues of SPF chickens in high titers.

### Conclusion

Our data indicated that HD1 is highly virulent and transmissible in SPF chickens. Meanwhile, HD1 could play a role in transmission in ducks without exhibiting any clinical symptoms.

*Keywords: H5N6;HPAI;Pathogenicity;Transmission*

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## VIRAL FACTORS IMPORTANT FOR THE NEUROTROPISM OF INFLUENZA A VIRUSES

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### Introduction and Objectives

Central nervous system (CNS) disease is the most common extra-respiratory tract complication of influenza A virus infections. Even though the ability to cause CNS disease in mammals varies between strains, it is currently poorly understood which viral factors are important for the ability to spread to and replicate in the CNS. Furthermore, it is unknown whether adaption occurs during CNS invasion in vivo. Therefore, we investigated which viral factors— intrinsic or acquired—are important for the neurotropic potential of influenza viruses.

### Methods

We determined the replication kinetics of different influenza virus strains in CNS cells in vitro. Furthermore, viruses isolated from the respiratory tract and CNS of H5N1 virus infected ferrets were compared, and phenotypic changes associated with the amino acid changes were determined.

### Results

In vitro, highly pathogenic H5N1 virus replicated more efficiently in CNS cells than H3N2 and pandemic H1N1 viruses, which was associated with efficient attachment and infection. Removal of the multi-basic cleavage site (MBCS) attenuated, but did not abrogate, replication. In vivo, H5N1 viruses isolated from the cerebellum and CSF acquired two mutations in PB1 and one in NP, which were not present in the respiratory tract. These mutations resulted in a cell specific increase in polymerase activity (3-8 fold) and replication efficiency.

### Conclusion

This study shows that the presence of a MBCS and efficient attachment and infection contribute to the ability to replicate in CNS cells. In addition, viruses isolated from the CNS were phenotypically different than viruses isolated from the respiratory tract. This suggests that within-host adaptation is important for the ability to spread to, replicate, and disseminate in the CNS, which is currently further investigated in vivo. Combined, these data show that multiple viral proteins, such as hemagglutinin and the polymerase complex contribute to the neurotropic potential of influenza viruses.

*Keywords: pathogenesis; CNS disease; neurotropism; H5N1 virus; adaptation*



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## Favipiravir-resistant virus shows potential for transmission

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### Introduction and Objectives

Favipiravir is a nucleoside analog which has been licensed to treat influenza in the event of a new pandemic. Our previous work has shown that two mutations combined to give influenza virus resistance to favipiravir in vitro. The first mutation, K229R in PB1, gave resistance to favipiravir at a cost to polymerase activity and the second mutation, P653L in PA, compensated for the cost of polymerase activity. However, the clinical relevance of these mutations is unclear: the mutations have not been found in sequenced isolates and it is unknown whether these mutations will transmit in a more complex environment found in vivo. Here, we tested whether favipiravir-resistant virus would transmit between ferrets in the absence of drug whilst maintaining drug resistance.

### Methods

A transmission study was performed using A/England/195/2009, a virus from the first wave of the 2009 A(H1N1) pandemic. Donor ferrets were infected with a mix of 5% wildtype virus and 95% favipiravir-resistant virus containing two mutations, K229R in PB1 and P653L in PA. Sentinel ferrets were used to measure contact transmission and respiratory droplet transmission. Viral titers were measured from ferret nasal wash samples and RNA was extracted and used for sequencing the population of virus.

### Results and Conclusion

Favipiravir-resistant virus successfully infected ferrets. In the absence of drug, favipiravir-resistant virus transmitted by contact (4/4 ferrets) and by respiratory droplet (3/4 ferrets) whilst maintaining resistance. Sequencing revealed that the K229R mutation, which gives resistance, decreased in frequency within some ferrets. Modelling revealed an unexpected fitness advantage for the P653L mutant in the absence of K229R, which caused the loss of K229R following reassortment. Therefore, whilst favipiravir-resistant virus can transmit in vivo, resistance may be lost in the absence of drug pressure.

*Keywords: Favipiravir; T705; Resistance; Transmission; Polymerase*

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission  
Abstract No: 11133

## FUNDAMENTAL CONTRIBUTION AND HOST RANGE DETERMINATION OF ANP32A AND ANP32B IN INFLUENZA A VIRUS POLYMERASE ACTIVITY

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**Introduction and Objectives:** The polymerase of the influenza A virus is part of the key machinery necessary for viral replication. Although the cellular protein ANP32A has been found to influence polymerase activity and interspecies restriction, the key mechanisms that determine viral polymerase activity and host range are poorly understood.

**Methods:** CRISPR/Cas9 system was used to establish a series of knockout and site-directed amino-acid-substituted 293T cell lines, and a minireplicon assay to determine the influenza viral polymerase activity in these cell lines. We tested the influenza virus production and infectivity in knockout cells by ELISA and TCID50. Then we used site-mutagenesis and co-immunoprecipitation to confirm the key sites of host factors.

**Results:** We found that either human ANP32A or ANP32B is indispensable for human influenza A virus RNA replication. The contribution of huANP32B is equal to that of huANP32A, and together they play a fundamental role in the activity of human influenza A virus polymerase, while neither human ANP32A nor ANP32B support the activity of avian viral polymerase. Two amino acid mutations at sites 129-130 in chicken ANP32B lead to the loss of support of viral replication and weak interaction with the viral polymerase complex, and these amino acids are also crucial in the maintenance of viral polymerase activity in other ANP32 proteins.

**Conclusion:** Here we provide evidence that ANP32A and ANP32B from different species are powerful factors in the maintenance of viral polymerase activity. Human ANP32A and ANP32B contribute equally to support human influenza virus RNA replication. However, unlike avian ANP32A, the avian ANP32B is evolutionarily non-functional in supporting viral replication because of a 129-130 site mutation. The 129-130 site plays an important role in ANP32A/B and viral polymerase interaction, therefore determine viral replication, suggesting a novel interface as a potential target for the development of anti-influenza strategies.

**Keywords:** ANP32A; ANP32B; influenza A virus; polymerase activity; interspecies transmission

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission

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## H6 CHICKEN INFLUENZA VIRUS RECOGNIZES SULFATED ALPHA2,3 SIALYLATED GLYCANS AS THE RECEPTORS

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### Introduction and objectives

Avian influenza viruses (AIVs) recognize sialic acid linked  $\alpha$ 2,3 to galactose (SA $\alpha$ 2,3Gal) glycans as receptors. In our previous study, fucosylated SA $\alpha$ 2,3Gal glycans were detected in chicken trachea, and these glycans were critical for recognition by an H5 low pathogenic AIV isolated from chicken. In the present study, the interactions between hemagglutinins (HAs) of AIVs and other modification of glycans, sulfated SA $\alpha$ 2,3Gal glycans, were analyzed in order to clarify the molecular basis of interspecies transmission of AIVs to chickens.

### Methods

Soluble recombinant HA proteins were prepared from duck influenza viruses, A/duck/Hong Kong/960/1980 (H6N2) and chicken influenza virus, A/chicken/Tainan/V156/1999 (H6N2). Binding specificity of these HAs and their mutants were analyzed by binding assays. To analyze virus growth in chickens, the duck virus and its mutants were prepared by reverse genetics and inoculated to chickens intranasally. After the inoculation of the viruses, virus titer in nasal and cloacal swabs were determined.

### Results

In these binding assays, an H6 chicken virus isolate, A/chicken/Tainan/V156/1999 (H6N2), bound to sulfated SA $\alpha$ 2,3Gal glycans, whereas an H6 duck virus isolate, A/duck/Hong Kong/960/1980 (H6N2), did not. Binding preference of recombinant HAs and recombinant duck viruses revealed that an E190V substitution is induced for the binding of sulfated SA $\alpha$ 2,3Gal glycans. Furthermore, A/duck/Hong Kong/960/1980 (H6N2) did not replicate in chickens, however, E190V substitution in the HA increased the infectivity of the virus in chickens, speculating that the virus recognized sulfated SA $\alpha$ 2,3Gal glycans expressed on chicken trachea.

### Conclusions

The binding of HA from H6 AIV to sulfated SA $\alpha$ 2,3Gal glycans contributed to virus growth in chickens, and E190V mutation in the HA was critical for the recognition to the glycans.

*Keywords: Hemagglutinin, Receptor, H6, avian, poultry*

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## Human Airway Organoids as a Platform for Influenza Pandemic Risk Assessment

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### Introduction

The active surveillance program carried out worldwide identifies influenza A viruses circulating in birds and other animals. Risk assessing these viruses for zoonotic and pandemic threat therefore remains a crucial next step. *Ex-vivo* human bronchus remains the gold standard for risk assessment of virus tropism and replication in conducting airways. However, they have limited availability of tissues, donor-to-donor variability and remain viable for only 3 days. Human airway organoids (AOs) retained morphological characteristics comparable to human airways overcome the limitations of *ex-vivo* bronchus cultures as novel platform for risk assessing influenza viruses.

### Methods

AOs were infected with influenza viruses having various transmissibility including pandemic H1N1 (pH1N1), H7N9, human H5N1 and human H5N6 viruses. The viral replication competence, and tissue tropism were monitored and the results were compared with human *ex-vivo* bronchus explants. We further assessed the virus replication of avian H5N6 and H5N8 viruses isolated from routine surveillance using AOs.

### Results

In both AOs and bronchus explants, influenza pH1N1 and H7N9 significantly replicated to higher titers than other subtypes that correlated well with their transmissibility. Tissue tropism of influenza was observed in ciliated and goblet cells. HPAI H5N1 induced higher levels of cytokines in AOs. Avian influenza viruses isolated from surveillance replicated to lower titers than pH1N1 suggesting that the H5Nx viruses tested have low transmissibility potential among human.

### Conclusion

Human AOs give results that correlate well with *ex-vivo* bronchus cultures for influenza A virus infection. Cellular tropism and cytokine induction can be monitored using AOs. Organoids from the same donor can be used repeatedly and can be efficiently retrieved from cryo-storage to generate adequate replicate cultures to provide statistical robustness. Therefore, human AOs would be an invaluable addition to risk assessment algorithms.

**Keywords:** Human Airway Organoids; H5Nx; Risk Assessment; Transmission; Cellular Tropism

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission  
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## PHENOTYPIC CHARACTERIZATION OF THE AIRBORNE TRANSMISSIBILITY OF AVIAN AND HUMAN INFLUENZA A H2N2 VIRUSES

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Influenza A viruses of the H2N2 subtype initiated a pandemic in 1957 and continued to circulate until 1968. A/H2N2 viruses emerged upon reassortment between a human A/H1N1 and avian influenza viruses. Avian A/H2N2 viruses are still circulating in wild birds worldwide and population immunity is low. As a result, A/H2N2 viruses can possibly be reintroduced into the human population. Here we assess the airborne transmissibility of old human and recent avian A/H2N2 viruses in the ferret model and determine the molecular and phenotypic basis of their transmissibility.

We assessed the airborne transmissibility of three human A/H2N2 viruses isolated in 1957, 1958 and 1968 and the contact and airborne transmissibility of six avian A/H2N2 viruses in the ferret model. Subsequently, acid- and temperature stability assays and modified red blood cell assays and glycan arrays were performed to study HA stability and receptor binding properties.

Only one of the three human viruses (1958) and none of the avian viruses was efficiently transmitted in the ferret model. Receptor binding studies showed obvious differences in binding patterns, with A/H2N2 from 1958 and 1968 displaying a human receptor binding preference. HA stability studies demonstrated that the 1958 H2 HA was more stable than HA from 1957 and 1968 strains.

Here we investigated phenotypic traits that help to fully understand the molecular basis of the emergence of the H2N2 pandemic. Our data show that H2N2 viruses early in the pandemic were not sufficiently adapted to humans for efficient airborne transmission and that both HA receptor binding preference and stability needed to change. These results will help to assess the risks and pandemic potential of currently circulating avian A/H2N2 viruses.

*Keywords: Transmission, H2N2, receptor binding*

Topic: Virology and Pathogenesis: Virus Pathogenesis & Transmission  
Abstract No: 11733

## PRIMARY CELL CO-CULTURES WILL CHANGE OUR UNDERSTANDING OF INFLUENZA VIRUS CELL BIOLOGY

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In recent years, more laboratories have begun using human lung cell lines or primary normal human bronchial epithelial cells when studying the cell biology of influenza virus infection. While a significant step forward from MDCK cells, these cells still do not recapitulate the complexity of *in vivo* epithelial cell infections. To overcome this barrier, we developed *in vitro* systems where primary human bronchial epithelial cells are co-cultured with primary human microvasculature or pulmonary endothelial cells, primary lung fibroblasts, or all three and allowed to differentiate and grow at the air-liquid interface. Epithelial cells co-cultured with endothelial cells or fibroblasts exhibited increased differentiation including tight junction formation and barrier permeability. Surprisingly, it also led to decreased viral replication and spread in the epithelial cells or complete viral clearance with co-cultured fibroblasts. This protection required direct cell-to-cell contact. Ongoing studies will define the cellular mechanisms for the decreased viral replication and determine the factors produced by fibroblasts and endothelial cells that protect infected epithelial cells. This work will not only lead to an improved understanding of the cell biology of influenza virus, but may result in new therapeutic targets.

Topic: Public Health: Disease Burden & Severity  
Abstract No: 10446

## THE EPIDEMIOLOGY AND BURDEN OF INFLUENZA B/VICTORIA AND B/YAMAGATA LINEAGES IN KENYA, 2012 – 2016

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**Introduction:** There is paucity of data describing the epidemiology and burden of influenza B in Sub-Saharan Africa.

**Methods:** We analyzed surveillance data from acute respiratory illness (ARI) associated hospitalizations and outpatient visits in 11 sites in Kenya. We compared the epidemiology and clinical features of influenza B/Victoria and B/Yamagata associated hospitalizations and outpatient visits, and estimated lineage and age-specific rates per person-years. Rates were adjusted based on ARI cases not tested for influenza and those whose influenza B lineage results were not available. We assessed matching of circulating influenza B lineages in Kenya with vaccine strain components by creating nine seasons corresponded to Northern Hemisphere (October to March), and Southern Hemisphere (April to September) vaccines.

**Results:** From 2012-2016, 24,268 patients (16,182 hospitalized and 8,086 outpatients) with ARI were enrolled and tested for influenza A and B viruses. Of these, 74% were children <5 years. There were 415 (3%) hospitalized patients, and 408 (5%) outpatients positive for influenza B. Overall, influenza B represented 31% of all influenza-associated ARI detected (annual range: 13% to 61%). Among hospitalized <5 years, influenza B/Victoria was more frequently associated with pneumonia, defined as cough or difficult breathing and tachypnea or chest in-drawing, or hypoxia, (64% [60/94] vs 44% [34/77], p=0.010) and in-hospital mortality (6% [6/94] vs 0% [0/77], p=0.042) compared to B/Yamagata. Rates of hospitalization and outpatient visits associated with influenza B were higher among those <5 years compared to those ≥5 years. The two lineages co-circulated in Kenya and there were mismatches with available trivalent influenza vaccines in 2/9 seasons assessed.

**Conclusions:** Influenza B contributes substantially to influenza morbidity in Kenya. In children <5 years, influenza B/Victoria may be associated with more severe disease compared to Yamagata. Our findings suggest a potential benefit of including both lineages when considering influenza vaccination in Kenya.

**Keywords:** Burden; hospitalization; incidence; influenza B; Kenya; lineage; Victoria; Yamagata

Topic: Public Health: Disease Burden & Severity  
Abstract No: 10570

## Influenza-associated effect on implementation of an emergency department length of stay policy in a state health system, New South Wales, Australia

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### Introduction and Objectives

Influenza outbreaks cause overcrowding in hospital emergency departments (ED). Using a state-wide electronic medical record database, we aimed to quantify the impact of influenza on the National Emergency Access Targets (NEAT) policy and on premature patient departures from ED in New South Wales, Australia.

### Methods

This was a retrospective observational study of around 11 million presentations to 115 hospitals during 2010 through 2014, using routinely collected administrative records. Time series methods typically used for estimating influenza burden were used in this policy evaluation across a state health system. A generalised additive regression model was used to assess the association between weekly influenza activity and the weekly proportion of all patients leaving the ED in >4 hours (NEAT policy target) and the proportion that departed before commencing or completing treatment ('did not wait'), after controlling for background winter and holiday effects.

### Results

During 2011-2014, peak annual circulating influenza was associated with the peak weekly proportion of all presentations that did not meet the NEAT policy target of being seen and treated within 4 hours. The maximum estimated absolute weekly change in that proportion was 3.88 (95% confidence interval 3.02-4.74) percentage points in 2014. For presentations that did not wait to be seen or treated, influenza circulation was associated with statistically significant increases in all years, with a maximum weekly value of 2.68 (95% confidence interval 2.31-3.06) percentage points in 2012.

### Conclusion

Increasing and sustained influenza circulation was associated with a higher and sustained proportion of total ED patients exceeding length of stay targets and of patients prematurely leaving the ED. Annual peak influenza circulation corresponded to annual peaks in patients exceeding length of stay targets. Monitoring of influenza surveillance information may assist in the development of health system, hospital and ED workforce planning and bed management strategies.



Topic: Public Health: Disease Burden & Severity  
Abstract No: 10586

## Overview of Severe Acute Respiratory Infections in the Country of Georgia, 2017-19

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### Introduction

Georgia has established sentinel-based surveillance system on Severe Acute Respiratory Infections (SARI) in 2011. It is used to identify influenza viruses and determine disease burden by routine collection of epidemiologic and laboratory data. Influenza virus subtypes and proportion of SARI cases among all hospitalizations varies from season to season, thus, it is crucial to analyze data continuously, especially due to low influenza vaccination coverage in Georgia.

### Methods

Total of five sentinel sites are established throughout Georgia. Physicians are using World Health Organization's SARI case definition (2014) for identification of patients. Nasal and throat swabs are collected and then tested at the National Center for Disease Control and Public Health / R. Lugar Center (NCDC) by using molecular methods for identification of influenza virus. Data has been extracted for two influenza seasons - 2017-18, and 2018-19.

### Results

During 2017-18, influenza virus type B virus was dominant, and accounted for a total 65.6% of all influenza confirmations, and influenza virus type A subtype H1p was found in 34.4%. A total of number confirmations during the season was 67. Peak percentage of SARI among all hospitalizations was seen during 1<sup>st</sup> week, with 19.9%. During 2018-19, A/H1p was found in 87.2% among all confirmations; A/H3 in 12.0%; and B in 0.8%. Influenza virus was confirmed in 753 cases. SARI peaked on 52<sup>nd</sup> week, at 41.6%.

### Conclusions

Influenza season of 2017-18 was considered as one of the mildest ones during previous years, while the following season had significant burden on public health in the country. Interestingly, there was no major difference in influenza vaccination coverage between the two seasons, however, as recent analysis suggests – the dominant strain during the season of 2018-19 was correctly included into the recommended vaccine composition. This highlights the need to improve vaccination policy and coverage in Georgia.

*Keywords: Influenza; SARI; Georgia.*

Topic: Public Health: Disease Burden & Severity  
 Abstract No: 10657

## Burden of influenza-associated severe acute respiratory infections in Vietnam, 2014-2016

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### Introduction

The annual circulation of influenza viruses causes substantial morbidity and mortality worldwide. To estimate the burden of influenza-associated severe acute respiratory infections (SARI) in Vietnam, we conducted a hospitalization admission survey (HAS) that leveraged the recent adoption of electronic medical records (EMR) in all Vietnamese public hospitals.

### Methods

We selected one province from each of the four regions of Vietnam with at least one SARI sentinel surveillance site conducting laboratory testing for influenza within January 2014–December 2016. From all public hospitals in selected provinces, hospitalization records with ICD-10 admission codes J06, J09-J18, and J20-J22 were collected as potential SARI admissions. To confirm these were SARI admissions, a random selection of 900 medical charts per province were reviewed to determine the proportion meeting the WHO SARI case definition of fever and cough onset within 10 days. Using the proportion of SARI cases that tested positive for influenza from sentinel surveillance and provincial census data, we estimated the rate of influenza-associated SARI hospitalizations and estimated 95% confidence intervals among patients in four age categories, <5, 5-40, 50-64 and ≥65 years.

### Results

Of 3,622 medical charts reviewed, 60% met the WHO SARI case definition. From sentinel surveillance, 20% of 6,647 SARI patients tested positive for influenza. We estimated an influenza-associated SARI hospitalization rate of 990/100,000 persons in 2014 (95% confidence interval [CI]: 828-1159), 1653/100,000 (95% CI: 1216-2081) in 2015, and 2067/100,000 (95% CI: 1803-2318) in 2016. Children <5 years had the highest rates of influenza-associated SARI (1207/100,000 [95% CI: 1001-1408]), followed by adults aged ≥65 years (233/100,000 population [95%CI: 174-292]).

### Conclusions

National adoption of EMRs simplified HAS methods and allowed influenza-associated SARI hospitalization rates to be estimated in four Vietnamese regions. Influenza contributed substantially to SARI hospitalizations, particularly among young children and older adults, underscoring the potential benefits of vaccination.

*Keywords: Influenza, Burden, Severed Acute Respiratory Infection, Vietnam*

Topic: Public Health: Disease Burden & Severity

Abstract No: 10675

## Comparative epidemiology of influenza and respiratory syncytial virus across five seasons – Washington State, 2011/12 – 2015/16

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**Background:** Influenza and respiratory syncytial virus (RSV) are respiratory viruses with annual seasonal epidemics that vary in intensity and in the dominant viral types/subtypes. We compared the incidence of influenza and RSV in ambulatory care settings across five winters.

**Methods:** The United States Influenza Vaccine Effectiveness (US Flu VE) Network conducted active surveillance for influenza among patients seeking care for acute respiratory illness (ARI) at multiple US sites, including Kaiser Permanente Washington (KPWA). Eligible and consenting patients aged  $\geq 6$  months were recruited into the study and provided paired nasal and oropharyngeal swab specimens. Respiratory swab specimens were tested for influenza by real-time polymerase chain reaction (RT-PCR). In the present study, specimens from 2011/12 through 2015/16 were retested for RSV via RT-PCR. Cohorts at risk were defined as KPWA enrollees whose home medical clinic was one where US Flu VE Network occurred. The number of influenza and RSV cases among US Flu VE Network enrollees was extrapolated to the full cohorts at risk.

**Results:** Each year, cohorts ranged from 82,266 to 162,633 individuals, and between 959 and 2,360 patients were enrolled in the US Flu VE Network. Average annual incidence was 25.8 cases per 1,000 for influenza and 19.1 for RSV. In children aged  $< 10$  years, the average cumulative incidence of medically-attended influenza was 42.3 cases per 1,000 population (annual range, 27.9 – 55.4), compared to 24.6 per 1,000 for children and adults aged  $\geq 10$  years (range, 17.2 – 30.2). For RSV, incidence in children aged  $< 10$  years was 80.7 per 1,000 (range, 53.2 – 93.3) compared to 14.3 for children and adults aged  $\geq 10$  years (range, 10.5 – 17.1).

**Conclusions:** Across multiple seasons, influenza and RSV caused a comparable burden of outpatient illness. On average, 2.6% of the populations sought care for influenza each year, compared to 1.9% for RSV.

*Keywords: influenza; respiratory syncytial virus; epidemiology; burden*

Topic: Public Health: Disease Burden & Severity  
 Abstract No: 10726

## GLOBAL BURDEN OF RESPIRATORY INFECTIONS ASSOCIATED WITH SEASONAL INFLUENZA IN YOUNG CHILDREN IN 2015 AND 2018: A SYSTEMATIC REVIEW AND MODELLING STUDY

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### Introduction and Objectives

Seasonal influenza virus (IFV) is a common cause of acute lower respiratory infection (ALRI) in young children. In 2008, we estimated 20 million IFV-associated ALRI and 1 million IFV-associated severe ALRI in children under five. Despite this substantial burden, most countries have achieved only low vaccine uptake. The appearance of 2009 pandemic H1N1 virus caused many deaths during the pandemic period. Since then, many new data are available. We aimed to update estimates of the global number of episodes, hospitalisations, and mortality from IFV-associated ALRI in young children in 2015 and 2018.

### Methods

We performed a systematic review of studies published between 1 January 1995 and 31 December 2018 and identified a further 62 high-quality unpublished studies (Figure 1). We estimated IFV-ALRI incidence rates, hospitalisation rates, and in-hospital case-fatality ratios (hCFRs) by case ascertainment, region, and age. The rate and hCFR meta-estimates did not differ between pre-2015 and 2015-2018. Thus, we estimated morbidity burden using the rate meta-estimates and 2015 (and 2018) population estimates. We estimated IFV-ALRI in-hospital deaths by combining IFV-ALRI hospitalisations and hCFRs, as the lower bound of IFV-ALRI mortality. We estimated the upper bound of IFV-ALRI mortality using in-hospital deaths, US paediatric IFV-associated deaths, and population-based pneumonia mortality data in six sites in developing countries.

### Results

Globally in children under five, we estimated that in 2015, 10.2 million (uncertainty range [UR] 6.8-15.2) IFV-ALRI episodes and 849 thousand (UR 532-1370) IFV-ALRI hospital admissions. Up to 34,900 (UR 13,600-93,600) deaths were associated with IFV-ALRI, with 15,300 (5,900-41,800) deaths occurring in hospitals. We estimated similar burden in 2018.

### Conclusion

IFV is associated with 7% of ALRI episodes, 5% of ALRI hospitalisations, and 2-4% of ALRI deaths. Of the in-hospital deaths, 38% were in infants under six months, and 80% were in low- and lower middle-income countries.

*Keywords: Acute respiratory lower infection, influenza, children, global burden*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 10771

## INFLUENZA EPIDEMIOLOGY AND BURDEN OF DISEASE IN MONGOLIA, 2013/2014 TO 2017/2018

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### Introduction and Objectives

Mongolia is located in Central Asia. Its harsh climate and nomadic lifestyle make the population vulnerable to acute respiratory infections, particularly influenza. Evidence related to the morbidity, mortality and socio-economic impact of influenza in Mongolia is scarce. However, routine surveillance for influenza-like illness (ILI), severe acute respiratory infection (SARI) and laboratory-detected influenza is conducted. This study sought to describe the epidemiology of influenza and estimate the burden of influenza-associated illness in Mongolia for the five years from 2013/2014 to 2017/18.

### Methods

Demographic and laboratory data from 152 sentinel surveillance sites on all patients meeting the ILI and SARI case definitions from October 2013 to May 2018 were extracted and analyzed following the methods described in the WHO Manual for Estimating Disease Burden Associated with Seasonal Influenza.

### Results

The estimated influenza-associated ILI and SARI incidence rates were 2,798 and 666 per 100,000 population, respectively. Children aged less than five years accounted for 67% of all ILI cases and 79% of all SARI patients. However, the annual specimen positivity for influenza was highest (11-30% for ILI and 11-31% for SARI) for those aged 5-15 years and children less than 5 years old, respectively. The annual mortality rate due to SARI was highest among children aged 0-2 years (15.8-54 per 100,000 population). Although the incidence of influenza-associated ILI and SARI was the lowest for the elderly aged 65 years and older, the mortality rate due to SARI was higher than those aged 15-64 years.

### Conclusions

Influenza-associated ILI and SARI burden is high in Mongolia with children aged less than five years. These findings provide support for decision-makers in Mongolia to consider implementation of targeted influenza vaccination of children and the elderly, other control measures for older children to prevent community influenza transmission, and building of a new children's hospital.

*Keywords: Influenza, influenza associated ILI and SARI, burden of seasonal influenza.*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 10810

## INFLUENZA-RELATED MORTALITY FOR HOSPITALIZED PATIENTS WITH AND WITHOUT COMORBIDITIES IN BRAZIL AND MEXICO

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**Introduction:** Seasonal influenza results each year in about 3-5 million cases of severe illness and about 290-650 thousand deaths worldwide. Although all persons are at risk of being infected, the risk of severe influenza is higher for people with comorbidities. The main objective of the study was to compare influenza-related mortality for patients with and without comorbidities in Brazil and Mexico.

**Method:** This cross-sectional study is based on national hospital databases from Brazil (2010-2018) and Mexico (2010-2014). Influenza cases were defined using ICD10 J09 to J11 and J12.9 codes. Seasonality was assessed using the Serfling method. In-hospital Case fatality rate (CFR) and odds-ratio (OR) were calculated and populations with and without comorbidities (cardiovascular, diabetes, immunodeficiency) were compared using the Fisher's exact test.

**Results:** Overall, 103 million hospitalized patients were included for Brazil and 14 million for Mexico. In Brazil, 327,572 influenza cases were identified and the week with peak number of admissions varied between week 15 and 27 according to season with some regional differences. CFRs increased from 0.3% to 0.6% (0-5 years) and from 11.6% to 23.2% (65+ years) when comparing those without and with comorbidities. In Mexico, 12,100 influenza cases were identified and the week with peak number of admissions varied between week 51 and 5. CFRs increased from 0.5% to 4.4% (0-5 years) and from 18.4% to 19.4% (65+ years) when comparing those without and with comorbidities. CFR in patients 50-65 years with influenza alone is comparable to the CFR in patients 65+ years old. Having a comorbidity increased significantly CFRs of hospitalized influenza patients by 2.0-6.2 and 1.8-11.9-fold compared to absence of comorbidities for >5-year-old Brazilian and <50-year-old Mexican patients respectively.

**Conclusion:** Comorbidities play an important role in hospitalized influenza mortality risk. Additional stratified analyses will support understanding of this association, particularly to understand CFR values.

**Keywords:** Influenza; attributable mortality; RWE

Topic: Public Health: Disease Burden & Severity  
Abstract No: 10813

## Influenza B-Associated Pediatric Mortality in the US Between 2010 and 2018

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**Introduction and objectives:** To better characterize the contribution of influenza B to mortality in the US pediatric population, we analyzed the proportion of influenza-associated pediatric mortality attributed to influenza A and B over the last eight influenza seasons using national surveillance data. The effectiveness of influenza vaccines against influenza B in the pediatric population was also assessed.

**Methods:** The study period was the 2010/11 to 2017/18 influenza seasons. The proportions of circulating strains and influenza-associated pediatric mortality for each season were obtained from annual Morbidity and Mortality Weekly Reports on influenza activity. Consolidated vaccine effectiveness (VE) for inactivated influenza vaccine (IIV) and live attenuated influenza vaccine (LAIV) against influenza B for the 2010/11 to 2015/16 seasons was obtained from a published meta-analysis. VE for IIV for subsequent seasons was from the annual US Flu VE Network studies; there was minimal use of LAIV in the US during the 2016/17 and 2017/18 seasons.

**Results:** During the 2010/11 to 2017/18 seasons, influenza B accounted for between 12.6% and 29.2% of circulating influenza strains each season. During the same period, influenza B accounted for 10.3% to 44.6% of pediatric influenza-associated mortality (**Figure**). Except for 2014/15, all predominantly H3N2 seasons had excess influenza B mortality. Point estimates of VE against influenza B ranged from 53% to 82% for LAIV between the 2010/11 and 2015/16 seasons, and from 42% to 70% for IIV between the 2010/11 and 2017/18 seasons.

**Conclusion:** Between the 2010/11 and 2017/18 seasons, influenza B accounted for a disproportionate percentage of pediatric mortality in the US relative to its overall circulation. These data refute the perception that influenza B is less severe than influenza A. IIV and LAIV showed moderate-to-good effectiveness against influenza B during this period, highlighting the importance of influenza vaccination to prevent influenza and associated complications.

*Keywords: influenza; influenza B; pediatric; mortality; influenza vaccine effectiveness*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 10814

## ETIOLOGY OF PNEUMONIA IN PATIENTS WITH SEVERE ACUTE RESPIRATORY INFECTION, MOSCOW EXPERIENCE, 2013-2019

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Russian Federation

**Introduction and Objectives.** In Russia since 2009 influenza A(H1N1)pdm09 virus dominated in etiology of the epidemics and co-circulated with influenza A(H3N2). High activity of influenza A(H1N1)pdm09 virus correlated with the increased indexes of morbidity, hospitalization, mortality and registered cases of pneumonia. The goal of this study was to find the main causes of pneumonia during epidemic activity of influenza viruses.

**Materials and methods.** Questionnaires collecting information on demographic, medical and vaccination history, clinical symptoms, treatment and outcomes were administered to cases meeting the case definition. Clinical materials were tested for different influenza and non-influenza viruses by RT-PCR, virus isolation, Hemagglutination Inhibition (HI), part sequencing.

**Results.** 289 hospitalized patients during 2013-2019 epidemics were included in study, 56% - men and 44% - women. The age distribution was depended on seasons, but group of 15-65 years old was involved the most (52%). Among the comorbidities there were chronicle diseases of heart, COPD, metabolic disorders, AIDS. Approximately all patients were not vaccinated. Pneumonia was diagnosed in 74% of patients in which the main role of 4 viruses was found: Influenza – 50%, Rs-virus – 15%, hRv – 12% and Adenovirus – 11%. Ten lethal outcomes were registered, and the influenza A(H1N1)pdm09 was the most (80%). Influenza strains isolated from these patients were similar to reference/vaccine viruses by antigenic and genetic properties. All of them were sensitive to inhibitors of neuraminidase, umifenovir and resistant to rimantadine.

**Conclusions.** Russian public health officials should continue collecting and analyzing surveillance data on pneumonia cases during epidemics of influenza viruses to guide prevention activities and patient care. Funding: Our investigations were supported by the Centers for Disease Control and Prevention, Atlanta, USA, CoAg: NU51IP000854-03-00.

*Keywords: Influenza A(H1N1)pdm09 virus, pneumonia*



Topic: Public Health: Disease Burden & Severity  
 Abstract No: 10819

## Incidence Density of Influenza-Associated Acute Respiratory Illness in Pregnant Women in Suzhou, China, 2015–2018

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### Introduction and objectives

Data on incidence of seasonal influenza-associated acute respiratory infections (ARI) in pregnant women in China are limited.

### Methods

From October 2015–September 2018, we conducted active surveillance for ARI among pregnant women by continuously enrolling pregnant women, living in and planning to deliver in Suzhou, from prenatal care facilities. For each participant, nurses conducted twice weekly follow-up, one phone call and one text message upon enrollment until delivery to identify ARI, defined as  $\geq 1$  respiratory symptom (cough, sore throat, stuffy nose, chest pain, difficulty breathing) and  $\geq 1$  systemic symptom (feverish, temperature  $\geq 38^{\circ}\text{C}$ , chills, headache) or  $\geq 2$  respiratory symptoms. Nasal and throat swabs were collected  $\leq 10$  days of illness onset. Monthly incidence density and 95% confidence intervals (CI) of RT-PCR confirmed influenza-associated ARI were calculated. We assumed a pregnant woman infected with one influenza subtype/lineage infection was still at risk infection with another subtype/lineage.

### Results

We enrolled 16,419 pregnant women. Of 6,163 ARIs reported, 92% were tested, and 8% (472/5902) were influenza positive. Among the enrolled, none reported receiving an influenza vaccination during pregnancy. During 2015–2016, influenza was detected in December–April (peak in January 2016) and in July 2016. During 2016–2018, influenza was detected in October–April 2017 (peak in December 2016) and in July 2017–April 2018 (late summer peak in September 2017 and a peak in January 2018) (Table 1). From all surveillance months 2015–2018, 4.5–4.9% of influenza-associated ARI cases required hospitalization.

### Conclusion

Influenza-associated ARIs in pregnant women in Suzhou were common with incidence density ranging from 1.1–3.0/100 pregnancy months during October 2015– September 2018. These data will help in decisions on timing of vaccination for this population given that twice annual circulation has been detected.

*Keywords: Influenza, Incidence Density, Pregnant Women*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 10837

## Estimating the burden of disease to inform vaccination policy in Australia

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Influenza is the most common vaccine preventable disease in Australia, causing significant morbidity and mortality in the community. We aimed to estimate the burden of influenza in Australia and determine differences between population groups.

We assessed the burden of influenza in terms of mortality and hospitalisation using national mortality data, hospital separations and surveillance data. Influenza-associated excess respiratory mortality and hospitalization rates from 2007 to 2015 were estimated using generalized additive models with a proxy of influenza activity based on syndromic and laboratory surveillance. Estimates were made for each age group and year within jurisdictions and then pooled to obtain national estimates.

The estimated mean annual influenza-associated excess respiratory mortality was 2.6 per 100,000 population (95% CI: 1.8 to 3.4 per 100,000 population). The excess annual respiratory hospitalisation rate was 53.3 per 100,000 population (95% CI: 29.6 to 76.9 per 100,000 population). The highest mortality rates were observed among those aged  $\geq 65$  years, and hospitalisation rates were also highest among older adults as well as for children aged  $< 6$  months. Annual variation was apparent, ranging from 1.0 to 3.9 per 100,000 population for mortality and 47.2 to 368.8 per 100,000 population for hospitalisations. Jurisdiction-specific estimates varied and had wide confidence intervals, especially in jurisdictions with small populations. Although overall mortality was similar, influenza-associated excess respiratory hospitalisation rates were 2.4 times higher in the Aboriginal and Torres Strait Island population compared with the overall Australian population.

Influenza causes considerable burden to all Australians. Although there was variation among age groups and years, much of this burden falls on the elderly and young children. The Aboriginal and Torres Strait Islands populations are also disproportionately affected. These estimates have been used in mathematical models that examined the potential benefit of various influenza vaccination strategies to inform policy.

*Keywords: disease burden; hospitalisation; mortality; Australia; proxy*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 10847

## Estimation of influenza and RSV attributable medically attended acute respiratory illness in Germany, 2010/11-2017/18

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Introduction and objectives:

We aimed to examine the impact of influenza by A subtype and B lineage and respiratory syncytial virus (RSV) on medically attended "acute respiratory infections" (MAARI) by age group in the German population.

Methods:

Data on MAARI and virological results of respiratory samples (virological sentinel) were available from 2010/11 until 2017/18 from the German sentinel system. The virological sentinel surveillance is performed by the German National Reference Laboratory for Influenza. Beside influenza virus detection and differentiation of A subtypes and B lineages, every sentinel sample was analysed also for RSV since 2010. We updated a previously published generalized additive regression model for influenza to include RSV.

Results:

The 8 seasons-cumulative burden attributable to all four influenza subtypes/lineages and RSV showed that A(H3) had the largest share (32%), followed by A(H1) and B(Yam) with each 24%. The cumulative burden of B(Yam) is thus three times higher than that caused by B(Vic)(8%). RSV contributed with 12 % of all medically attended illnesses. Regarding B(Yam) and B(Vic) there were three seasons with substantial B(Yam)-circulation (2012/13, 2014/15 and 2017/18) and one where B(Vic) circulated strongly (2015/16). We found that the proportion of MAARI due to RSV is substantial only in the 0-1 and 2-4 year old age groups; in the 0-1 year old age group RSV leads in almost all seasons to a higher burden than any influenza, but this is reversed in the age group 2-4 year old.

Conclusion:

In summary, we extended our burden of disease model for the estimation of MAARI due to RSV in addition to influenza in primary care. This will allow us to monitor the effect of present and future prevention concepts such as vaccination for certain circulating respiratory viruses and to better understand interactions between influenza and RSV.

*Keywords: primary care; Burden of Disease; RSV; Influenza subtypes; sentinel surveillance;*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 10921

## CHILDREN HOSPITALIZED WITH INFLUENZA IN NORWAY, 2008-16.

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### Introduction and objectives

Children are more likely to be hospitalized with influenza compared to adults. Vaccination policy in Norway advises yearly influenza vaccine to risk groups, and only children with risk conditions are recommended routine flu vaccinations. We aimed to investigate all children hospitalized for influenza in Norway for the presence of risk factors for severe disease.

### Methods

We retrieved data from the Norwegian Patient Registry on all patients below 18 years of age, hospitalized with influenza in the period 1.1. 2008-31.12.2016. Based on other registered diagnoses the children were classified as being in a risk group or non-risk group for severe influenza. We compared the groups in terms of age, sex and length of hospital stay.

### Results

A total of 2889 children (55% boys) were hospitalized with influenza. The majority (78%) did not have an underlying risk factor for severe disease. Lung disease, neurological disease and heart disease were the most frequently reported risk groups. The average age in risk group vs. non-risk group patients was 6 years vs 5 years, respectively. More boys than girls were seen in both groups (55 vs 58%). Of the boys, 23% were in a risk groups compared to 21% of the girls. The risk group patients were hospitalized longer than the non-risk groups patients (seven vs three days). Among the 14 fatal cases, nine were classified as a risk group for severe influenza disease.

### Conclusion

The majority of children hospitalized with influenza were previously healthy children with no indication for seasonal influenza vaccine. The risk group patients were older and stayed longer in the hospitals. This information should be considered when making recommendations for yearly influenza vaccination.

*Keywords: Children; hospitalizations; influenza; risk groups;*

Topic: Public Health: Disease Burden & Severity  
 Abstract No: 10956

## HOW MUCH SARI IN INTENSIVE CARE UNITS IS ATTRIBUTABLE TO COMMON RESPIRATORY VIRUSES?

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### **Introduction and Objective**

Severe acute respiratory infections (SARI) are a common cause of intensive care unit (ICU) admissions and are associated with high mortality rates. Exhaustive laboratory testing of all SARI patients does not often occur. The burden that different common respiratory pathogens pose to SARI is therefore unclear. We aimed to estimate how many SARI admissions to adult ICU are attributable to common respiratory viruses by using a modelling approach.

### **Methods**

Number of SARI admissions to adult ICU (aggregated per week; July 2007-June 2017) was retrieved from the National Intensive Care Evaluation registry (NICE) of the Netherlands. The weekly number of positive diagnoses of respiratory viruses were extracted from the national virological laboratory surveillance: Influenza A & B, Rhinovirus, Respiratory syncytial virus, Adenovirus, Coronavirus, Parainfluenza and Human metapneumovirus (hMPV). A binomial regression model was used to associate the number of SARI admissions with respiratory virus counts.

### **Results**

SARI admissions to adult ICU were significantly associated with Influenza A&B, Rhinovirus, Coronavirus and hMPV. On average, 580 SARI admissions (13%) were attributed to these viruses yearly, but varying by year from 4% to 21%. The highest proportion (21%) and incidence (942 attributed cases) were observed in season 2015/2016, when influenza A(H1N1)pdm09 dominated, followed by influenza B (Victoria lineage). The largest contributors varied per season: Influenza A (4-6%, 5 out of 10 seasons), influenza B (6%, 1 season – together with influenza A also 6%), Coronavirus (6%, 1 season) and Rhinovirus (1-3%, 2 seasons).

### **Conclusion**

While 5% of SARI in ICU were coded as viral pneumonia upon admission this percentage may be an underestimation: on average 13% of SARI was estimated attributable to viruses. In many seasons influenza A was the largest viral contributor, with 4-6% of SARI admissions attributed. But Influenza B, Rhinovirus and Coronavirus occasionally play relatively large roles too.

*Keywords: Intensive Care, SARI, Pneumonia, influenza, respiratory infections*

Topic: Public Health: Disease Burden & Severity  
 Abstract No: 10958

## INFLUENZA ILLNESSES AMONG PATIENTS HOSPITALIZED IN BANGLADESH CARDIOLOGY UNITS, 2018

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**Introduction:** Life threatening cardiovascular events (CVEs), such as ischemic or coronary heart disease are more likely to cause deaths in low-income countries with limited resources compared to high-income countries. Although influenza illnesses can trigger CVEs, little is known about their prevalence in low-income countries like Bangladesh. We estimated the prevalence of laboratory-confirmed influenza among patients hospitalized with CVEs in Bangladesh.

**Method:** During January—December 2018, we enrolled patients admitted with any CVE and <10 days of feverishness and cough to cardiology units in eight tertiary level hospitals. Each week, from Saturday—Thursday, surveillance physicians enrolled CVE patients with respiratory illnesses. We collected demographic, clinical, and discharge information using standardized surveys. Nasopharyngeal and throat swabs were tested for influenza viruses through real-time rt-PCR. We compared the case fatality proportions among all CVE patients with respiratory illnesses and influenza using Chi-square tests.

**Result:** Of 41,207 CVE patients, we identified 603 (1.5%) with respiratory illness and enrolled 518 (86%). Median age was 58 years (IQR 47–66) and 75% were male. Influenza was detected among 10% (n=54) and was most common during peak season in July (44%) and August (33%). Among those with influenza, 85% (n=46) had influenza A (76% H1N1; 24% H3N2) and 15% (n=8) influenza B (87% Yamagata; 13% Victoria). None received oseltamivir. Almost half of CVE patients with influenza were admitted with ischemic heart disease (26, 48%); 50% of whom had myocardial infarctions. Twenty-five (5%) CVE patients with respiratory illnesses died in the hospital; four deaths (16%) were CVE patients with influenza. Death was not statistically associated with influenza among CVE patients (p=0.35).

**Conclusion:** Influenza was commonly identified among hospitalized CVE patients with respiratory illness. Our findings suggest the need to assess the potential cost benefits of influenza vaccination for preventing influenza and hospitalizations related to CVEs with respiratory illness.

*Keywords: Cardiovascular events; Influenza; Severe acute respiratory illness, Cardiology Units*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 11000

## SEASONAL INFLUENZA EPIDEMIC SEVERITY ASSESSMENTS USING THE WHO PISA GUIDANCE

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### Introduction

Influenza severity assessments categorize current data into 5 levels compared to historical data, for indicators of transmissibility, seriousness of disease and impact. Weekly reports of two indicators received since 2015 from temperate countries applying WHO's pandemic influenza severity assessment (PISA) framework are summarized below.

### Methods

The distribution of country reports by level above seasonal threshold were analyzed by season (northern hemisphere/NH and southern hemisphere/SH) and indicator and complemented with summary virological information.

### Results

Since 2015, 24 countries/areas/territories have reported at least one weekly assessment to WHO, 10 of which are considered temperate and experiencing seasonal epidemics. For transmissibility, the proportion of reports by level ranged from: 21% to 81% for low, 16% to 67% for moderate, and 0 to 26% for high. Two reports of extra-ordinary intensity were recorded during the 2017-2018 NH season.

For the impact indicator, the proportion of reports by level ranged from: 14% to 100% for low, 11% to 44% for moderate, and 0 to 38% for high. Four and 2 reports of extra-ordinary intensity were recorded during the 2017-2018 and 2018-2019 northern hemisphere seasons, respectively.

Reports of transmissibility and impact levels at high or extra-ordinary intensity were more frequent during the 2017-2018 NH and 2016 SH seasons. Relatively greater numbers of reports of high and extra-ordinary impact were reported during the 2018-2019 NH season.

In the countries reporting PISA assessments, influenza A(H1N1)pdm09 viruses predominated during the 2015-2016 NH, 2018-2019 NH and 2018 SH seasons. Influenza A(H3N2) viruses predominated in the 2016-2017 NH and 2017 SH seasons. Consistent patterns in other seasons were less clear.

### Conclusion

A collation of national severity reports provides a snapshot on global influenza activity, complementing virologic information. With more countries reporting on seasonal severity, global trends may become more clear and informative, increasing capacity to assess a future pandemic's severity.

*Keywords: influenza; severity; impact; thresholds; World Health Organization;*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 11004

## Mapping the Global Burden of Influenza and RSV: the BIRD Project

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**Introduction and objectives:** The Burden of Influenza and Respiratory syncytial virus (RSV) Disease (BIRD) project aims to estimate the global and regional burden of influenza and RSV infections in terms of hospitalizations and mortality. The assessment will take into consideration age groups, comorbidity, vaccination coverage (influenza) and direct versus indirect causality (attribution vs contribution).

**Methods:** In a first phase, BIRD catalogued published literature, clinical data, and grey literature from a globally representative sample of 26 core countries. In the second phase, data were synthesized to provide: 1) country-specific estimates; 2) an assessment of the direct and indirect impact of each virus on hospitalizations and mortality. In order to perform the modeling exercise, we are collecting two types of studies: 1) studies that report the proportion of laboratory-confirmed RSV or influenza among respiratory hospitalisations; and 2) studies that report excess hospitalisations or mortality using time series models. The analysis will be expanded beyond the 26 core countries in later stages.

**Results:** The cataloging of influenza was first completed for papers reporting excess hospitalizations or mortality. For mortality, the review resulted in a total of 73 papers for the 26 core countries, with the most frequently cited countries being: USA (19 papers), China (8), UK (7), Australia (5) and South Africa (5). For hospitalizations, the cataloging is still ongoing. The cataloging of RSV was completed for papers reporting the proportion of RSV among respiratory hospitalizations. This included 218 studies in young children and 19 studies in older adults. The majority of studies (202/237) came from the 26 core countries. The data cataloging of excess hospitalizations and mortality is still ongoing.

**Conclusion:** The BIRD project is working on a comprehensive global and regional assessment of the burden of influenza and RSV. All data will be made public on the BIRD website.



Topic: Public Health: Disease Burden & Severity

Abstract No: 11029

## INFLUENZA IN OLDER PATIENTS IS ASSOCIATED WITH INCREASED HEALTHCARE UTILIZATION

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### Introduction and Objectives:

Data on healthcare utilization and mortality associated with vaccine uptake and disease outcomes in influenza-associated upper respiratory tract infections (URTI) in Southeast Asia remains sparse, while age is considered a predictor of poor prognosis. We aimed to determine the above and to identify the influence of age on clinical presentation and outcomes.

### Methods:

A retrospective case-note analysis was conducted on a cohort of 3,935 patients attending primary care at the University Malaya Medical Centre, Malaysia from February 2012 till May 2014 with URTI symptoms. Demographics, clinical characteristics, medical and vaccination history were obtained from the electronic medical records, and mortality data from the National Registration Department. Comparisons were made between those aged <25, ≥25 to <65 and ≥65 years.

### Results:

470 (11.9%) patients had PCR-confirmed influenza virus infection. Six (1.3%) received prior influenza vaccination, none of which were aged ≥65 years. Those aged ≥65 years (n=53) were more likely to have ≥2 comorbidities (p<0.001) and were less likely to present with fever (p=0.004). Almost one-third (28.3%) of those aged ≥65 years experienced hospitalization, intensive care admission or death within a year compared to 10% in the ≥25 to <65 year age group. Compared to individuals aged <25 years, those age ≥65 years were significantly more likely to experience hospitalization and death after adjustment for known confounders (OR=9.97; 95% CI=3.11 to 31.93).

### Conclusion:

Older patients in our cohort were more likely to have comorbidities and present with atypical features, with older age being an independent predictor of poor health outcomes. Our findings will now inform future health policies on the older persons and economic modelling on funding of adult vaccination programmes.

*Keywords: Influenza; healthcare utilization; mortality; elderly; Southeast Asia*

Topic: Public Health: Disease Burden & Severity  
 Abstract No: 11049

## Influenza Disease Burden and Cost Estimates in Indonesia

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**Introduction and Objectives.** Influenza is a leading cause of morbidity and mortality worldwide affecting particularly young children and elderly. While influenza disease and cost burden has been partially quantified in Indonesia through its contribution to Severe Acute Respiratory Tract infections (SARI), its contribution to lower respiratory tract infection (LRTI) burden is not clearly defined. However, data from other countries report a significant proportion of influenza in hospitalized LRTI patients. This study generated national cost and disease burden estimates for 2017 in Indonesia.

**Methods.** Through experts consensus, incidence of influenza-LRTI was estimated based on the review of influenza-associated SARI studies conducted in 1999-2003, 2003-2007 and from the Indonesia Hospital Based SARI sentinel surveillance data from 2013-2016. Using the Global Burden of Disease tool, that incorporates the impact of Influenza LRTI illness, disability and premature death, the macroeconomic burden was estimated. Direct health care expenditures for ambulatory and hospitalization services were also calculated from LRTI associated cost collected from the National Social Health Insurance (BPJS).

**Results:** The annual incidence of LRTI attributable to influenza was 1,285 episodes per 100,000 pop., with 3,358,418 episodes in total; 1,204 hospitalizations per 100,000 pop., with 40,435 hospitalizations; case fatality rate was 0.122%, with 4,097 deaths. The estimated total medical expenditure (out/in-patient) due to influenza LRTI in 2017 was US \$ 19,2 M (250.05 billion rupiah) and the years of healthy life lost due to premature death and disability (DALYs) was 220,301 amounting to US \$ 847,5M (11.01 trillion rupiahs). Thus, the total economic costs (years of healthy life lost and medical expenditures) due to Influenza in Indonesia in 2017 were estimated to be US \$ 866,7M (11.26 trillion rupiahs)

**Conclusions:** There were substantial health and economic costs due to influenza LRTI in Indonesia. Enhancement of influenza prevention and control strategy, including increased immunization, can reduce this burden.

*Keywords: Influenza, disease burden, cost estimates, Indonesia*

Topic: Public Health: Disease Burden & Severity

Abstract No: 11102

## Community mortality due to influenza in Melghat, India

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**Introduction:** Influenza-associated lower respiratory tract infections (LRI) are a major cause of morbidity and mortality in developing countries, yet few studies have simultaneously examined its impact on young children in the community and hospital. We sought to (1) estimate the age-specific incidence of influenza associated severe LRI and mortality, and (2) compare the incidence of influenza mortality in the community vs. hospital.

**Methods** Nasal swabs were collected from all infants or children less than 2 years of age, in 96 villages in Melghat, India over 3 years, who had severe LRI or who died in the community or at one of 11 village health centers and hospitals. A respiratory PCR panel was performed to detect influenza (sub-types A, B, H1N1, H3N2). Only infants and children 7 -730 days of age were studied. For child-years of observation, the denominator was calculated by determining the number of active days in the study.

**Results** 91 children in the community and 42 children in the hospital had severe influenza LRI. There were 27, 14 and 44 children with H1N1 2009, H3N2 and B in the community 13, 7 and 20 hospitalized subjects respectively. The incidence of community and hospital influenza LRI was 12.3 and 5.8 per 1000 child-years of observation respectively. There were 69 LRI-related deaths among 2470 ALRI cases (CFR 2.79). 5 (7.2%) of the ALRI deaths were influenza associated 4 community and 1 hospital -related (4 influenza B; Case fatality ratio - CFR 6.25%) and 1 H1N1 2009 death (CFR - 2.55) and. The incidence of community and hospital influenza-related deaths was 0.6 and 0.1, respectively, per 1000 child-years of observation.

**Conclusions** The community burden of severe influenza LRI is twice that of the hospital burden and mortality is 4 times higher in the community than in the hospital, in rural Melghat.

Topic: Public Health: Disease Burden & Severity  
Abstract No: 11125

## THE USE OF HEALTH SERVICES BY POPULATIONS FACING ACUTE RESPIRATORY INFECTION IN COTE D'IVOIRE FROM 2016 TO 2017

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### Introduction and Objectives

Estimating the burden of the disease associated to influenza is a key foundation of vaccine policy's introduction. The methods for calculating influenza burden estimates are complex and required reliable specific data from Illness Like Influenza (ILI) or Severe Acute Respiratory Infection (SARI) sentinel sites. We conducted a study to determine the utilization rate of health services by populations presenting Acute Respiratory Infection (ARI).

### Method

The study covered 6,800 households including 28,308 people. The individuals were interviewed in face to face to gather information on the occurrence of SARI or ILI. For those who reported SARI or ILI episodes, data on health service utilization were collected. A logistic regression analysis was performed to determine the factors associated with health services attendance for ARI.

### Results

Among the interviewees, 32.7% (9258/28308) reported having an episode of acute respiratory infection (ARI) of which 27.1% (766) of ILI and 5.6% (1593) of SARI. The attendance rate of health services for ARI was (4990/9258 = 53.9%). The hospital that represents the sentinel site for influenza was (2532/9205 = 27.5%). Age, education and socio-economic status were significantly associated with attendance at health services in IRA.

### Conclusion

The study showed a relatively moderate attendance rate of health services and a low use of the influenza sentinel site by populations in case of ARI. It is therefore critical that national authorities continue to encourage the use of health services in case of ARI to enable better estimate the burden of influenza.

*Keywords: Health Utilisation Survey ; ARI ; Influenza ; Côte d'Ivoire*

Topic: Public Health: Disease Burden & Severity

Abstract No: 11144

## RSV-associated respiratory hospitalization in Hong Kong, 1998-2015

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### Abstract

**Background:** Respiratory Syncytial Virus (RSV) is a common respiratory virus, which could cause severe diseases in both children and adults. The importance of RSV-associated respiratory hospitalizations has not been generally recognized in Hong Kong. We examined the impact of RSV on respiratory hospitalized people by age in Hong Kong from 1998 to 2015.

**Method:** We obtained weekly hospitalization data by age and cause in Hong Kong from Hospital Authority, and used these to derive weekly hospitalization rates for respiratory causes, respiratory and cardiovascular causes combined, and all causes. We used the product of influenza-like illnesses and laboratory detections of RSV as a proxy for weekly incidence of RSV infections in the community. We applied linear regression models to respiratory hospitalization rates from 1998 to 2015, and used the fitted models to estimate the corresponding RSV-related excess hospitalizations.

**Results:** We estimated that an annual average of 5.9 (95% confidence interval (CI): 3.5, 8.3) excess respiratory hospitalizations per 10,000 person-years were associated with RSV, which was 3.0% of all respiratory hospitalizations from 1998 to 2015. RSV was associated with 192.0 (95% CI 167.1, 219.8) respiratory hospitalizations per 10,000 person-years in children less than 1 year, which accounted for 16.2% of all respiratory hospitalizations among this age group during the years studied, and was also attributed to 22.1 (95% CI 9.7, 33.9) respiratory hospitalizations per 10,000 persons per year in older adults aged  $\geq 65$  years.

**Conclusion:** RSV has substantial impact on respiratory hospitalizations in Hong Kong, especially in children age less than 1 year and older adults. Our results suggested that RSV preventive interventions might be beneficial for the high-risk groups, cost-effective analysis on specific interventions could be considered in the future.

Topic: Public Health: Disease Burden & Severity  
 Abstract No: 11186

## HEALTH AND ECONOMIC BURDEN OF INFLUENZA-ASSOCIATED ILLNESS IN SOUTH AFRICA, 2013-2015

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### Introduction

Economic burden estimates are essential to guide policy-making for influenza vaccination, especially in resource-limited settings.

### Methods

We estimated the cost of medically and non-medically attended influenza-associated illness in South Africa including mild and severe respiratory, circulatory, and non-respiratory/non-circulatory illness. Additionally, we restricted the analysis to influenza-associated severe acute respiratory illness (SARI) and influenza-like-illness (ILI) (subsets of all respiratory illness). This study uses data collected during 2013-2015 and a modified version of the World Health Organization (WHO) tool for estimating the economic burden of seasonal influenza. Absenteeism and years of life lost (YLL) were also estimated. We reported mean annual estimates during the study period.

### Results

The estimated mean annual cost of influenza-associated illness was \$270.5 million, of which \$111.3 million (41%) were government-incurred costs, 40.7 million (15%) were out-of-pocket expenses and \$118.4 million (44%) were indirect costs. The cost of influenza-associated medically attended mild illness (\$107.9 million) was 2.3 times higher than that of severe illness (\$47.1 million). Influenza-associated respiratory illness costs (\$251.4 million) accounted for 93% of the total cost. Estimated absenteeism and YLL were 13.2 million days and 304,867 years, respectively. Among patients with influenza-associated WHO-defined ILI or SARI, the costs (\$95.3 million), absenteeism (4.5 million days) and YLL (65,697) were 35%, 34% and 21% of the total economic and health burden of influenza.

### Conclusion

The economic burden of influenza-associated illness was substantial from both a government and societal perspective. Models that limit estimates to those obtained from patients with WHO-defined ILI or SARI substantially underestimated the total economic and health burden of influenza-associated illness.

*Keywords: influenza; health burden; economic burden; Africa*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 11213

## Influenza surveillance in hospitalized patients with acute respiratory symptoms in 2 Klang Valley hospitals in Malaysia, 2018-2019

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### Introduction:

Influenza is a major cause of hospitalization and death worldwide. In Malaysia, the virus circulates throughout the year with no clear seasonal trend. However, there is limited published evidence on the disease burden of influenza in Malaysia.

**Objective:** This study intends to estimate the incidence of severe laboratory-confirmed influenza among hospitalized patients with acute respiratory conditions, explore the risk factors associated with influenza and describe the distribution of the circulating influenza virus strains in Malaysia.

### Methodology

A prospective epidemiological active surveillance study is ongoing in 2 major hospitals located in the Klang Valley in Malaysia. Community-dwelling adults hospitalized with acute respiratory conditions had oropharyngeal/nasopharyngeal swabs collected and tested for influenza viruses by real-time PCR. Subjects' socio-demographic, health history and health outcomes data were collected using a standardized questionnaire. Descriptive and univariate preliminary (chi-squared test and logistic regression) analysis were conducted.

### Results

From July 2018 to February 2019, 350 subjects were enrolled (247 from Hospital Selayang and 103 from University Malaya Medical Centre). The mean age was 60.2 years old (range, 18-89), and 55% were female. The overall influenza positivity rate was 14%, with 89.8% influenza A and 10.2% influenza B. The vaccination coverage rate was low with 0.3% individuals vaccinated in the past 12 months. Preliminary results showed that presence of fever and absence of hospitalization within the last 12 months were associated with a significantly increased risk of laboratory-confirmed influenza with odds ratios of 2.8 (95%CI 1.2-6.5) and 2.47 (95% CI 1.2 – 5.1), respectively.

### Conclusion

Influenza is a significant (14%) contributor to hospitalization in this population. The study is ongoing and additional analysis will be conducted to better describe and understand the risk factors associated with influenza positivity in this population.

*Keywords: Influenza, Disease Burden, Klang Valley, Acute Respiratory Conditions, Hospitalization*

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## Moving from influenza burden estimates into policy: a tool to support decision makers

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### Introduction and Objectives

The World Health Organization (WHO) through the Influenza Burden of Disease (BoD) project with the Partnership Contribution of the Pandemic Influenza Preparedness Framework programme aims to ensure that national, regional and global influenza burden estimates are available and communicated effectively so that they are used by decision-makers. Understanding the challenges and constraints of the decision process as well as learning from successful countries' experience will help developing best practices for the use of BoD data for policy generation.

### Methods

Since 2013, WHO has provided technical guidance and financial support to countries to estimate influenza burden and encouraged countries to share their estimates through publication and contribution to the global influenza disease estimates. A consultation is planned on 25-27 June 2019 to gather country experience on moving forward in a decision process after influenza disease estimates are available. Aim is to develop tools that will support decision makers in understanding influenza disease estimates and using these to develop effective policies to reduce the BoD in their country.

### Results

Since 2013, 55 new influenza disease estimates have been published. In January 2018, a special edition of *Influenza and Other Respiratory Viruses* journal on influenza disease burden was published with 23 articles. For the consultation in June, 42 abstracts were received. The challenges of moving from the estimates to decision generation has been remarked clearly. Starting from the experiences collected during the consultation, a toolkit will be developed. The tool will guide through different scenarios and will enable to identify the best way to move forward in the decision process (ways to overcome challenges, need for additional data, etc).

### Conclusion

The translation of influenza estimates into policy can be challenging. The toolkit aims to direct the use of BoD data for decision in influenza disease prevention and mitigation strategies.

*Keywords: Burden of disease; Policy; toolkit*



Topic: Public Health: Disease Burden & Severity

Abstract No: 11268

## ECONOMIC BURDEN OF SEASONAL INFLUENZA-ASSOCIATED SEVERE ACUTE RESPIRATORY ILLNESS HOSPITALIZATIONS IN MADAGASCAR, 2016.

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### Introduction and Objectives

Influenza is responsible for substantial economic burden, global morbidity and mortality in young children and elderly individuals. However, there was no data on this economic burden in Madagascar, although this data can help the health authorities to prioritize resources and implement a better control and prevention strategy. Our goal was to estimate the annual economic burden of influenza-associated severe acute respiratory illness (SARI) hospitalizations in Madagascar.

### Methods

The World Health Organization method to estimate the economic impact of seasonal influenza was used. In 2016, we identified all influenza-positive patients in a tertiary hospital in Antananarivo. A questionnaire for these patients was administrated. We estimated direct medical costs (the cost of hospitalization, medicines, diagnostic test, and self-medication), direct non-medical costs (the costs of hospital transport, food, additional accommodation for accompanying persons) and indirect costs (loss of income for the patient and caregivers). Total cost per episode was estimated adding direct costs and indirect costs. We used the estimation of national numbers and rates of influenza-associated SARI hospitalizations and the mean costs per episode to estimate annual economic burden of influenza-associated SARI hospitalizations in 2016.

### Results

The estimated mean annual total economic burden of influenza-associated SARI hospitalizations in Madagascar in US dollars (\$) was \$5.2 million (\$1.5-\$24.1 million) representing around 0.05% of Madagascar GDP. The estimated mean direct medical cost was \$4.0 million (\$1.2-\$14.8 million) and indirect cost was \$1.5 million (\$0.3-\$9.3 million). The total charge related to influenza-associated SARI hospitalization per patient is estimated at \$536.

### Conclusion

Influenza-associated hospitalization is a heavy economic charge for the patient, his family and the Malagasy health system, considering the GNI per capita in Madagascar (\$406) and the budget of the Ministry of health excluding salary (\$25 million).

*Keywords: Influenza, Economic Burden, Hospitalization, Costs, Madagascar*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 11270

## ASSESSING THE SEVERITY OF SEASONAL INFLUENZA IN MADAGASCAR THROUGH SURVEILLANCE AND DEATH CERTIFICATES COLLECTION IN ANTANANARIVO, MADAGASCAR.

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### Background and Objectives

Lessons learned from the 2009 influenza A (H1N1) pandemic demonstrated the need to establish surveillance of influenza severity in Madagascar. Indeed, despite a functional influenza surveillance system in place, impact of seasonal influenza epidemics in particular influenza-associated mortality is still unknown. We aimed at assessing severity of seasonal influenza in Madagascar.

### Methods

Since 2016, we have implementing death surveillance in the urban community of Antananarivo (the capital of Madagascar). Under this surveillance, we have collected all cases of deaths that occurred in the city. Causes of death were coded according to the 10<sup>th</sup> revision of the International Classification of Diseases (ICD-10).

We estimated severity of Influenza using different sources including: the ARI (acute respiratory infection)-associated death rate (Code J00-J22), the Influenza-associated hospitalization rate, the mortality curve during influenza seasonal epidemic periods, and influenza sentinel surveillance data.

### Results

From January 2016 to December 2018, we recorded 27,179 deaths, 1,488 (5.5%) of which were coded as ARI. Most of deaths recorded 218 (73.6%) occurred at home. Amongst children less than 5 years and adults more than 65 years, the mean annual rates of ARI-associated death were respectively 45.5 and 299.5 per 100,000 populations. The rate of influenza-associated hospitalization in these same age groups were respectively 168 and 76 per 100,000 populations. In addition, we found a positive association ( $r=0.71$ ,  $p=0.0001$ ) between the number of collected deaths and the number of influenza-associated severe acute respiratory illness (SARI) during specific periods for patients aged less than 5 years.

### Conclusion

Our first estimates highlight the potential burden of seasonal influenza-associated death in age group under five years and more than 65 years. Despite that SARI and mortality surveillance is only functional in Antananarivo, this study provided additional information to address the severity and the burden of seasonal influenza.

*Keywords: Influenza, Mortality, Surveillance, Madagascar*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 11280

## Effect of socio-economic status on Influenza risk in English households

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### Introduction and Objectives

Influenza causes a wide spectrum of disease outcomes from asymptomatic infection to hospitalisation and death. Current literature suggests socioeconomic status (SES) is associated with moderate and severe influenza outcomes, but few studies have investigated milder outcomes. This analysis uses data from the Flu Watch study to investigate whether area-level socio-economic deprivation affects the risk of PCR-confirmed influenza in the community.

### Methods

Flu Watch was a prospective community cohort that followed up English households during periods of influenza circulation between 2006 and 2011. Participants completed weekly surveys to identify respiratory illnesses and submitted self-administered nasal swabs on day two of any illness for PCR testing. SES was measured using national quintiles of the Index of Multiple Deprivation (IMD). The outcome was PCR-confirmed influenza. Incidence rate ratios were calculated using multivariable poisson regression with robust standard errors to account for clustering. Age group, influenza season, ethnicity, handwashing and geographic region were investigated as potential confounders and influenza vaccination status was tested as an effect modifier.

### Results

5484 participants contributed 4937 person-seasons of follow up. After adjusting for age, season, ethnicity, handwashing and region, IMD was found to be associated with PCR-confirmed influenza ( $p < 0.001$ ). The rates of PCR-confirmed influenza in the most deprived quintile was between 1.7 and 2.5 times higher than the rates in the four least deprived quintiles. There was little variation in rate ratios between the four least deprived quintiles. There was no evidence that vaccination status was an effect modifier.

### Conclusions

In this community-level study, individuals residing in the most deprived areas had higher rates of PCR-confirmed influenza illness. These results agree with other studies showing that low SES increases the risk of more severe influenza outcomes. This strengthens the case for making highly deprived areas a target group for public health interventions.

*Keywords: influenza; socio-economic status; PCR; deprivation; burden*

Topic: Public Health: Disease Burden & Severity

Abstract No: 11290

## DYNAMICS OF SEASONAL INFLUENZA AT A TERTIARY CARE HOSPITAL IN SOUTH INDIA

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**Introduction:** Influenza viruses (influenza A and B) are responsible for significant disease burden, hospitalization and mortality, globally. In lower-middle income countries (LMICs), disease burden is many folds higher than the developed world. Despite the high burden, the epidemiology and clinical course and outcomes of infection in LMICs are relatively understudied.

**Objectives:** i) To detect influenza A and B viruses among cases of influenza-like illness (ILI) or severe acute respiratory infection (SARI). ii) Determine epidemiological characteristics of influenza type/subtype

**Materials/methods:** Patients presenting to outpatient clinics or admitted in wards and intensive care units. of Christian Medical College with ILI / SARI from 2009 to 2018 were included. Respiratory samples collected were tested for influenza A and B viruses using an in-house triplex real-time PCR (Influenza A/B/GAPDH). Samples positive for influenza A were subtyped using a duplex HA-specific real-time RT-PCR (H1N1/H3N2). Metadata including age, gender, ward/ICU admission were gathered to study temporal trends in influenza virus detection.

**Results:** A total of 22,294 samples were received from 2009-2018. Influenza viruses were detected in 5157 (23.1%) of which 2952(13.2%),1266 (5.7%) and 939(4.2%) were A/H1N1, A/H3N2 and influenza B, respectively). Distinct seasonal patterns were seen with detection peaking between August and March with variable intensity and timing of virus detection. From 2015, unsubtypeables were detected necessitating updating of A/H1N1 primers. All 3 viruses showed similar age distribution among non-admitted and admitted cases. However, influenza B and A/H3N2 infections resulting in ICU admission were mainly among the elderly, while A/H1N1 ICU admissions peaked among adults(40-50 years).

**Conclusion:** Influenza presents as mild illness as well as severe disease necessitating ward admission and ICU care. A high disease burden is seen in the population. Severe disease frequently affects the elderly and young adults.

**Keywords:** influenza; temporal trends; seasonality; hospitalisation;

Topic: Public Health: Disease Burden & Severity  
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## Estimating the excess mortality of influenza in the Philippines

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Introduction: Little is known about the burden of influenza mortality in the Philippines.

Objectives: We estimated the burden of excess influenza mortality in individuals aged 0 to <5, 5 to <10, 10 to <20, 20 to 59, and 60 and above.

Methods: A set of negative binomial regression models were run on 2006–2015 data with all-cause mortality from the civil registry as the dependent variable, and influenza A and B positivity rate from FluNet, meteorological controls, time, and cyclical trends as independent variables. The difference between predicted all-cause deaths of the full model and the predicted all-cause deaths of the model holding influenza positivity rate as zero served as the predicted number of influenza deaths.

Results: Estimated excess mortality rate (EMR) of influenza per 100,000 individuals was 5.25 (95% CI: 2.77–7.44). Highest EMR was in individuals aged ≥60 years with 44.29 (95% CI: 25.82 – 64.38) deaths per 100,000, while ages 10 to <20 has the lowest EMR of 0.81 (95% CI: –0.25–1.47). Young children also had a higher risk of death. More than 50% of fatalities occur in the 60+ population, consistent with global data. These mortality rates equate to >5,300 annual influenza deaths in the Philippines, 4,400 from influenza A and 900 from influenza B viruses. Mortality estimates are about 50-fold higher than official influenza death statistics.

Conclusion: This study is the first empirical evidence on flu excess mortality in the Philippines based on country-specific data and may provide policymakers an alternative source of information aside from official death statistics that are likely to be underreported. This study's findings are consistent with the established fact that the older and younger individuals, who may have weaker immune systems, are more susceptible to death following influenza infection.

Funding: Sanofi Pasteur

Keywords: *influenza, excess mortality, Philippines*

Topic: Public Health: Disease Burden & Severity

Abstract No: 11294

## The mortality impact of the 1918 influenza pandemic in Greenland

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### **Introduction and Objectives**

The 1918 pandemic was particularly devastating in Arctic/remote populations in Newfoundland, Alaska and the Samoa islands. We studied spatial/temporal patterns of influenza deaths in Greenland, using all individual death records from archival parish books for 1914-1921.

### **Methods**

We digitalized each death, retaining date and cause of death, age, gender and settlement. We combined pneumonia and influenza (P&I) as deaths listed as influenza, Spanish flu, cold-fever, pneumonia, and "sting". We divided the population into 5 geographical regions and 15 parishes; population census for 1911 and 1921 were used to compute parish populations in 1916.

### **Results**

Out of a population of 13917, 3546 deaths occurred during 1914-1921; hereof 709 influenza deaths. In May 1916 a severe influenza outbreak occurred in the cape region where 4.9% of the population died. In the August 1919 pandemic wave all areas were affected and 1.3% of the population died across Greenland (range, 0.3% to 4.2% in individual parishes). During the recurrent pandemic wave in August 1921, 1% died of influenza (range, 0% to 4.4% across individual parishes). We could not identify an age shift in influenza or respiratory deaths in 1919, relative to 1916; all age groups were affected.

### **Conclusion**

The pandemic impact in Greenland was lower than that reported in other Arctic settlements. The overall mortality impact of 1.3% was similar to the global mortality rate (1-2%). Interestingly, the 1916 seasonal influenza outbreak was equally severe nationally with a mortality rate of 1.3% due to highly severe outbreaks in a few parishes in southern Greenland. The influenza pandemic reached Greenland in 1919, a full year after its emergence in Copenhagen. Why Greenland was not as severely affected as other Inuit communities may be explained by Greenland being better connected through ships and thus enjoying higher levels of pre-pandemic immunity to influenza.

*Keywords: Pandemic mortality; Arctic populations; disease burden; remote populations*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 11322

## Loose Ends in the Epidemiology of the 1918 Pandemic: Explaining the Extreme Mortality Risk in Young Adults

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### Introduction and Objectives

In the century since the 1918 influenza pandemic, insights have been sought to explain the pandemic's signature pattern of high death rates in young adults and low death rates in the elderly and infants. Our understanding of the origin and evolution of the pandemic has shifted considerably. We review evidence of the characteristic age-related pattern of death during the 1918 pandemic relative to the "original antigenic sin" hypothesis. We analyze age-stratified mortality data from Copenhagen around 1918 to identify break points associated with unusual death risk.

### Methods

We review clinical, epidemiological and phylogenetic evidence of the origin and evolution of the 1918 influenza pandemic. In addition, we looked at the death patterns of different age cohorts in Copenhagen in 1918 and sought to place these patterns in the context of long time series of outpatient records of influenza-like illness. For this we used detailed, long time series of age-stratified monthly death records along with population census statistics searching for break points in the age profile of cases.

### Results

Whereas infants had no meaningful risk elevation, death risk gradually increased, peaking for young adults 20–34 years of age before dropping sharply for adults ages 35–44 years, suggesting break points for birth cohorts around 1908 and 1878. Taken together with data from previous studies, there is strong evidence that those born before 1878 or after 1908 were not at increased risk of dying of 1918 pandemic influenza.

### Conclusion

An increasing number of interdisciplinary studies covering fields such as virology, phylogenetics, death, and serology offer exciting insights into patterns and reasons for the unusual extreme 1918 pandemic mortality risk in young adults. Although the peak death risk during the 1918 pandemic coincided with the 1889–1892 pandemic, the 1908 and 1878 break points do not correspond with known pandemics.

*Keywords: 1918 Spanish flu; age patterns; antigenic sin; excess mortality; pandemic influenza*

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Abstract No: 11329

## Influenza infections factors: active epidemiological surveillance in a pediatric hospital 2000-2018

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Background: Influenza is an important cause of acute lower respiratory tract infection (ALRI), hospitalization, and mortality in children. The aims of this study were to describe the clinical-epidemiologic pattern and infection factors associated with influenza, and to compare case features of influenza A and B.

Methods: prospective, cross-sectional study of patients admitted for ALRI 2000–2018, diagnosed with respiratory syncytial virus, adenovirus, influenza, or parainfluenza by fluorescent antibody (FA) or real-time polymerase chain reaction (RT-PCR) assay of nasopharyngeal aspirates.

Results: From a total of 16,018 patients included, 13,545 were tested for respiratory viruses and 44.6%(6,047) had positive samples identifying Influenza in 7.5%(456; 89%[406] influenza A, 11%[50] influenza B). Influenza frequency followed a seasonal epidemic pattern (May-July, the lowest average temperature months). The median age of influenza cases was 12 months (IQR: 6-23 months); 21% <6 months, 47% <1yo, 76% <2yo, 90% <5yo; 55.7% of cases were male. The most frequent clinical presentation was consolidated pneumonia (58.1%). Almost half of influenza cases had previous admissions for respiratory causes; 9% were readmissions; 61.2% had comorbidities; 25.7% (115/447) had complications. The average case fatality rate was 2%(9/450). The following were independent predictors for influenza infection: age ≥6 months, odds ratio(OR): 1.8(95% CI: 1.4-2.4); p<0.001; presence of chronic neurologic disease, OR:1.4 (95%CI: 1.0-2.1); p=0.04; previous admissions for respiratory causes, OR:1.5 (95%CI: 1.2-1.9); p<0.001; readmissions, OR:1.70 (95%CI: 1.2-2.4); p=0.005; clinical pneumonia, OR:1.6 (95% CI: 1.3-1.9); p<0.001; immunodeficiency, OR:1.7(95%CI:1.1-2.7); p=0.02. No significant association was found when comparing cases of both influenza A and B infection.

Conclusion: Influenza infection showed an epidemic seasonal pattern (May-July), with higher risk in children aged ≥6 months, pneumonia, previous admissions for respiratory causes or certain comorbidities.

*Keywords: Argentina; Child, Epidemiology, Influenza A; Influenza B; Seasonal Variation*



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Abstract No: 11331

## 2018 Influenza season in Argentina: multicenter study

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**Background:** Respiratory disease is 3<sup>rd</sup> cause of death in Argentina. Influenza vaccine is mandatory for children between 6-24 months. The objectives of this study were •to describe the clinical and epidemiological pattern of acute lower respiratory infection (ALRI), influenza (Flu) cases and to identify risk factors associated with Flu infection.

**Methods:** A prospective, multicenter cross-sectional study of patients admitted for ALRI in four Argentina regions (Buenos Aires province, Buenos Aires city, Rosario and Mendoza) between June and November 2018. Virological diagnosis: RSV, adenovirus(AV), influenza(IF) and parainfluenza(PI) was made by fluorescent antibody assay of nasopharyngeal aspirates or real time-PCR. A multivariate analysis was performed to found independent predictors of influenza infection factors comparing with others viruses.

**Results:** A total of 1,220 ALRI were included; 97,8% tested and 43,8%(523) had positive samples. Viral distribution: VSR:84.1%, IF:7.5%(56% type A, 44% type B), PI:5.5%, AV:2.9%. Median age:8 months (RI=3-17mo). ALRI lethality: 0.1%(2/1220). Influenza vaccination coverage (6-24 months):37% (over 475 vaccination cards evaluated). Influenza(n=39) showed a seasonal epidemic pattern (late winter). Median age:17 months(IR:10-38 months). Age distribution:<6 months(7.7%), 6-23 months(53.8%), 2-5 yo(23.1%), >5 yo(15.4%). Most frequent clinical feature was consolidated pneumonia(66.7%); 49% recorded previous ALRI hospitalization, 21% were born preterm, 69% had comorbidities; 20% required intensive care. No influenza death recorded. From 6-24 months influenza cases (n=21), 16 had vaccination card and 4 had complete influenza vaccine schedule. Independent factors of IF infection: age ≥6 months OR:7.1(CI95%=2.1-23.9)p<0.001 and pneumonia as clinical presentation OR:3.49(CI95%=1.6-6.9)p<0.001.

**Conclusions:** Half of IF cases had <17 months of age and 23% had 2-5 yrs (group of age involved in transmission). IF was equally distributed between types A and B with no difference in their clinical-epidemiological pattern. Influenza vaccine coverage was low in the studied population. IF infection was associated with children older than 6 months of age and diagnosis of pneumonia.

*Keywords: ALRI; Influenza; pediatrics; influenza vaccine; epidemiology*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 11353

## The impact respiratory syncytial virus and influenza viruses on hospitalization in Mongolia from 2017 to 2019

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**Background:** Respiratory syncytial virus (RSV) and influenza is the most common viral respiratory pathogens in hospitalized children with severe acute respiratory infections (SARI). Worldwide, it is estimated that RSV is responsible for approximately 33 million lower respiratory tract illnesses, three million hospitalizations, and up to 199,000 childhood deaths; the majority of deaths are in resource-limited countries like Mongolia. This study aimed to estimate the prevalence of RSV and influenza among the hospitalized patients with SARI from 7 district hospitals in Ulaanbaatar city from May 2017 to March 2019.

### Materials and methods:

1475 nasopharyngeal swabs were collected and were screened for RSV and influenza using real time reverse transcription-polymerase chain reaction in Virology Laboratory of National Center for Communicable Diseases. We were calculated the estimation of SARI on positivity rate of RSV and Influenza.

**Results:** 1302/1475 samples (88.3%) were from children under 5 years of age. There were 521/1475 (35.3%) samples positive for either RSV or influenza. 397/521 (76.2%) were positive for RSV, 124/521 (23.8%) were positive for influenza, 20/521 (3.8%) were co-infection. 124 samples were positive for influenza subtype tested: 94/124 (75.8%) were positive for A(H1N1)pdm, 17/124 (13.7%) were positive for A(H3N2), 13/124 (10.5%) were positive for influenza B. RSV positive rate in 0-5 months, 6-11 months, 12-23 months, 2-4 years were 33%, 29%, 26%, 30% respectively. By estimated, SARI hospitalization rates for RSV was 30.3 (24%) and influenza was 10.1 (8%) per 10,000 population. The RSV season preceded influenza season and continued for 18-19 weeks in 2017/2018 and 2018/2019 season. No significant difference in the onset of season in different age groups. The results shown the high impact of RSV on SARI hospitalizations during cold season in Ulaanbaatar.

### Conclusions:

It is important to have surveillance data updated on the impact of RSV and influenza related hospitalization at national level.

*Keywords: respiratory syncytial virus, influenza, hospitalization, SARI, impact*

Topic: Public Health: Disease Burden & Severity  
Abstract No: 11713

## The burden of influenza-associated respiratory hospitalizations in Bhutan, 2015- 2016

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**Introduction and Objectives:** Influenza burden estimates help provide evidence to support influenza prevention and control programs. In this study, we estimated influenza-associated respiratory hospitalization rates in Bhutan, a country considering influenza vaccine introduction.

**Methods:** Using real-time reverse transcription-polymerase chain reaction laboratory results from severe acute respiratory infection (SARI) surveillance, we estimated the proportion of respiratory hospitalizations attributable to influenza each month among patients aged <5, 5-49, and ≥50 years in six Bhutanese districts for 2015 and 2016. We divided the sum of the monthly influenza-attributed hospitalizations by the total of the six district populations to generate age-specific rates for each year.

**Results and Conclusions:** In 2015, 10% of SARI patients tested positive for influenza (64/659) and 18% tested positive (129/736) in 2016. The incidence of influenza-associated hospitalizations among all age groups was 50/100,000 persons (95% confidence interval [CI]: 45-55) in 2015 and 118/100,000 persons (95% CI: 110-127) in 2016. The highest rates were among children <5 years: 182/100,000 (95% CI: 153-210) in 2015 and 532/100,000 (95% CI: 473-591) in 2016. The second highest influenza-associated hospitalization rates were among adults ≥50 years: 110/100,000 (95% CI: 91-130) in 2015 and 193/100,000 (95% CI: 165-221) in 2016.

Influenza viruses were associated with a substantial burden of severe illness requiring hospitalization especially among children and older adults. The burden findings were presented to the National Immunization Technical Advisory Group (NITAG) leading to a NITAG recommendation for the use of influenza vaccine. The government has taken up the NITAG recommendation as is currently planning to vaccinate frontline healthcare workers and pregnant women, and is considering other risk groups such as children under 2 years of age and the elderly.

**Keywords:** Bhutan, burden, influenza, severe acute respiratory infections, surveillance

Topic: Public Health: Disease Burden & Severity  
 Abstract No: 11753

## Hospitalization Burden of Severe Acute Respiratory Infections Associated with Influenza in Niamey, Niger, 2015-2018

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### Background

Influenza burden estimates are challenging to calculate in middle-, low-income, and tropical climate countries. Few influenza burden estimates are available for these populations, including Niger. To address this limitation, we estimated the burden of severe acute respiratory infection (SARI) hospitalized influenza in Niamey, Niger using SARI surveillance and hospital admission data.

### Methods

To estimate the burden of influenza-associated hospitalizations, we conducted a Hospital Administrative Survey (HAS) in the capital city of Niamey. We estimated influenza-associated hospitalizations for 2015–2018 in Niamey using methods from the WHO Burden Manual. There are five admitting hospitals in Niamey; three are SARI sentinel surveillance sites. Admission logbooks from the five admitting hospitals were reviewed to identify patients admitted with any respiratory illness. The monthly proportion of SARI patients testing positive for influenza by RT-PCR from three surveillance sites was applied to all respiratory hospitalizations for the same month to estimate the number of SARI hospitalizations and estimate influenza-associated SARI hospitalizations from all five Niamey sites. Since all admitting hospitals in Niamey were included in the HAS, the Niamey population was used as the catchment population. Rates were calculated by dividing the estimated number of SARI hospitalizations in Niamey due to influenza by the catchment population.

### Results

During 2015–2018, there were 81,400 adult and pediatric admissions, including 6,087 respiratory admissions, at the five Niamey hospitals. We found that respiratory hospitalizations represented 1%-10% of total hospital admissions, depending on the hospital. Children <5 years represented 76% of total respiratory admissions. Through sentinel surveillance, 11% percent of SARI patients tested positive for influenza during the study period. We estimated an influenza-associated SARI hospitalization rate of 29.2, 8.8, 22.2 and 15.8 per 100,000 persons in 2015, 2016, 2017, and 2018, respectively. The mean influenza-associated SARI hospitalization rate between 2015–2018 was 13.0/100,000 (95% CI: 9.5-16.5).

### Conclusion

Influenza contributes to the burden of hospitalizations in Niamey, Niger. Further work to estimate the burden for the entire country is planned to help provide useful information for the Ministry of Health that could be considered for future prevention and control efforts.

*Keywords: Hospitalization, Burden, SARI, Niger*

Topic: Public Health: Epidemiology & Transmission  
 Abstract No: 10115

## Knowledge, Attitudes, and Behaviours (KAB) of Influenza Vaccination in China: Telephone Survey Study in 2017-2018 Influenza Season

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**Introduction:** The 2017-2018 influenza season was more severe than that of recent years in China, but the national sentinel surveillance system only measures the number seeking treatment at health facilities rather than the total population affected. With low vaccination coverage, individuals age<5 and the elderly particularly jeopardized by influenza for risk of severe illness and even death.

**Objectives:** This study aimed to estimate influenza like illness prevalence and influenza vaccination uptake during the 2017-2018 influenza season.

**Methods:** A retrospective cross-sectional study based on a randomized population-based telephone survey was conducted during the October 2017 to March 2018 influenza season to assess influenza-like illness (ILI) prevalence and prevention uptake among different demographic groups. The survey included questions about ILI symptoms, influenza vaccine uptake, healthcare seeking behaviors, prevention awareness, and vaccination willingness.

**Results:** 10045 individuals were enrolled with consent and completion of questionnaire and 2834 were self-reported to have suffered from ILI (28%). The ILI rate of those under age 5 and those age 5-14 were significantly higher ( $p<0.001$ ), and self-reported ILI of the economically developed urban metropolis increases as latitude increases. The overall willingness of vaccination rate is 45%. Hospitalization and seeking medicines from pharmacy is less frequent in urban metropolis. Age < 15 and age > 65 seek treatment more often and from outpatient/ emergency and hospitalization.

**Conclusion:** The increase of self-reported ILI rate in urban metropolis' as latitude increases may result from long lasting influenza season. The urban metropolis' had relatively higher vaccination coverage, but more needs to be done to ensure key populations are aware of their influenza risk, know prevention strategies and are vaccinated to prevent costly morbidity and mortality from influenza. Improving vaccination coverage through targeted policies, subsidizes and free vaccinations campaigns is necessary for those over age 60 as their vaccine coverage is relatively low.

*Keywords: influenza like illness, China, telephone survey, KAB*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10244

## EPIDEMIOLOGY OF INFLUENZA B VIRUS IN SINGAPORE, 2011-2017

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### Introduction and Objectives

Singapore is situated in the tropics where the epidemiological features of influenza are less distinct. Quadrivalent influenza vaccines (QIVs) containing a second influenza B strain have been available in Singapore since 2016. Understanding of the pattern of influenza virus circulation is paramount for effective prevention and control strategies. We described the epidemiology of influenza B virus from 2011 to 2017.

### Methods

We analyzed laboratory-confirmed virological data collected under the national influenza surveillance programme. Influenza activity was measured by the proportion of influenza-positive specimens from outpatients with influenza-like illness.

### Results

Influenza positivity ranged from 40.8% in 2011 to 51.9% in 2016 with peaks observed around middle and start/end of the year. Of the positive samples tested during the 7-year period, influenza A(H3N2) constituted the majority (45%) followed by influenza B (31%). The proportion of influenza B ranged from 18.5% in 2015 to 47.6% in 2012. Influenza B was the predominant virus type in 2012 and it was predominantly circulating with influenza A(H3N2) in 2014.

The annual proportion of influenza B among influenza-positive samples was highest in older children aged 5–14 years compared to other age groups. Among influenza B positive samples with the virus lineages characterized, a higher proportion of B/Victoria was detected in age groups of 5–14 years and 15–44 years from 2011 to 2012 and 2016. Influenza B/Yamagata lineage was more frequently characterized in age groups of 45–64 years and ≥65 years in the majority of years.

### Conclusion

The two influenza B lineages co-circulate in the community. While QIVs may provide added benefits over trivalent influenza vaccines, particularly in older children who are more affected by influenza B, there is a need for further studies to estimate the disease burden of influenza B during the years of mismatches and cost-effectiveness of QIVs in local context.

*Keywords: influenza; virus lineages; surveillance; Singapore*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10257

**PREVALENCE OF INFLUENZA AND CO-INFECTION WITH OTHER VIRAL PATHOGENS IN A SELECTED SAMPLE OF CHILDREN WITH ACUTE RESPIRATORY TRACT INFECTIONS IN SRI LANKA**

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**Introduction and Objectives:** Acute respiratory tract infections (ARTI) due to influenza virus is a common cause of mortality and morbidity globally. The purpose of this study was to determine the distribution of influenza and co-infections with other pathogens among children with ARTI.

**Methods:** A total of 502 nasopharyngeal aspirate samples (NPA) were collected from May 2016 to July 2018 from hospitalized children with  $\leq 4$  days' history of ARTIs in the paediatric ward of the General Hospital, Kegalle Sri Lanka. All NPA samples were tested for influenza A and B using immunofluorescence assay (IFA) (D3 UltraTM, USA). Samples positive for influenza A were further typed using a multiplex polymerase chain reaction (mPCR)

**Results:** Of the 502 NPA, 10/502 (1.99%) were influenza A H1N1pdm09 (Inf-A), 18/502 (3.58%) were influenza A H3N2 and 26/502 (5.17%) were influenza B (Inf-B). Co-infections have been noted in 13 children with detection rates of 2.58% (13/502). Of the 18 children infected with Inf A H3N2, 4 had co-infection with the respiratory syncytial virus (RSV) and one each had co-infection with Inf-B and Human parainfluenza 3 (HPIV-3). Of the 26 Inf-B 6 had co-infection with the RSV and one had co-infection with HPIV-3. None of the Inf-A H1N1pdm09 had co-infections with other pathogens. Most Influenza infection and co-infections were observed among boys between 6-18 months than girls (61.53%, 8/13). Children with both mono and co-infections had mild to moderate bronchiolitis.

**Conclusion:** HPIV and RSV were frequently detected as co-infecting viruses with Inf-A and B. ARTI is more common among boys below 18 months than girls. Majority of the coinfection were observed among children less than 12 months. However, no significant difference in disease severity was observed between children with mono and coinfection. Identifying the etiology of ARTI would be valuable to minimize the irrational use of antibiotics.

*Keywords: Influenza; children; immunofluorescence assay; epidemiology; co-infection.*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10321

## SEROPREVALENCE OF SWINE INFLUENZA A VIRUS IN LIVE PIG MARKET, KAFANCHAN SOUTHERN KADUNA

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Influenza virus is shared between humans and swine since the 1918 Spanish flu pandemic. It is a virus of public health implication. Pigs play an important role in the ecology of Influenza as the mixing vessel for the emergence of a novel pandemic strains. Three factors supporting the mixing vessel hypothesis are: susceptibility of swine to avian and human viruses; reassortment of swine/avian/human viruses' occurring in pigs which can transmit reassortant influenza virus to humans through occupational exposures. In the study area most farmers keep pigs in backyard and free range, and pigs are sold at live animal markets that holds at regular. This study provides information on the serotypes of some influenza virus in the pig market in Kaduna State.

A total of 305 samples were collected from December, 2017 to early February and in June, 2018. Rectal temperature before slaughter was recorded and blood were collected from exsanguinated pigs at Katsit slaughter slab. Serum were separated and kept at -20°C until further analysis. The sera were tested for swine influenza by competitive ELISA and positive samples were subtyped for swine influenza virus (H1 and H3) by haemagglutination Inhibition according to protocol described in the OIE manual.

The result showed overall seroprevalence of 28.5% (n=86). Of this, male animals' represented 9.4 % and the female 18.36% of the total percentage. The breakdown results for each month was as follows: [December; 11 positives (3.61%), January; 22 positives (7.21%), February 20 positives (6.56%), June; 33 positives (10.82%)]. There were 16 coinfections from the positive samples. This study revealed Swine Influenza subtype H1 and H3 are in circulation in pigs in Kaduna State and the co-infection observed is an indication of potential reassortment through joint infection of the host.

*Keywords: Swine Influenza; Seroprevalence; Coinfections; Southern Kaduna; Subtype*



Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10343

## FATAL SEASONAL INFLUENZA CASES IN RUSSIA IN 2015-2019

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Introduction and objectives.

This study aimed at monitoring fatal seasonal influenza cases in Russia in 2015-2019.

Methods.

RT-PCR. Virus isolation in MDCK cells. Hemagglutination inhibition assay. Neuraminidase inhibition assay. Sanger and NGS sequencing.

Results.

There have been 501 fatal seasonal influenza cases in 2015-2016 in Russia (497 H1N1pdm09 and 4 H3N2), 49 in 2016-2017 (42 H3N2, 1 H1N1pdm09 and 6 influenza B) and 84 in 2017-2018 (56 H1N1pdm09, 13 H3N2 and 15 influenza B). 66 A(H1N1)pdm09 and 22 A(H3N2) influenza-associated deaths were reported since December 2018 until February 2019.

In 2015-2018 132 A(H1N1)pdm09, 2 A(H3N2), 2 B/Victoria and 3 B/Yamagata viruses were isolated from fatal cases.

All isolated A(H1N1)pdm09 viruses were antigenically similar to A/Michigan/45/2015. Out of 35 A(H1N1)pdm09 viruses subjected to genetic analysis 3 belonged to 6B.2 clade, the rest were from 6B.1 clade. In hemagglutinin of 13 A(H1N1)pdm09 strains 222D/G/N polymorphism was detected, which has been previously associated with enhanced tropism of influenza viruses to the human lower respiratory tract.

One characterized A(H3N2) virus (2018) belonged to 3C.2a3 subclade and did not agglutinate guinea pig red blood cells in the presence of oseltamivir.

Antigenic properties of 3 isolated influenza B/Yamagata viruses from clade 3 were similar to B/Phuket/3073/2013.

Two B/Victoria strains belonged to clade 1A and were genetically similar to vaccine strain, though titers of ferret anti-B/Brisbane/60/2008 serum with these viruses were 8 times lower than the homologous titer.

Neuraminidase inhibitors susceptibility assessment revealed 3 A(H1N1)pdm09 strains which were resistant to oseltamivir and had H275Y mutation. The rest A and B influenza viruses were susceptible to oseltamivir and zanamivir.

Conclusions.

The majority of fatal influenza outcomes in Russia during 2015-2019 was caused by A(H1N1)pdm09 viruses. 3 A(H1N1)pdm09 strains with H275Y-associated oseltamivir resistance were identified during surveillance.

*Keywords: influenza; fatal; epidemiologic; surveillance*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10415

## Results of sentinel epidemiological surveillance of severe acute respiratory infections (SARI) in Ust-Kamenogorsk, Kazakhstan during seasons 2016/2017 – 2017/2018

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**Introduction:** The goal of this work was to describe the SARI morbidity during the last two epidemic seasons (2016/2017 - 2017/2018) in Ust-Kamenogorsk, Republic of Kazakhstan.

**Methods:** Comparative analysis was performed of SARI morbidity during weeks 40-20 of seasons 2016/2017 (season 2016) and 2017/2018 (season 2017) in our online database (<http://ses.dec.kz>).

**Results and discussion:** During the last epidemic seasons 3,306 patients were hospitalized in sentinel hospitals during weeks 40-20 of season 2017-2018, among them 1,139 cases (34.4%) who met the standard definition of the SARI case.

The hospitalization rate of SARI patients varied from 14.5% in 2016-2017 (*total hospitalized – 3,474, among them with SARI - 505*) to 34.5% in 2017-2018 (*total hospitalized – 3,306, among them with SARI – 1,139*). The growth was explained by introduction of the new standard definition of the SARI case.

227 patients were laboratory tested during epidemic season 2016-2017, and 334 patients during epidemic season 2017-2018.

The share of flu positives among SARI patients who were laboratory tested was 15.8 % (36) during epidemic season 2016-2017, and 27.8% (92) during epidemic season 2017-2018.

Circulation of viruses A(H3N2) and B was noted during epidemic season 2016-2017. The number and share of flu virus A(H3N2) was 21 (58.3%), and flu virus B was 15 (41.7%).

During epidemic season 2017-2018 flu viruses A(H1N1)09pdm, A(H3N2) and B circulated, with domination of A(H1N1)09pdm. Total 92 flu cases were registered, among them flu A – 76 (82.6%), by subtypes A(H1N1)09pdm – 39 (42.4%), A(H3N2) – 37 (40.2%), and B – 16 (17.4%).

Compared to the previous seasons, epidemic season 2017-2018 started late, during week 1 with flu B, while during the previous season the first flu cases were registered during week 48 with flu B.

### Conclusions:

During season 2016-2017 we observed prevalence of circulation of flu virus A(H3N2). During season 2016-2017 we did not note circulation of flu virus A(H1N1)09pdm among SARI patients. During season 2017-2018 we noted circulation of mostly flu virus A(H1N1)09pdm among SARI patients.

The number of counted SARI cases increased 2.4 times (34.4%) during 2017-2018, compared to 14.5% during 2016/2017, which was explained by introduction of the new standard definition of the SARI case.

Topic: Public Health: Epidemiology & Transmission  
 Abstract No: 10416

## Influenza in Taraz, Zhambyl region, Republic of Kazakhstan during epidemiological seasons 2013-2018.

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**Introduction:** The national program “Sentinel Epidemiological Surveillance” (SES) was introduced in the territory of Taraz City, Zhambyl region in 2009, recommended by World Health Organization, which allowed ensuring year-round examination of the materials collected from out-patient patients with symptoms of influenza-like illnesses (ILI) and hospitalized patients with symptoms of severe acute respiratory infection (SARI).

**Methods:** 3 hospitals and 4 polyclinics participate in SES in Taraz City, they ensure selection of the patients who meet the standard definitions of ILI and SARI cases. Swabs from nose/pharynx collected during the first three days from the onset of clinical symptoms were the subject of examination. Examinations of materials for influenza were conducted according to the algorithm: all samples were tested by using the method of polymerase chain reaction (PCR) in real time with «Amplisense» reagents. Then PCR positive, subtyped samples were retrospectively sampled for influenza virus isolation by using MDCK cell culture.

**Results:** Based on the results of laboratory diagnostics 2,358 samples from ILI and SARI patients were examined by using PCR method during seasons 2013-2018, among them 429 (18.1%) were positive for flu, including 174 (7.3%) – type A(H<sub>3</sub>N<sub>2</sub>), 111(4.7%) – type A(H<sub>1</sub>N<sub>1</sub>)pdm09, and 118 (5.0%) – type B. Among PCR positive samples total 54 (12.5%) isolates were isolated by using virology method on cell culture, including 27 (6.2%) – strains of flu type A(H<sub>3</sub>N<sub>2</sub>), 18 (4.1%) – strains of flu type B, and 9 (2.0%) – strains of flu type A(H<sub>1</sub>N<sub>1</sub>)pdm09. During each of five seasons flu type A/H3N2 and type B circulated annually, during season 2014/2015 flu type A/H3N2 (6.3%) prevailed, and during season 2016/2017 flu type B (10%) prevailed. Flu type A/H1N1pdm09 circulated alternating every other season. During seasons 2014/2015 and 2016/2017 flu type A/H1N1pdm09 was not detected. It should be noted that during 2013/2014 all three flu types circulated, with prevalence of A/H1N1pdm09 (11%), and the same picture was observed during 2015/2016, when the share of flu A/H1N1pdm09 was 10.3%. On the contrary, during season 2017/2018 all three flu types circulated, but with prevalence of A/H3N2 (14%).

**Conclusions:** Thus, during the last five years the epidemic process of each season was characterized by simultaneous circulation of flu viruses of different subtypes with domination of one subtype during the season. During five seasons 2013-2018 the annual increase of the number of examined patients was noted, as well as the number of positive results. Use of PCR testing in the system of SES of influenza allowed detecting the main influenza pathogens, effectively complementing the classical virology methods.

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10441

## Epidemiological surveillance of acute respiratory infections in the Intensive Care Units, Kazakhstan, 2017-2019.

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**Introduction.** The share of severe acute respiratory infections (SARI) was 16% from the total number of hospitalized patients with acute respiratory infection (ARI) in infectious diseases hospitals of Almaty and Astana cities, the Republic of Kazakhstan. 30% of patients with ARI went to the Intensive Care Units (ICU) of these hospitals. The goal of this study was to determine the morbidity and mortality rates among patients with SARI, flu and other respiratory viruses in ICU of infectious diseases hospitals of Almaty and Astana cities.

**Methods.** The design of this study was prospective epidemiological surveillance. Patients were included aged 0 and older, who were hospitalized in ICU and met the SARI case definition. Questionnaires were filled for all SARI patients, also samples were collected from nose and pharynx for flu and other respiratory viruses.

**Results.** 1,534 patients with all diagnoses were hospitalized in ICU during two flu seasons 2016-2018, among them the share of ARI was 27%. The share of SARI patients among ARI patients was 98%. 98% (444) of patients were covered by tests. In the etiology of SARI development the share of flu virus AH1N1pdm09 was 50%, AH3N2 – 19%, and B – 11%. 250 samples were tested for other non-influenza viruses, among them 97 were positive. The following non-influenza viruses prevailed: rhinovirus– 40.6%, respiratory-syncytial virus – 35.4% and adenovirus – 12.5%. 98.8% of patients were not vaccinated against flu. The main symptom among males was shortness of breath 76.5% (CI -1.58 – 5.34; p=0.0002), and the main symptom among females was headache 45.6% (CI – 0.1 – 0.4; p=0.00001). 12 mortality cases were registered among SARI patients, the mortality rate was 2.7%. 63.6% (7) of cases were children under one, two cases were aged 30-44, and one case aged 1-4, 5-14, and 15-29. Based on the test results, flu virus B was isolated in one patient, rhinovirus was isolated in four patients, and adenovirus was isolated in one patient. Other patients' results were negative for ARI.

**Conclusions.** Flu mortality among SARI patients in ICU was 11%. Total 1.2% of patients were vaccinated against flu, which indicates the need to increase the vaccination coverage. High share of rhinoviruses among patients with mortality demands further monitoring of other non-influenza viruses.

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10569

## Redefining influenza seasonality and aligning each country's immunisation policy to the influenza vaccine manufacturing cycle

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### Introduction and Objectives

The burden of influenza in each country varies during the year, and vaccine distribution should be aligned with when the most burden occurs. However, annual seasonal influenza vaccine manufacturing cycles align with temperate country seasonality in each hemisphere, yet influenza seasonality is poorly defined for many countries. The study introduces a novel and universal approach to defining and classifying seasonality that can be used to classify any country's influenza vaccine cycle alignment.

### Methods

Countries reporting to the World Health Organization's FluNet influenza virology database in 90% of weeks during 2011 through 2017 were included. A smoothed, standardised, average proportion of influenza occurring in each week of the year was used to determine degree of seasonality based on the range of average weekly variation. The proportion of activity occurring May through October was used to align influenza activity with a hemisphere's vaccine manufacturing cycle.

### Results

From 84 included countries, there were 2,239,208 positive influenza results, of which 26% were influenza type B. Degree of seasonality was moderately positively correlated with absolute value of latitude ( $r = 0.69$ ,  $p < 0.0001$ ). Latitude was strongly negatively correlated with the proportion of influenza occurring during May through October ( $r = -0.83$ ,  $p < 0.0001$ ). Thirteen countries (12% of the included global population), mainly in tropical zones, had influenza occurrence aligned with the opposite hemisphere's influenza vaccine manufacturing cycle. In tropical zones, concordance in the degree of seasonality and vaccine cycle alignment within regions and between adjacent countries was limited. In temperate zones, on average, influenza B peaked four weeks later than A.

### Conclusions

The study provides evidence for different population dynamics of influenza B compared with A. These findings highlight the challenge of optimising influenza vaccine recommendations that best serve countries which are in, or extend into, the tropics.

*Keywords: Influenza; seasonality; latitude; global; tropics*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10579

## EPIDEMIOLOGICAL STUDIES OF INFLUENZA INFECTION AMONG CHILDREN IN MALAYSIA

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### INTRODUCTION

Influenza is one of the most significant causes of acute respiratory tract infections (ARI) worldwide. Its ability to spread easily may cause seasonal epidemics. Children aged <6 years and those with underlying medical conditions are particularly at risk. We aimed to study the epidemiology of seasonal Influenza virus infection among children in Malaysia.

### METHOD

10155 nasopharyngeal swab samples from paediatric patients (age ≤18) suspected with respiratory tract infections were collected from Jul 2016-Feb 2019. 4 Influenza subtypes (non-specific influenza A, influenza A/H1, influenza A/H1-2009 and influenza A/H3) and influenza B were identified qualitatively by PCR method using Luminex NxTAG RPP reagent kit.

### RESULTS

2027 samples were positive for influenza infection, which gave prevalence of 20%. Data showed that children below 6 years old were susceptible to influenza infection, especially influenza A. 76% of the influenza positive samples came from children below 6 years. The predominant influenza A subtype was influenza A/H1-2009, followed by influenza A/H3, non-specific influenza A and influenza A/H1 in the descending order. Influenza A/H3 was the most common strain in 2016. Influenza A prevalence was higher than influenza B.

There were fewer recorded cases of influenza from the months of June - September. However, seasonal influenza infection was consistently much higher throughout the other months. There was no specific month whereby influenza infection peaks. Slight variations may be observed from year to year. 39% of patients infected with influenza were admitted to hospital.

### CONCLUSION

Influenza is one of the main causes for ARI in pre-school children. With this data, public health authorities should look at introducing influenza vaccination to pre-school children below 6 years to reduce child morbidity as well as the economic burden to family and society. Parents should also consider vaccinating their children before travelling to tropical countries.

*Keywords: epidemiology; influenza; children*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10716

## QUAIL REARING PRACTICES IN HOUSEHOLDS AND THE POTENTIAL FOR TRANSMISSION OF AVIAN INFLUENZA VIRUSES FROM QUAILS TO HUMANS, DHAKA, BANGLADESH

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In Bangladesh, avian influenza virus (AIV) A/H9N2 is endemic in quail, which may have a role in generating reassorted AIVs. In February 2015, population-based influenza surveillance detected a human infection of A/H9N2 with a history of exposure to sick quail reared in a Dhaka household. A qualitative study was conducted to explore quail rearing practices at the household level among households participating in our surveillance system.

We identified 52 (7.3%) of 716 households raising quail within the surveillance system. We purposively selected 20 households with the longest history of raising quail, conducting in-depth interviews with the household member who had raised quail longest within their household. We transcribed interviews, developed a coding system, and summarized coded data.

All participants reported raising quail, with a median of 3 months (range, 1-36 months). All respondents were women, average age of 28 years; most (90%) reported no primary education. Most respondents (75%) reported keeping quail with other birds in cages. Household members, including children, fed and bathed quail, separated sick quail, cleaned feeding pots and cages, and discarded feces. Most respondents (85%) reported children played with and often kissed quail. Sixty-percent reportedly slaughtering sick quail and 35% reported children assisting women when slaughtering quail. Twenty-five percent reported discarding dead quail in a waste receptacle at home. Household members washed hands with running water only after cleaning waste, slaughtering quail, and disposing of dead quail, with 20% reporting using soap. Few participants (15%) used polythene bags during handling of dead quail; none wore personal protection.

Women and children frequently had unprotected contact with quail while handling, slaughtering, or disposing of quail. Quail were frequently raised in cages with other birds, potentially increasing the risk of cross-species infection. Education on proper hygiene practices in households may decrease potential AIV transmission from quail to humans.

*Keywords: avian influenza virus; quail; household; transmission; human*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10734

## Occurrence of influenza viral RNA on surfaces in university campus

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### Introduction and objectives

School settings could facilitate transmission of influenza due to extensive mixing of students and sharing of facilities, but the contribution of indirect contact route to influenza transmission in university settings has not been studied before. The aim of this study is to examine the occurrence of influenza virus RNA on surfaces in university campus and to assess the potential role of contaminated surfaces in influenza transmission.

### Methods

We conducted surface sampling on commonly touched, less frequently touched and frequently passed surfaces in shuttle buses, amenities centre and lecture halls in a university in Hong Kong. For each collection episode, we collected a paired set of 5-15 surface swab samples in early morning (before-class) and in the evening (after-class). Polyester swabs were used to sample the targeted surfaces and swab samples were stored in viral transport media. Influenza A or B viral RNA from the surface swab samples were identified by a quantitative reverse transcriptase polymerase chain reaction assay.

### Results

From September 2017 to February 2018, we conducted 83 collection episodes (shuttle bus: 26; lecture hall: 32; amenities centre: 25) and collected a total of 1508 surface swab samples. We identified influenza A/B virus on at least one surface in 12/83 (14.5%) before-class and 15/83 (18.1%) after-class episodes. We identified influenza A viral RNA from these surfaces during Hong Kong's non-influenza season. And both influenza A and B viral RNA were detected during the influenza season, although based on local surveillance data the predominating strain was influenza B.

### Conclusion

We found influenza viral RNA on commonly-touched and less frequently touched surfaces in university campus. Influenza viral RNA was recovered on surfaces in both after and before period of expected high usage, indicating that influenza virus might be frequently inoculated and remain for a long time on these surfaces.

*Keywords: Influenza; viral RNA; surface; university campus; transmission*



Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10735

## Contamination of influenza virus on hands and objects of laboratory-confirmed influenza patients

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### Introduction and objectives

Hand and object contamination with influenza virus are essential steps of indirect contact transmission of influenza. Few studies reported the occurrence of influenza virus on human hands and objects in naturally occurring influenza infection. The objective of this study is to examine the prevalence of influenza virus contamination on hands and objects of influenza infected individuals.

### Methods

This study was conducted in out-patient clinics to recruit adults within 48 hours from ARI symptom onset. For each participant, we collected three pairs of hand swab samples, including baseline (without any intervention), after-cough and after-disinfection swab samples. Two object swab samples were collected, one from the pen provided for participant to sign the informed consent form and another from participant's personal object. All collected samples were tested by a quantitative reverse transcriptase polymerase chain reaction (qPCR) assay. Cell culture was performed on samples with qPCR positive results.

### Results

From January to April 2018 we have recruited 51 laboratory-confirmed influenza cases (18 influenza A and 33 influenza B cases). Influenza A/B virus RNA was detected on 22 (21.6%), 48 (47.1%) and 28 (27.5%) of 102 baseline, after-cough and after-disinfection hand samples respectively, while 3 (2.9%) and 6 (5.9%) of 102 baseline and after-cough hand samples were culture positive. RNA recovery rate was 13.7% (7/51) on provided objects (pen), but no virus could be cultured. 21.6% (11/51) of personal objects were qPCR positive and 3.9% (2/51) of them were culture positive.

### Conclusion

We detected influenza virus RNA on all types of hand and object swab samples and viable influenza virus on baseline and after-cough hands and personal objects of laboratory-confirmed influenza patients. To prevent and reduce indirect contact transmission of influenza, it is recommended that individuals should pay particular attention to proper hand hygiene practice and frequently disinfection of shared objects.

*Keywords: Influenza; hand; object; detection; transmission*

Topic: Public Health: Epidemiology & Transmission  
 Abstract No: 10737

## Influenza-like illness surveillance in Khovd, Orkhon, Darkhan, and Dornod provinces in Mongolia from 2016 to 2019

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**Background:** Epidemiological and virological surveillance of influenza in Mongolia is performed by a national network covered 115 sentinel sites coordinated by the National Influenza Center (NIC) in collaboration with District and Provincial Health Centers. The purpose of this study was to investigate the viral causal agents of influenza-like illness (ILI) in Khovd (Western region), Dornod (Eastern region), Orkhon, and Darkhan (Central region) provinces using the reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assays.

**Method:** Nasopharyngeal specimens were collected from 911 patients with ILI between January 2016 and March 2019. RT-LAMP assays were performed to detect influenza viruses (FluV: FluA, FluB, H3, and H1pdm), respiratory syncytial virus (RSV) A and B, human parainfluenza virus (HPIV)-1, -2, and -3, coronavirus (CoV) OC43, NL63, rhinovirus A (RVA), and human metapneumovirus (HMPV) in the Virology Laboratory of Regional Diagnostic Treatment Centers in 4 provinces in Mongolia. The collected data were analyzed in the NIC.

**Results:** The age distribution of the 911 patients were as follows: 610 (67.0%), 85 (9.3%), 91 (10.0%), and 125 (13.7%) were aged younger than 5 years, 6-9 years, 10-24 years, and older than 25 years, respectively. During the study period, 337/911 (37.0%) samples tested positive for any viruses, and of those, 148, 77, 50, 34, 33, and 2 samples tested positive for FluV, RSV, CoV, RVA, HPIV, and HMPV, respectively. In the group aged younger than 5, respiratory viruses other than FluV were detected predominantly (67.0%), whereas FluV were detected mainly (73.5%) in those aged 6-9 years. The majority of the detected viruses in Darkhan were FluV. FluV were mostly detected and followed by RSV in Orkhon and Khovd. In Dornod, FluV, RSV, CoV, and HPIV were detected almost equally.

**Conclusions:** During the study period, the prevalence rates of respiratory viruses differed in 4 provinces.

**Keywords:** ILI, Surveillance, RT-LAMP

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10784

## Evaluation of Influenza Case Definitions in a Primary Care Database for Use in Real World Evidence Research

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### Introduction & Objectives

Electronic medical records (EMRs) are increasingly being used to generate real world evidence as this data is more complete and has a lower risk of up-coding. Whilst diagnostic codes are routinely entered in primary care EMRs, lab-confirmation (gold standard influenza diagnostic) is not systematically performed and may therefore not be available. This study compares diagnostic code sets for influenza to the Centers for Disease Control (CDC) flu surveillance data and evaluates their concordance within a cohort of vaccinated individuals in the U.S.

### Methods

National incidence of lab-confirmed influenza in the 2017-18 season was obtained from the U.S. CDC. Influenza diagnostic code sets were obtained from the Armed Forces Health Surveillance Center (AFHSC). Code Set A, intended for broad surveillance, identifies ILI (International Classification of Diseases [ICD-10] codes: B97.89, H66.9\*, J00, J01.9\*, J06.9\*, J09.\*, J10.\*, J11.\*, J12.89, J12.9, J18.\*, J20.9, J40, R05, or R50.9 in any diagnostic position) whilst Code Set B, intended for specific epidemiological investigations, identifies influenza diagnoses (ICD-10 codes: J09\*-J11\* in any diagnostic position). The national incidence rate of influenza, derived from the CDC data, was compared to the incidence of flu in a population of influenza vaccinees identified from a large ambulatory EMR.

### Results

The incidence of influenza as defined by the diagnostic Code Sets A and B followed the same distribution as the CDC's surveillance data for both ILI (Figure 1A) and influenza diagnosis (Figure 1B). Influenza defined by Code Set B aligned nearly perfect with the surveillance data for confirmed cases of A/H3N2, the predominant strain during the 2017-18 season.

### Conclusion

There is strong concordance between the AFHSC diagnostic code sets for influenza and the incidence of lab-confirmed influenza in a vaccinated cohort. Use of diagnostic codes in a large ambulatory EMR dataset provides a reliable alternative to lab-confirmed influenza.

*Keywords: electronic medical records, influenza, ICD codes, vaccines, case definition*

Topic: Public Health: Epidemiology & Transmission  
 Abstract No: 10789

## Strengthening Pediatric Influenza Vaccination Offering and Acceptance: Findings from The Pediatric Influenza Vaccination Optimization Trial (PIVOT)

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### Introduction and Objectives

The Pediatric Influenza Vaccination Optimization Trial (PIVOT) consists of four integrated studies concerned with strengthening pediatric influenza vaccination offering and uptake.

### Methods

Studies I, III & IV involved prospective cohort research with 24 health-care providers (HCPs) and 207 parents of infants. Clinicians provided parents information about pediatric influenza and vaccine options and were asked about their perceptions of parents' intentions to vaccinate their children. Structured surveys assessed parents' attitudes, perceptions, and intentions to vaccinate their infant. Predictors of parental intention to vaccinate were estimated using multivariable logit models.

Study II randomized physicians to an accredited, theoretically-based continuing medical education (CME) program (n=33) or to no CME (n=35). Physicians in each study arm communicated with parents (n=614) concerning pediatric influenza and vaccine choice. The proportions of influenza vaccinations administered were compared between the two study arms.

### Results

Study I established that brief provider-parent communication resulted in high levels of parental acceptance (66.6%) of adjuvanted seasonal pediatric influenza vaccine (aTIV). Acceptance was driven by parental perceptions of safety, efficacy and importance (OR=79.25; 95% CI (6.05-1037.50)). Study II demonstrated that theoretically informed CME strengthened providers' pediatric influenza vaccination offering and elevated vaccination uptake (OR=1.52, 95% CI (1.09 to 2.12)). Study III established that approved-but-unfunded status served as a negative heuristic: parents agreed that public health would fund aTIV if it were: really safe (90.0%), really effective (86.0%) and really important (87.9%). Study IV determined that the correlation of clinicians' assessment of parents' intention to vaccinate ranged between 0.15 and 0.48 depending on vaccine cost and accounted for a small proportion of the variance.

### Conclusions

Findings from this integrated series of studies provide information about vaccine offering and acceptance for parents of young children and HCPs. This research identifies key modifiable factors related to vaccine hesitancy at both the parental and physician levels.

*Keywords: influenza, vaccine hesitancy, pediatric, parental acceptance*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10808

## Age-specific burden of influenza hospitalization in infants and young children in Singapore, 2005-2015

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### Background

Infants and young children are at particularly high risk of influenza, but the incidence of hospitalisation in tropical settings with year-round influenza transmission is not well described. We estimated hospitalisation rates in children in Singapore between 2005 and 2015. We also compared the epidemiology between pandemic and non-pandemic years.

### Methods

We used laboratory-confirmed influenza hospitalisations data from KK Women's and Children's Hospital, the single largest maternal and child specialist public hospital in Singapore, to construct a model to estimate age- and sex-specific influenza A and B admissions for the whole of Singapore between 2005 and 2015. We estimated hospitalisation rates per 100,000 children up to age 29 months using population estimates derived from published birth and mortality statistics. We used Monte Carlo simulation to account for parameter uncertainty.

### Results

Influenza A hospitalisation rates per 100,000 children over the 11-year period were 509 in infants <6 months, 301 in those 6-11 months of age, and 226 in children aged 12-29 months. Rates were around 25% higher in males than females at all ages. Infants aged one and two months had the highest hospitalisation rates, at 877 and 1199 per 100,000 respectively in 2015. Corresponding influenza B hospitalisation rates were much lower at 67, 59 and 41 per 100,000 respectively. There was no apparent difference in age-specific rates between pandemic and non-pandemic years.

### Conclusions

Our analysis indicates a high burden of paediatric influenza hospitalisation in Singapore, particularly among very young infants <6 months. Improving maternal influenza vaccination coverage even with trivalent seasonal influenza vaccine could have substantial impact on influenza burden in neonates in light of the low burden of influenza B in the first two months of life.

*Keywords: influenza, disease burden, hospitalisation, children, epidemiology*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10809

## Acceptance and feasibility of school-based seasonal influenza vaccination in Singapore

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### Introduction and objectives

Influenza is a major cause of disease in children. Seasonal vaccination of school children has been shown to be cost-effective to improve vaccine uptake and reduce disease burden, and provide indirect protection to unvaccinated groups. The acceptance and feasibility of school-based influenza vaccination are likely to be highly context-specific, but limited data exist from tropical settings with year-round transmission. This study aimed to assess acceptability and feasibility of a school-based, seasonal influenza vaccination programme in Singapore.

### Methods

We conducted qualitative in-depth interviews with key stakeholders, including healthcare professionals, representatives of relevant ministries, pre-school principals and parents to assess their perspectives on a potential school-based, seasonal influenza vaccination programme. Interviews were transcribed verbatim and analysed using thematic analysis. We coded interview transcripts using a standardised coding scheme and grouped codes into higher-order themes.

### Results

We conducted 40 interviews. Overall, participants across stakeholder groups were supportive of school-based, seasonal influenza vaccination in Singapore. However, stakeholders pointed to the need for context-specific cost-effectiveness analyses and more comprehensive evidence on local influenza transmission patterns, to stimulate political will for a school-based programme. Other major challenges identified by stakeholders included the complex logistics and cost of sustaining the programme over time. The three most common recommendations included i) implementing the programme in primary and/or secondary schools, where existing vaccination infrastructure and vaccination of older children would facilitate logistics; ii) offering free or subsidised vaccination to increase uptake, ensure equitable vaccine access and increase the programme's acceptability; and iii) complementing the programme with age-appropriate public education and awareness campaigns.

### Conclusions

In Singapore, school-based influenza vaccination is likely to be more feasible and acceptable in older children not currently in a recommended group for vaccination. A better informed, evidence-based rationale is required to estimate the programme's impact and gain full support across stakeholder groups.

*Keywords: influenza, influenza vaccine, school-based vaccination*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10816

## Influenza Surveillance And Out Break Investigation In Ethiopia, 2016: Establishing and Maintining Strong Laboratory Surveillance can help Timely Detect Unusual Influenza Activity

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**Introduction:** Laboratory based influenza sentinel surveillance is a key to undergo influenza surveillance. Influenza virus has focused attention worldwide due to their rapid molecular changes and its nature of high rate of transmission. Confirming the type and subtype of influenza viruses has critical advantages for early prevention and control of potential outbreak and for better case management. Influenza sentinel surveillance in Ethiopia has started in November, 2008 with one ILI site and then expanded to 8 sites.

**Methods:** In 2016, we received 929 swab samples from seven out of eight surveillance sites (3 ILI sites plus 4 SARI) from ARI cases fulfilling case definitions and 140 samples from ARI outbreak that occurred in different regions of the country and Addis Ababa. RNA was extracted manually by QIAGEN RNA extraction mini kit and analyzed using RT-PCR for typing and sub typing of the influenza virus by CDC protocol. **Result:** Among 929 sentinel surveillance samples tested, 274 (29.5%) samples were positive for influenza and Influenza A/AH3 accounted for 120(12.92%), H1N1pdm09 77(8.28%) and Influenza B 77(8.28%). From 120 ILI outbreak samples tested 79(65.8%) were positive for influenza A of which 75(97.4%) were due to H1N1pdm09. The SARI outbreak of Addis Ababa also showed 8/20(35%) H1N1pdm09 positivity. No influenza B was detected in any of the outbreak samples.

**Conclusion and Recommendation:** We confirmed significant number of samples for influenza virus during the period 2016 and influenza A/H3, H1N1pdm09 and influenza B were the circulating influenza viruses. The early 2016 ILI and SARI outbreak was also identified to be due to H1N1pdm09 virus. This showed that influenza virus can be a threat for public health at any time and the importance of influenza surveillance has a paramount importance for early detection and right response plan. The surveillance system should be strengthened to assess any influenza virus activity.

*Keywords: influenza, surveillance, outbreak, Laboratory, RT- PCR, Ethiopia*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10829

## THE EVOLUTIONARY DYNAMICS OF INFLUENZA A VIRUSES CIRCULATING IN MALLARDS IN DUCK HUNTING PRESERVES IN MARYLAND, USA

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### Introduction:

Duck Hunting Preserves (DHP) have high densities of dabbling ducks that are raised locally or transported from other locations, providing potential breeding grounds for the evolution of influenza A virus (IAV).

### Methods:

Through an eleven-year collaboration (2003-2013) with seven DHPs in Maryland, USA, we studied the genetic diversity and spatial dynamics of IAVs circulating in the DHPs in captive-reared mallard ducks over this time period, using the Bayesian genealogical framework.

### Results:

We detected 8% of IAV frequency in the DHPs (290/3705). The IAVs observed in the DHPs had high genetic diversity, including 12 HA subtypes, 9 NA subtypes, and 32 HA-NA combinations. The diversity was predominantly generated by new viruses being introduced from wild birds into the DHPs each year, with no evidence of viral persistence across years and relatively little viral gene flow between DHPs. Maryland is situated in the Atlantic flyway, and the IAV population circulating in the DHPs derives predominantly from viruses circulating in the Atlantic and neighboring Mississippi flyways, and from birds that share a similar habitat and belong to the same Anatidae family.

### Conclusion:

Overall, we find that sentinel sites import many genetically diverse viruses from similar wild birds each year, but we do not observe a role for DHPs as breeding grounds for long-term viral persistence, or as an important source of viruses for wild birds in the Atlantic flyway.

*Keywords: influenza A virus; bird flu; evolution; phylogenetic analysis; Bayesian analysis*



Topic: Public Health: Epidemiology & Transmission  
 Abstract No: 10836

**UNDERLYING CARDIOPULMONARY CONDITIONS AS A RISK FACTOR FOR INFLUENZA AND RESPIRATORY SYNCYTIAL VIRUS INFECTION AMONG COMMUNITY-DWELLING ADULTS AGED ≥65 YEARS IN THAILAND: FINDINGS FROM A TWO-YEAR PROSPECTIVE COHORT STUDY**

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**Introduction:** Adults with cardiopulmonary conditions are at increased risk for influenza and respiratory syncytial virus (RSV) infection but few data are available from middle-income countries. Using longitudinal prospective cohort data from a study of influenza vaccine effectiveness in Thai adults aged ≥65 years, we compared the incidence of influenza and RSV in those with and without cardiopulmonary conditions.

**Method:** During May 2015-May 2017, adults in a rural province were followed weekly with year-round surveillance for acute respiratory illness (ARI), defined broadly as new onset or worsening of cough with or without fever, and hospitalized ARI. When ill, participants self-collected nasal swabs (validated elsewhere) for reverse-transcription polymerase chain reaction for influenza and RSV. Multi-variable Poisson regression was used to calculate incidence rate ratios (IRR) after adjusting for age, sex, and influenza vaccination status.

**Results:** Overall, 3,220 adults were enrolled with a median age of 71 years (IQR 68-76); 1,324 (41%) were male and 983 (31%) had ≥1 underlying cardio-pulmonary condition; most commonly hypertension (755; 76.8%), chronic obstructive pulmonary disease (131; 13.3%), asthma (73; 7.4%), and chronic bronchitis (68; 6.9%). Influenza A(H3N2) was the predominant influenza strain each year. The median time from symptom onset to swab collection was 1 day (IQR 1–2). Overall, 105 (3.3%) participants had an influenza virus infection and 81 (2.5%) had an RSV infection. Compared to adults without cardiopulmonary conditions, those with cardiopulmonary conditions had a higher incidence of ARI (443/1000 person-years vs 352/1000, p<0.001) and hospitalized ARI (31.4/1000 vs 3.8/1000, p<0.001). Adjusted IRR for RSV, influenza A, and influenza B were 1.64 (95%CI 1.04-2.58), 1.59 (95%CI 1.04-2.45), and 1.16 (95%CI 0.25-3.71), respectively.

**Conclusion:** Older adults in rural Thailand with cardiopulmonary conditions are at increased risk for influenza A and RSV infections. This population should have access to influenza vaccines and other respiratory illness prevention measures.

*Keywords: Influenza, respiratory syncytial virus, incidence, older adult, Thailand*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10838

## RISK FACTORS FOR HOSPITALIZATION AMONG INDIVIDUALS WITH INFLUENZA IN BANGLADESH, 2007-2017

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**Introduction and Objective:** The Ministry of Health in Bangladesh is concerned about the dual burden of infectious and non-communicable diseases in its population. Influenza virus illnesses may lead to serious complications for individuals with pre-existing chronic conditions. We investigated prevalence of influenza and pre-existing chronic conditions and identified risk factors of hospital admission among them.

**Methods:** During 2007–2017, we identified outpatient influenza-like illnesses (ILI), defined as subjective or measured fever  $\geq 38^{\circ}\text{C}$  and cough within 10 days of symptom onset and severe acute respiratory infections (SARI) as hospitalizations with history of ILI in 14 tertiary level hospitals. We collected demographic and clinical information and obtained nasopharyngeal and throat swabs to detect influenza viruses through real time rt-PCR. We calculated odds ratios to identify factors associated with hospitalization (i.e., SARI versus ILI) using multivariable logistic regression adjusted for year, peak influenza circulation months and hospital location.

**Results:** Influenza was detected in 16.8% (954/5,693) of ILI episodes and 19.6% (2,473/12,645) of SARI episodes. The median age of among ILI cases was 28 years (IQR: 21–40); 51% were male. The median age among SARI cases was 40 years (IQR: 25–50) and 66% were male. None of the case-patients had seasonal influenza vaccination or were treated with oseltamivir for their illness prior to hospitalization. Age  $>65$  years (AOR=5.1, 95% CI: 3.8–6.9), male sex (AOR=2.0, 95% CI: 1.01–3.9), history of asthma (AOR=4.9, 95% CI: 2.5–9.6), chronic obstructive pulmonary disease (AOR=7.8, 95% CI: 1.1–53.8), diabetes (AOR=3.8, 95% CI: 2.3–6.4), and cardiovascular disease (AOR=4.3, 95% CI: 2.4–7.6) were independently associated with SARI case-status.

**Discussion:** Men, persons aged  $>65$  years, and with pre-existing conditions like asthma, chronic obstructive pulmonary disease, diabetes, and cardiovascular disease were at greater odds of requiring hospitalization during their influenza illnesses. Bangladesh might benefit from an evaluation to determine if influenza vaccination would be cost-effective in preventing hospitalizations in these subpopulations.

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10874

## PRESENCE OF INFLUENZA VIRUS ON TOUCH-SURFACES IN PRIMARY SCHOOLS AND KINDERGARTENS

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**Introduction:** School as a semi-closed setting where children spend prolonged hours provide a favourable environment for heightened influenza transmission, however transmission in school is not well understood. Indirect contact transmission may be more significant amidst crowding of children due to the presentation of infection and heightened contact behavior. Presence of influenza virus on touch-surfaces serve as a pre-requisite for the feasibility of indirect contact transmission in such settings.

**Method:** 4 primary schools and 3 kindergartens were enrolled as study sites for this observational study, each site was visited weekly for 6 weeks during the winter influenza season 2017/18. During each visit, environmental samples were collected before and after school day. Samples were collected using pre-moistened polyester-tipped swab applicators from selected high-touch surfaces including desk, chair, bookshelf, handrail, and low-touch surfaces such as extra furniture. Samples were screened for influenza A and B virus by qPCR; samples with Ct value <40 were considered positive and subjected to viability testing.

**Results:** 1352 samples were collected from 29 sampling sessions during the peak of influenza B transmission in Hong Kong according to local surveillance data. Overall, 7/11 classrooms sampled had at least 1 positive touch-surface. 4 and 8 samples (<1%) were positive for influenza A and B virus respectively with Ct values ranging 25 - 39 (median 39), from high- and low-touch student desks, chairs, bookshelves and doorknobs. 8/12 (67%) positive samples were collected at the beginning of school day. None of the positive samples was viable.

**Conclusion:** The presence of influenza virus on touch-surfaces in schools and viral load of positive samples are low. Such presence is more frequently found on commonly-touched surfaces. Presence of virus on touch-surfaces before school day may indicate insufficient disinfection effort, or the settling of airborne virus particles after school day.

*Keywords: Influenza virus; Schools; Surfaces; Environment*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10876

## INFLUENZA TRANSMISSION IN HOUSEHOLDS: PRELIMINARY RESULTS FROM THE INFLUENZA TRANSMISSION EVALUATION STUDY (FluTES)

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**Introduction and Objectives:** Few contemporary studies have characterized the transmission of influenza in households. We conducted a case-ascertained study of influenza transmission within households.

**Methods:** Patients who tested positive for influenza and were the first person with symptoms in a household (index cases) were eligible for the study. Index cases together with other household members were enrolled and followed through household visits. Information on respiratory symptoms and mid-turbinate nasal swab samples were collected daily from household members until seven days after the most recent acute respiratory disease onset in the family. Verified influenza vaccination status, antivirals use, and social contacts within households were systematically collected. Respiratory samples were tested with RT-PCR for identification of influenza infection, including virus type, subtype and lineage.

**Results:** During the 2017-2018 season, we enrolled 116 households in Nashville, TN, encompassing 330 total household members. The median age of the 116 index cases was 33.5 years (interquartile range: 12.5 – 54.5), 55% were female, and 82% were white and 8% were Hispanic. After the diagnosis of influenza infection in index cases, antivirals were used by 65% of index cases and by 11% of other household members. Influenza vaccination was verified in 47% of index patients and in 50% of other household members, for an overall 49% vaccination coverage. Secondary laboratory-confirmed influenza infections occurred in 25 (22%) of study households. Study enrollment will continue for two additional seasons. Multivariable assessments of the effectiveness of vaccination and antivirals are planned.

**Conclusion:** This preliminary assessment indicates a moderate risk of secondary transmission of influenza within households, in the context of moderate vaccination and antiviral use.

**Keywords:** household; transmission; influenza; vaccine; antiviral

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10907

## IS FRAILTY A RISK FACTOR OF INFLUENZA-LIKE ILLNESS? A PROSPECTIVE COMMUNITY COHORT STUDY OF OLDER PEOPLE IN HONG KONG

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**Introduction and objectives:** Older people suffer from a heavy disease burden of influenza. Few studies have evaluated the relationship between frailty and influenza, despite of an increasing public health concern on aging populations. We conducted a prospective cohort study in community-dwelling older people in Hong Kong, with the aim to evaluate the impact of frailty on the risk of influenza-like illness (ILI) in the community setting.

**Methods:** 231 older people aged 65+ years were recruited from December 2016 - April 2017, and 227 participants (98%) were successfully followed up until April 2018. Individual frailty was measured by Fried frailty index (FFI). The ILI incidence in the participants was collected by monthly phone calls. We used a logistic regression model with adjustment of age, BMI, vaccination history, chronic diseases, indoor temperature, absolute humidity, and housing characteristics, to estimate the odds ratio (OR) and 95% confidence interval (CI) associated with influenza-like illness.

**Results:** The mean age of participants was 77.2 years, ranging from 65 to 95 years. There were more female participants (81%) in this cohort. The participants were classified into three groups according to the FFI criteria: 58 as robust, 154 as pre-frail and 19 as frail groups. Of them, 163 (70%) received influenza vaccines during the study period, and 68 reported a total of 87 ILI episodes. We found that the frailty groups had a higher risk of ILI incidence compared with the robust group, with an OR estimate of 4.57 ( $p = 0.036$ ). The pre-frailty group also had a higher risk of ILI (OR=2.19), but not statistically significant ( $p = 0.082$ ).

**Conclusion:** The findings demonstrated that frail older people might have a higher chance of ILI. More interventions should be engaged among this high-risk population. Further analyses will be conducted when more follow-up data are collected.

*Keywords: Influenza, elderly, frailty, community-dwelling, epidemiology*

Topic: Public Health: Epidemiology & Transmission  
 Abstract No: 10909

## Characterising the role of absolute humidity on influenza transmission and seasonality in a subtropical city, Hong Kong

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**Background:** Unlike temperate regions, influenza virus epidemics can have weak seasonality in tropical and subtropical locations. The underlying mechanisms of influenza seasonality remain difficult to disentangle, particularly in the tropics, where several drivers simultaneously modulate influenza seasonality. Ambient absolute humidity is one of such potential extrinsic drivers, perhaps because of its correlation with indoor relative humidity, and our aim was to characterize the role of absolute humidity on influenza transmission in Hong Kong.

**Methods:** We analyzed surveillance data on influenza virus activity and meteorological factors in Hong Kong during 1998-2017. We used a branching process model to estimate the instantaneous reproduction number ( $R_t$ ), a real-time measure of transmissibility, which is highly driven by depletion of susceptibles ( $S_t$ ) along with extrinsic drivers. Hence, we estimated the transmission rate  $\beta_t = R_t / S_t$  and investigated the underlying association between  $\beta_t$  and extrinsic factors through multivariable nonlinear regression approach and quantify the influence of various extrinsic factors on transmissibility.

**Results:** We identified a total of 54 distinct influenza seasons for different influenza type/subtypes over 20 years (1998-2017). We found a non-linear U-shape association between transmissibility and absolute humidity across all influenza type/subtypes in Hong Kong. The improved model could explain 10%-17% (for seasonal influenza) and 5% (for pandemic influenza) of variance in transmissibility by absolute humidity over the basic model. Sensitivity analysis indicated the fact that whole population was not initially susceptible during each epidemic.

**Conclusions:** The non-linear (U-shape) effect of humidity on influenza transmission may contribute to the distinct irregular patterns of influenza seasonality observed in subtropical areas, including the occurrence of summer epidemics.

**Keywords:** Influenza virus; transmissibility; absolute humidity; statistical models; sub-tropical city

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10919

## Meteorological drivers of respiratory syncytial virus infections in Singapore

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Background: Meteorological drivers are known to affect the transmissibility of respiratory viruses, including respiratory syncytial virus (RSV), but there are few studies quantifying the role of these drivers.

Methods: We used daily RSV hospitalization data to estimate the daily effective reproduction number ( $R_t$ ), a real-time measure of transmissibility, and examined its relationship with environmental drivers in Singapore during 2005-2015. We used multivariable regression models to quantify the proportion of the variance in  $R_t$  explained by each driver, by comparing these improved models to a basic model including depletion of susceptible and inter-seasonal effects.

Results: The median  $R_t$  value across 11 epidemics was 1.03, and ranged from 2.32 at the start of epidemic to 0.40 at the end of epidemic. Most of the variance was explained by the intrinsic factors in the basic model. Meteorological drivers explained a further 15% of the variance in RSV transmissibility. While higher mean temperature and diurnal temperature range (DTR) were associated with increased RSV transmissibility, higher maximum wind speed and precipitation were correlated with decreased transmissibility. The time series of mean temperature, DTR, maximum wind speed and precipitation could explain a maximum of 3.40%, 2.29%, 4.85% and 4.13% of the variance in  $R_t$  respectively.

Conclusions: We found that meteorological drivers were associated with RSV transmissibility. The negative association of maximum wind speed on transmissibility indicates the virus might circulate more in lower wind flow. Further lower DTR was associated with lower RSV transmissibility, indicating that smaller temperature fluctuations over short periods may limit RSV spread. These findings could help to predict surges in community RSV incidence and could have implications for the design of ventilation features in hospital settings to mitigate nosocomial transmissions.

*Keywords: Respiratory syncytial virus; transmissibility; meteorological drivers; statistical models; Singapore*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10922

## THE IMPACT OF INDOOR ENVIRONMENTAL FACTORS ON INFLUENZA-LIKE ILLNESS IN THE COMMUNITY-DWELLING OLDER POPULATION OF HONG KONG

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**Introduction and Objectives:** The relationship between environmental factors and influenza virus activities is still unclear in tropical and subtropical regions. The controversy might be due to lack of indoor environmental data, because people spend more time in indoors. We conducted a prospective cohort study in community-dwelling older population to evaluate the impact of indoor temperature, relative humidity (RH) and absolute humidity (AH) on the risk of influenza-like illness (ILI) in a subtropical city.

**Methods:** 231 participants aged 65+ years were recruited during 12/2016 – 04/2017, and followed up until 04/2018. One digital data logger was installed in the living room of each household to collect hourly indoor temperature and RH during the study period. Influenza-like symptoms were tracked by monthly phone calls. We used a time-stratified case-crossover analysis to estimate the accumulative excess risk (ER) of ILI over preceding four days that was associated with temperature, RH and AH in univariate and multivariate conditional logistic models.

**Results:** There were 87 episodes of influenza-like illness occurred in 68 participants (annual incidence rate: 29.4%) during the study period. Annual mean and range of indoor temperature, RH and AH were 25.1°C (9.2-42.4), 68.4% (15.0-100.0) and 23.3g/m<sup>3</sup> (2.4-56.4), respectively. We estimated that per unit increment of AH was associated with 17.3% (95% confidence interval 1.1%, 31.0%) decrease of ILI in winter season. Negative but non-significant associations with indoor temperature (ER -25.2%, 95%CI -46.2%, 4.1%) and RH (ER -6.54%, 95%CI -13.7%, 1.3%) were found in winter season. None of the indoor environmental factors showed significant association with ILI in summer season.

**Conclusion:** Our study provides evidence that older people living in the home with lower indoor AH had a higher risk of ILI in winter season. Further analysis with consideration of daily activity patterns will be conducted to investigate the impacts of indoor environments.

*Keywords: temperature, humidity, indoor, influenza, elderly*



Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10954

## NOTHING SECOND-RATE ABOUT INFLUENZA B

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### Introduction

Influenza B viruses are regularly perceived as a lesser public health problem than influenza A. It is commonly stated that they mostly affect the young, and typically cause outbreaks of lesser magnitude and severity than outbreaks with influenza A viruses.

We here describe experiences from the 2017/2018 influenza season in Norway, which indicate that such perceptions of influenza B epidemiology fail to capture the full picture.

### Methods

The influenza outbreak was studied using data from the following influenza surveillance components: influenza-like illness (ILI) in primary health care, virological sentinel and non-sentinel sources, including influenza detections in hospitalised patients.

### Results

The ILI surveillance revealed that the 2017/2018 outbreak was among the largest in several years both for all ages and in the elderly. This was due more to the cumulative effect of its longevity at medium intensity than to high incidence in any single week. Influenza B/Yamagata lineage viruses predominated, followed by A(H3N2), with much fewer A(H1N1)pdm09 cases and virtual absence of influenza B/Victoria lineage. B/Yamagata and A(H3N2) viruses showed a similar age profile, with frequency of laboratory verified infection increasing with old age. Correspondingly, in the elderly the number of influenza B-associated hospitalisations was also high. The age profiles for the different viruses were consistent with previous seasons, where we have also observed that the influenza B Victoria and Yamagata lineages affect different age segments with B/Victoria being more prominent in those 35 years and younger.

### Conclusion

The 2017/2018 influenza season in Norway clearly illustrates that there is nothing second-rate about the impact of influenza B. Even though caused by a non-drifted strain, the outbreak was the largest in many years, and comparable in severity to influenza A-driven outbreaks. Furthermore, the B/Yamagata-lineage resembled A(H3N2) viruses in its age profile, differing profoundly from the age profile for B/Victoria lineage.

*Keywords: Influenza B virus; influenza B lineage; epidemiology; age*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 10955

## ASSESSING FACTORS INFLUENCING INFLUENZA VACCINE CHOICE IN U.S. NURSING HOMES

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### Introduction

Influenza vaccination is recommended by the Advisory Committee on Immunization Practice (ACIP) and covered free of charge (primarily through Medicare) for all adults  $\geq 65$  years of age. Available influenza vaccines include standard trivalent (TIV) or quadrivalent (QIV), and two enhanced vaccines approved specifically for adults  $\geq 65$  years of age (MF59 Adjuvanted and High-dose). Decisions on influenza vaccine for nursing home (NH) residents (and staff) in the U.S. are made by the NH facility or their corporate management. Understanding what factors influence influenza vaccine choice for NH residents and staff is essential.

### Methods

Questionnaires were administered to 598 NHs throughout the U.S. (August-December 2018). NH leadership responded to measures of spontaneously elicited beliefs concerning positive and negative aspects of influenza immunization, influenza vaccines, sources of support for vaccinating their residents and staff, knowledge about influenza, and enhanced influenza vaccines.

### Results

Most NHs used TIV or QIV for both the 2017-18 (39.8%) and the 2018-2019 (50.0%) seasons. The choice of an enhanced influenza vaccine *declined* from 40.5% in 2017-18 to 25.7% in 2018-19. The majority of respondents indicated that the choice of flu vaccine for each season was a corporate decision (58.4%), while 33.3% indicated that recommendations from leadership were important, and only 18% listed cost. NHs reported high vaccination rates for residents (82%) and somewhat lower rates for staff (60%). Respondents overwhelmingly felt moderately or very well informed about seasonal flu (80.7%) and outcomes (77.6%), but fewer felt moderately or very well informed about enhanced influenza vaccines (48.6%).

### Conclusion

In a study involving over 500 U.S. nursing home decision makers, there was good knowledge about influenza illness and vaccines though, less so for enhanced influenza vaccines of special relevance to adults  $\geq 65$  years of age, and utilization of enhanced vaccines was relatively low and declining.

*Keywords: influenza vaccine; nursing home; enhanced influenza vaccine; attitudes and beliefs; influenza knowledge*

Topic: Public Health: Epidemiology & Transmission  
 Abstract No: 10994

## SETTING UP A NETWORK OF LABORATORIES TO ESTIMATE THE BURDEN OF REAL TIME POLYMERASE CHAIN REACTION (RT-PCR) CONFIRMED INFLUENZA AMONG OLDER ADULTS IN THE COMMUNITY IN A LIMITED RESOURCE SETTING

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**Introduction:** There are limited community-based influenza burden studies especially among older adults in low-and middle-income countries. We present the processes and experience in setting up multi-institutional laboratory network for estimation of influenza burden.

**Method:** We established a multi-institutional network of molecular diagnostic laboratories in All India Institute of Medical Sciences (AIIMS), New Delhi; National Institute of Virology (NIV), Pune; National Institute of Epidemiology (NIE), Chennai; and National Institute of Cholera and Enteric Diseases (NICED), Kolkata. AIIMS has one field laboratory for specimen testing and main laboratory is the central coordinating unit (CCU-Lab). We drafted common standard operating procedures (SOP) for influenza testing using RT-PCR. The CCU-Lab trained scientists and technicians of all site laboratories. We procured testing kits centrally and supplied to the sites to ensure uniformity. All sites performed two proficiency testing panels for influenza, one from AIIMS (6 samples) and one from US CDC (9 samples) before commencing study specimen testing. Each site sent 20% of their positive and 5% of negative samples at the CCU-Lab for re-confirmation.

**Results:** AIIMS and NIV had prior experience testing for influenza using RT-PCR, while project teams at NIE and NICED began testing for influenza as part of this study. All laboratories completed the influenza proficiency panels for seasonal influenza testing scoring  $\geq 90\%$ . During 2018, the network tested 2,394 nasal and oropharyngeal swabs collected from community-dwelling adults aged  $\geq 60$  years with acute respiratory infection. Influenza virus was detected in 109(5%) samples which included influenza A(H1N1)pdm09 (40/109;37%), A(H3N2) (29/109;27%) and B (40/109;37%). Overall, 139 samples were re-tested in the central laboratory and concordance was 98%(136/139).

**Conclusion:** We demonstrated the viability of establishing a network of laboratories with capacity for influenza testing using RT-PCR across India. Such multi-institutional laboratory-backed platforms can be useful for estimating the community-based burden of influenza associated ARI among older adults.

*Keywords: Network of Laboratories, Older adults, RT-PCR, Influenza, ARI*

Topic: Public Health: Epidemiology & Transmission  
 Abstract No: 11006

## HIGHLY PATHOGENIC AVIAN INFLUENZA IN EUROPE, SEASONS 2017-18 AND 2018-19

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The 2016-2017 epidemic of highly pathogenic avian influenza (AI) viruses in Europe required a better understanding of relevant epidemiological determinants to improve preparedness, risk assessment and early detection. The European Food Safety Authority (EFSA) collected structured, primary epidemiological data from outbreaks in poultry and wild birds to provide regular reports together with the European Centre for Disease Prevention and Control (ECDC) and the European Reference Laboratory (EURL) that describe the AI situation within the EU and worldwide focussing particularly on zoonotic viruses. Here, we describe the epidemiological situation in Europe for the seasons 2017-2018 and 2018-2019.

Data reported to the Animal Disease Notification System (ADNS) or submitted to EFSA between October 2017 and April 2019 were used. The epidemiological situation was described by temporal and geographical patterns, virus subtype, affected host population, and the characteristics of the affected establishments. ECDC collected information on public health measures and exposure data from the animal and human influenza surveillance network.

Overall, 187 HPAI outbreaks were detected in birds in 2017–2018 (13 countries) and 2018–2019 (three countries). Of those outbreaks, 98 (52%) were due to A(H5N6) virus and 89 (48%) to A(H5N8) virus. Of the A(H5N6) outbreaks, 90 (92%) affected wild birds, four (4%) poultry and four (4%) captive birds; of the A(H5N8) outbreaks, 82 (92%) affected poultry, six (7%) wild birds and one (1%) captive birds. A(H5N6) viruses were confined to north Europe whereas A(H5N8) were circulating in south/south-east Europe. Hundred-twenty humans were reported to have been exposed during outbreaks, but no transmission has been reported.

Continued monitoring for AI virus in wild birds and poultry in Europe and worldwide with timely generation and sharing of viral genome sequences between animal and human health sectors are crucial to be able to early detect and respond to threats relevant to animal and public health.

*Keywords: avian influenza; monitoring; poultry; wild birds; humans*

Topic: Public Health: Epidemiology & Transmission

Abstract No: 11036

## A VARIED GENETIC MATCH RATES OF CIRCULATING INFLUENZA B VIRUSES COMPARED TO THE VACCINE STRAINS DURING THE 2014-2016 SEASONS IN REPUBLIC OF KOREA

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### Introduction and Objectives

In Republic of Korea influenza virus is prevalent during winter season, with the type B accounting for about one-fourth of all infections. During influenza season, influenza B viruses of both Victoria and Yamagata lineage are co-circulating in varying ratios. It is known that these two B lineages have little cross-protection by vaccine-induced immunity. The most commonly used trivalent influenza vaccines (TIV) include only one B lineage virus. The co-circulation of two B lineage viruses lead to mismatch between the B virus strain in TIV and the circulating B virus. It is important to investigate the molecular evolutions of two B lineage viruses for establishing optimized influenza vaccine strategy.

### Methods

This study analyzed the lineages of prevalent influenza B strains through hemagglutinin gene sequencing over the 2014-2016 influenza seasons in Republic of Korea. A total of 397 influenza B samples were collected from patients who visited an emergency room due to influenza-like illness and hospitalized adult influenza patients in Hospital-based Influenza Morbidity and Mortality study network. In total 198 samples were eligible for HA sequencing analysis.

### Results

In the 2014-2015 season, all 146 samples (100%) matched the B strain of the Yamagata lineage included in TIV. In the 2015-2016 season, only 13 (33.3%) out of 39 samples matched the vaccine strain of the Yamagata lineage. In the 2016-2017 season, only 2 (15.4%) out of 13 samples matched the vaccine strain of the Victoria lineage.

### Conclusion

The genetic match rates between the vaccine strains and the circulating influenza B viruses were varied ranges from 15.4% to 100% during the 2014-2016 seasons in Republic of Korea.

*Keywords: Influenza B virus; Influenza B lineage*

Topic: Public Health: Epidemiology & Transmission  
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## OPTIMAL TIME TO VACCINATE AGAINST INFLUENZA AND TREAT SEVERE RESPIRATORY ILLNESS EMPIRICALLY WITH ANTIVIRALS IN BANGLADESH, 2008-2017

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**Introduction and Objectives:** Characterizing the timing of influenza is important for targeting prevention and control measures. We explored the influenza seasonality in Bangladesh to identify the optimal months to vaccinate against influenza and to use empiric antiviral treatment for influenza illness.

**Methods:** During 2008-2017, we identified WHO defined influenza like illness and severe acute respiratory illness patients from 14 tertiary care hospitals across Bangladesh and community children aged < 5 years with cough and/or runny nose. We collected demographic and antiviral use data and tested nasopharyngeal and oropharyngeal swabs for influenza using rRT-PCR. We calculated the percent of samples testing positive (PP) for influenza and defined epidemic onset as >2 consecutive weeks when the PP exceeded the annual mean and the end of epidemic as >2 consecutive weeks when PP was below annual mean. The peak was the week where the maximum weekly PP during the epidemic. We used median and interquartile range to calculate summary virus activity for the 10-year period.

**Results:** We tested 41,556 specimens of which 56% were from hospitalized patients and 41% from children aged <5 years in the outpatient setting. Approximately 1 in 5 (7,823 [19%]) were positive for influenza. Influenza epidemic typically started in May (week 20, IQR: 16-24), peaked in July (week 29, IQR: 25-37) and ended in October (week 40, IQR: 35-43). Start week varied by geographic region and by year from April to June (week 18-24). Epidemics typically started 6 weeks earlier in central Bangladesh than in Northern Bangladesh. Only 13 (0.03%) patients received antivirals during epidemics.

**Conclusion:** Although Bangladesh currently recommended Northern Hemisphere influenza vaccines for Hajj pilgrims, our findings suggest April-May is the optimal time to vaccinate Bangladeshis with a Southern Hemisphere formulation. Empiric antiviral treatment for severe respiratory illnesses may be useful during May-October to reduce complications and death.

*Keywords: influenza, Bangladesh, epidemic, seasonality, vaccination time*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11119

## INFLUENZA ASSOCIATED WITH SUBSEQUENT VIRAL AND BACTERIAL PNEUMONIA AMONG NICARAGUAN CHILDREN: A NESTED MATCHED CASE-CONTROL STUDY

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### Introduction:

Pneumonia is a leading cause of mortality worldwide and is commonly associated with influenza. While this association has been established ecologically, individual-level evidence remains sparse.

### Methods:

We conducted a case-control study within a cohort of Nicaraguan children aged 0-14 years between 2011 and 2018. Physician diagnosed cases of pneumonia using Integrated Management for Childhood Illness (IMCI) guidelines. Cases were matched to 4 controls on age (months) and study week. Conditional logistic regression was used to calculate odds of preceding laboratory-confirmed influenza infection within 5 time-periods: 0-7, 7-13, 14-20, 21-27, and 7-30 days prior. We presumed pneumonia episodes preceded by influenza infection within 7 days to be primary viral pneumonia, and those occurring >7 days after influenza infection to be secondary bacterial pneumonia. The Bonferroni method was used to adjust for multiple testing.

### Results:

We identified 1154 pneumonia cases, of which 61 (5.3%) had RT-PCR confirmed influenza infection in the 30 days prior to pneumonia diagnosis. Cases had greater odds of influenza A infection (matched OR [mOR]: 6.9, 95% Confidence Interval [CI]: 3.7, 12.7) and influenza B (mOR: 12.4, 95% CI: 4.0, 38.3) in the previous 7 days compared to controls. Similar results were obtained for both influenza A subtypes. Pneumonia was also associated with influenza A (mOR: 2.3, 95% CI: 1.0, 5.0) and H1N1 (mOR: 4.0, 95% CI: 1.2, 13.8) infection within the previous 14-20 days. Following Bonferroni correction, only associations within the previous 7 days remained significant ( $\alpha = 0.013$ ).

### Conclusions:

On an individual level, influenza infection is strongly associated with development of pneumonia during the week following infection. We also found that influenza A, particularly H1N1, was associated with secondary bacterial pneumonia within 14-20 days after influenza infection. Additional longitudinal analysis is underway to assess the potential causal relationships highlighted in this study.

*Keywords: Pneumonia; influenza; children; severe*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11120

## INFLUENZA SEASONAL AND INTENSITY THRESHOLDS ESTIMATION IN CÔTE D'IVOIRE.

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### Introduction and Objectives

Since 2007, a sentinel influenza surveillance network has been set up in Côte d'Ivoire to monitor trends and early detect outbreaks for timely action. Over the past decade, Illness Like Influenza (ILI) and Severe Acute Respiratory Infection (SARI) surveillance have been functional and generates sufficient quality data. The objective of this study is to determine influenza thresholds towards transmission dynamics in Côte d'Ivoire.

### Method

Weekly series analysis of the proportion of specimens tested positive for influenza was carried out using ILI and SARI data from 2013 to 2018. The average curve method was used according to the two-wave model to estimate the seasonal threshold and intensity thresholds for influenza activity. These intensity thresholds were classified as moderate (40%), high (90%) and extraordinary (97.5%). The data was analyzed using the R software.

### Results

Two waves were observed while performing the analysis, the first wave from the epidemiological weeks (epi-week) 43 to 10 and the second wave from epi-weeks 16 to 43. Among the category of moderate intensity related to selected parameter (proportion of positive influenza cases), the intensity thresholds for influenza activity were 12% during the first wave and 23% during the second phase. The estimation shown the thresholds of 34% during the first wave and 39% during the second wave for high intensity category. Finally, the extraordinary intensity category has been determined at 51%. In overall, the seasonal threshold calculated was 8%.

### Conclusion

The analysis enables to estimate two main types of threshold that are important for better understanding of the transmission dynamics of influenza in Côte d'Ivoire. This finding is critical to support public health prioritization for necessary preventive measures. Furthermore, the thresholds are very useful to monitor the different phase of the influenza season.

*Keywords: Threshold; Influenza; Côte d'Ivoire*



Topic: Public Health: Epidemiology & Transmission  
 Abstract No: 11148

## SEASONALITY OF INFLUENZA VIRUSES IN SOUTH AND SOUTHEAST ASIA: IMPLICATIONS FOR THE TIMING OF SEASONAL VACCINES

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**Introduction and objectives.** Annual influenza transmission patterns in Asia are complex and poorly understood, complicating the timing of vaccination programs. We describe seasonal patterns of influenza transmission from sentinel surveillance programs over a 10-year period. Our objective was to identify optimal months for annual influenza vaccination programs, based upon when the majority of cases tended to occur.

**Methods.** Individuals with influenza-like illness (ILI; fever >38°C and cough or sore throat) were recruited from sentinel sites in Bhutan, Nepal, the Philippines, and Thailand from 2009-2018. Nasopharyngeal swabs were tested by RT-PCR for influenza subtypes. For each month and each site, the total number of influenza cases occurring over the subsequent 12 months was calculated. The proportion of annual influenza cases occurring within the presumed window of vaccine effectiveness (5 months, following a one-month period post-vaccination where immunity may be incomplete) was estimated.

**Results.** 46,094 individuals experiencing ILI were enrolled, with 39.5% confirmed as influenza. The majority of ILI cases were from Nepal (36.1%) and Thailand (30.3%). 33.9% of influenza cases were influenza A/H3N2, 34.7% influenza A/H1N1, and 31.3% influenza B. Influenza transmission in all four countries was positively correlated, with two peaks of transmission occurring in Thailand (in sync with the other countries and following a six-month lag). Based upon the average monthly distribution of influenza cases, the optimal months for vaccination were March for Bhutan (with 72.7% of cases occurring within a window of presumed effectiveness beginning in March, 95% CI: 71.0–74.3%), June for the Philippines (77.5%, 75.4–79.3%), May for Nepal (67.4%, 66.4–68.4%), and June for Thailand (60.4%, 59.0–61.8%).

**Conclusions.** Many Asian countries have yet to introduce influenza vaccines or are in early stages of introduction. These results indicate that the optimal windows for routine vaccination campaigns in Asian countries may be identified and that they may differ.

*Keywords: Influenza; Epidemiology; Vaccine Effectiveness; Transmission; Seasonality*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11149

## Hemagglutination-inhibition antibody titers and protection against influenza virus infection: a systematic review and meta-analysis

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### Introduction

An antibody titer of 40 by the hemagglutination-inhibition (HAI) assay has been correlated with 50% protection against influenza virus infections in some studies, while other studies have suggested alternative levels of protection. We performed systematic review and meta-analysis using data from studies in humans on the association of HAI titers with protection against influenza virus infections.

### Methods

We searched 5 databases to identify human studies that reported estimates of the association between pre-infection HAI titers and protection against influenza virus infection, or relevant raw data that permit these calculations. Fixed-effects meta-analysis was used to derive pooled estimates of protection by recent vaccination status. Mixed-effects meta-regression was used to assess relationships between covariates and protection. Potential publication bias was assessed by funnel plots and Egger's test.

### Result

We screened 21416 titles, 2507 abstracts, 73 full-texts and included 25 studies in the meta-analysis. We estimated that an HAI titer of 40 in individuals with recent matched influenza vaccination was associated with 61% (95% CI 52%, 69%) protection after adjusting missing studies, while the same titer was associated with just 53% (95% CI 45%, 60%) protection in those without recent vaccination. We did not identify any publication bias in no recent influenza vaccination group. Recent influenza vaccination status was associated with the level of protection against influenza virus infection at a particular HAI titer.

### Conclusions

An HAI titer of 40 was associated with different levels of protection for recent vaccinated individuals, possibly due to heterogeneous immune responses elicited from recent or past vaccinations or infections, possibly affected by antibody specificity or quality, or because of correlations with other immune mechanisms which were typically not measured.

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11194

## EPIDEMIOLOGY OF INFLUENZA VIRUS INFECTION AMONG INFLUENZA-LIKE-ILLNESS PATIENTS IN THE OUT-PATIENT SETTING

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**Introduction:** Influenza is one of the most common cause of acute respiratory tract infection globally. Influenza virus infection causes a clinical syndrome indistinguishable from other respiratory infection. We conducted this study to describe the epidemiology of Influenza virus infection among persons with Influenza-like-illness (ILI) cases in out-patient setting in Indonesia.

**Method:** In this preliminary report, all patients diagnosed as having ILI according to WHO definition, who visited Sukajadi Primary Health Center were documented using standardized research form approved by Research Ethics Committee, Universitas Padjadjaran. Nasopharyngeal swab specimens were collected and examined using RT-PCR for Influenza.

**Result:** From 8 October 2018 until 6 January 2019, 215 ILI patients enrolled, 88/215 (40.93%) were positive for Influenza virus consisted of 75 (34.88%) Influenza A, 11 (5.12%) Influenza B, and 2 (0.93%) positive for Influenza A and B. Fifty percent of persons with confirmed influenza were female and there was no significant difference in sex ratio by influenza strain ( $P=0.447$ ). According to each age group, the highest positivity rate were in 12-18 year group (49%), 18-60 year (43%), and 6-12 year (42%). Of 75 Influenza A infection, the most common were in 18-60 year (36%), 6-12 year (35%), and 12-18 year (24%), while of 11 Influenza B were in 18 - 60 year (64%) and 12 - 18 year (27%) ( $p=0.262$ ). According to weekly period, the most common of 75 Influenza A infection were in week 6<sup>th</sup> (17%), and week 10<sup>th</sup> and 11<sup>th</sup> (each 15%), while of 11 Influenza B were in week 10<sup>th</sup> (27%). The difference between Influenza A and B was highly significant ( $p<0.001$ ).

**Conclusion:** There were no difference between Influenza A and B virus infection according to gender and age group. However, there was a high significantly different according to time period.

*Keywords: epidemiology;ILI;Influenza,out-patient setting*

Topic: Public Health: Epidemiology & Transmission

Abstract No: 11219

## Incidences and transmission of influenza A and B in a cohort population, Ulaanbaatar, Mongolia, 2013-2017

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### Introduction and Objectives

Influenza epidemic is observed during wintertime in temperate region. In Mongolia, we observe clear seasonality of influenza epidemic and that occur simultaneously over country in spite of low population density. To characterise transmission pattern of influenza in a remote community, a cohort study was conducted to collect data.

### Methods

We conducted field study in Baganuur district, Ulaanbaatar, Mongolia from 2013 to 2017. Baganuur district is one of suburb district of the capital city and has 27,440 populations in 2013. We selected one of 4 family practitioner's catchment area in the city. Prior to each influenza season, we enrolled all households in the area then observe influenza like illness (ILI) activity during season. In addition to catch up patients at health facilities, phone survey had been regularly operated and field staffs visited to symptomatic patients with point-of-care test kit. We pooled annual dataset for analysis.

### Result and Conclusion

Overall, 10438 persons were enrolled in this study and 5533 of them were participated throughout 4 seasons period. There were 23.5% that were less than 10 years old and female was slightly high (52.2%). We detected 465 influenza A and 132 influenza B cases. Influenza A virus was detected for all 4 seasons while influenza B virus was detected in the first and third season. For influenza A, overall incidence was 2.1% and the highest rate was found in children of 1-4 years (6.9%). For influenza B, overall incidence was 0.6 and the highest rate was found in children of 1-4 years (1.9%). Spatio-temporal analysis was then applied.

We could observe influenza epidemics for 4 seasons in one cohort. Both influenza A and B showed the highest incidence among children of 1-4 years. Age specific incidence is useful to evaluate influenza disease burden in the country.

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11222

## Survey for avian influenza among crows in Bangladesh

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### Introduction

Earlier studies have demonstrated that domestic poultry sold in the live markets throughout Bangladesh carry a wide range of avian influenza virus (AIV) strains. Poultry sold at the market are usually slaughtered on site, and the offal is disposed of without any containment. As a result, crows get exposed to this potentially infected waste. Our objective was to assess the presence of AIV among crows associated with these markets.

### Method

We sampled crows from April through May, 2014 from Dhaka, the capital of Bangladesh. We collected 5000 crow fecal environmental swabs and made a pool of 10 (total 500 pool) in viral transport media for testing of AIV using real time reverse transcriptase polymerase chain reaction. Partial sequencing of all eight segments of AIV was conducted for strain identification. Result

A single pool was tested positive for AIV and the sequence data identified it as H9N2. From 1998 to 2016, 28 laboratory confirmed human cases of H9N2 virus was reported to WHO of which three were from Bangladesh.

### Conclusion

As this strain has the potential to reassort with H5N1, H7N9 and H10N8 viruses, and can cause human infections, presence in a ubiquitous bird(s) as crows can potentially be a significant public health risk.

*Keywords: Avian influenza, H5N1, Bangladesh, crow*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11230

## Mobile poultry vendors in Bangladesh: practices and movement patterns

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### Background:

The distribution of mobile poultry vendors and their practices are currently unknown. The objective of this study was to learn about the practices and movement patterns of mobile poultry vendors in Bangladesh from December 2015 to August 2016.

### Methods:

Vendors were enrolled and interviewed using structured questionnaires. Global Positioning System data loggers were provided to selected vendors to track their movement while selling poultry. Tracheal and cloacal swabs from one randomly selected poultry and environmental swabs from cages and surrounding areas were collected from each selected vendor. Samples were tested using real-time reverse transcription-polymerase chain reaction assays. Quantile regression was used to compare the distance covered by the vendors in the different sites.

### Results:

514 vendors were interviewed and 34 vendors were tracked using data loggers. 48% of vendors reported selling poultry door-to-door. The median number of households visited by the vendors per day was 60 (IQR: 30-100). Of 301 healthy poultry sampled, 8/299 swabs (3%) in the summer, 55/301 swabs (18%) in the monsoon season, and 40/271 swabs (15%) in winter were positive for influenza A viruses. H5 was identified in all seasons, and 18/871 (2%) swabs from the healthy poultry were positive for H5. The median distance covered by the vendors was significantly higher ( $p < 0.01$ ) for Manikgonj (44km), Netrokona (43km) and Tangail (36km) compared to Dhaka (15km).

### Conclusions:

Many households are visited by the vendors each day, which may increase the risk of poultry-to-human transmission of zoonotic influenza to vendors and household members in contact with infected poultry. Educating the vendors regarding poultry infection control may reduce the risk of spreading influenza viruses through these practices.

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11234

## Detection of airborne influenza virus in pediatric wards

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### Introduction

Relative importance of transmission mode in influenza especially the contribution of fine particle aerosols remains controversial. In this study, we collected air samples in hospital rooms and determine whether influenza viruses could be detected in fine particles.

### Methods

Air collection was conducted in 5-bed pediatric patient rooms that had at least one patient with influenza-like symptoms or laboratory-confirmed influenza infection at local hospitals in Guangzhou, China. Two-stage cyclone air samplers was used for air collection in the room for 4 hours continuously. The samplers collected air into 3 size fractions: >4 $\mu$ m, 1-4 $\mu$ m and <1 $\mu$ m.

### Results

In the 5-bed pediatric rooms, influenza A virus was recovered from all size fractions (>4 $\mu$ m, 1-4 $\mu$ m and <1 $\mu$ m) of air particles in 22/26 (86%) air sampling occasions and influenza B virus was only recovered in <1 $\mu$ m and 1-4 $\mu$ m size-fractions.

### Discussion

Detection of influenza virus RNA suggests healthcare workers and visitors could be frequently exposed to influenza virus in patient rooms. A limitation of this study is that we did not investigate the viability of the recovered influenza RNA, but high levels of influenza virus RNA in other similar studies have been correlated with higher levels of infectious virus.

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11238

## THE EVOLUTION OF INFLUENZA A/H3N2 VIRUS ACROSS INDONESIAN ARCHIPELAGO, 2008-2017

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### Introduction:

Since its first occurrence in 1968, Influenza A/H3N2 virus has evolved both genetically and antigenically in an attempt to escape host immune pressure. Until now, there are 28 Influenza A/H3N2 vaccine strains have been recommended by WHO. However, only few data regarding evolution of Influenza A viruses within long period of time in tropical countries is available. This data is valuable for investigating the influenza virus activities in accordance with WHO Pandemic Influenza Preparedness Framework. The study describes the genetic changes in the surface glycoproteins of influenza A/H3N2 virus across Indonesian archipelago, from 2008 to 2017.

### Methods:

Archived clinical samples or virus isolates from ILI Surveillance during 2008 to 2017 were subjected for sequencing. A total of 176 of Hemagglutinin (HA) and 171 Neuraminidase (NA) complete coding sequences (CDS) of H3N2 virus were analysed. The phylogenetic analysis using neighbor-joining with 1000 replications was applied to investigate the evolution of the virus.

### Results:

The phylogenetic trees both of HA and NA genes show the "ladder-like" phylogenetic tree suggesting the genetic drift of Indonesian from 2008 to 2017 without specific group of geographical origin across Indonesian archipelago. The HA sequences underwent mutations at the antigenic sites and grouped into several clades in which relevant with WHO recommended vaccine strains. No mutation found in NA gene that related to oseltamivir resistance among Indonesian strains.

### Conclusion:

This study signifies the sequential mutations found in the surface glycoproteins of Indonesian Influenza A/H3N2 virus, marking its evolutionary pattern across time.

*Keywords: Evolution, Influenza A virus, H3N2, Indonesia*



Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11248

## INFLUENZA WAS NOT ASSOCIATED WITH RESPIRATORY HEALTHCARE-ASSOCIATED INFECTIONS IN CHILDREN HOSPITALISED IN A PAEDIATRIC HOSPITAL IN SOUTH AFRICA, 2016-2017

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### Introduction and objectives

The epidemiology and aetiology of respiratory healthcare-associated infections (rHAIs) in Africa is poorly understood. We sought to assess the risk factors for rHAIs and role of influenza in rHAIs among hospitalised children aged <15 years in a paediatric hospital in South Africa, accounting for potential colonization through the use of controls.

### Methods

We enrolled children with rHAIs, defined as onset of fever ( $\geq 38^{\circ}\text{C}$ ) or hypothermia ( $< 35^{\circ}\text{C}$ ) and respiratory symptoms (cough, shortness of breath or difficulty breathing)  $\geq 2$  days after admission or hospital-onset radiographic pneumonia. For each enrolled rHAI case we enrolled the first patient <15 years old without respiratory symptoms and/or fever identified in the same ward and week as the rHAI case. Nasopharyngeal specimens were tested for influenza and other pathogens using TaqMan Array Card real-time PCR assay.

### Results

During January 2016—December 2017, 151 rHAIs, median age 1 month (IQR 0.4-3.7) and 152 controls, median age 0.4 months (IQR 0.2-1.9) were enrolled. Among rHAI, common viral pathogens detected were rhinovirus, enterovirus (27/153; 18% each) and respiratory syncytial virus (20/151; 13%). Influenza was detected in 3% (4/151) of rHAI. Among controls, rhinovirus and enterovirus were frequently detected (24/152; 16%) and influenza was detected in 5% (7/152) (Figure 1). Cases were not more likely to be infected with influenza compared to controls (unadjusted OR 0.6, 95%CI 0.2–1.9). The influenza virus attributable fraction among rHAI was -9% (-393 to 75.9). rHAI cases were more likely to be admitted or transferred to ICU vs paediatric ward (aOR)10.6, 95% confidence interval (CI) 4.9–22.8, to be premature (aOR 2.9, 95%CI 1.6–5.4); to be aged 3–11 months vs <3 months (aOR 4.8 95%CI (2.1–10.9); and to die during the admission (aOR 10.6 (95%CI 2.8–40.5).

### Conclusion

Influenza was an uncommon cause of rHAI in patients admitted to a paediatric hospital in South Africa.

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11273

## MOLECULAR EPIDEMIOLOGY AND SEASONALITY OF INFLUENZA VIRUSES CIRCULATING IN NEPAL

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Diseases / Japan (日本)

**Introduction:** Influenza is one of the major public health problems in Nepal. The data on epidemiology and seasonality are scarce and insufficiently described. The objective of this study was to describe the molecular epidemiology and seasonality of influenza virus types and subtypes circulating in Nepal.

**Materials and Methods:** A descriptive cross sectional study was conducted at National Influenza Center, Nepal during the year 2012 to 2016. A total of 9,435 throat & nasopharyngeal swabs were collected from influenza like illness cases according to WHO case definition. Total nucleic acid was extracted using Pure Link viral RNA/DNA mini kit (Invitrogen) and real-time RT-PCR assays were performed.

**Results:** Of the total, influenza viruses were detected in 3517 (37.3%) specimens. Influenza A virus was detected in 2163(61.5%) samples; of which 1032(29.3%) were influenza A /H1N1 pdm09 and 1131 (32.1%) were influenza A/H3N2 subtype. Influenza B virus was identified in 1323 (37.6%) cases. Co-infection of A/H3N2 and A/H1N1 pdm09 with influenza B was found in 6 (0.2%) and 25 (0.7%) specimens, respectively. Phylogenetic analysis of influenza A/H1N1 pdm09 viruses revealed two major subclade 6B.1 and 6B.2 with minor diversity. Similarly, A/H3N2 viruses of this study fell into subclade 3C.3a and 3C.3b. The Yamagata lineage of influenza B viruses were predominantly circulated in Nepal and belonged to clade 2 and 3. Influenza A /H1N1 pdm09, A/H3N2 and B virus isolates of Nepal were antigenically similar to the influenza vaccine viruses recommended by World Health Organization in 2012 to 2016 year, respectively. The influenza virus was found year-round with peak incidence in winter season in Nepal.

**Conclusion:** The influenza viruses circulated in Nepal were similar to tropical and subtropical countries of the world. Regular surveillance and monitoring of influenza viruses could be useful for introduction of vaccine and reduction of annual morbidity and mortality.

*Keywords: Acute respiratory infection, Etiology, Influenza like illness, Nepal, Seasonality*

Topic: Public Health: Epidemiology & Transmission  
 Abstract No: 11278

## Incidence of influenza virus and respiratory syncytial virus infections in older adults in eastern China: Findings from the China Ageing Respiratory infections Study (CARES)

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### Introduction

Limited data exists on the incidence of laboratory-confirmed influenza virus and respiratory syncytial virus (RSV) infections in community-dwelling older adults.

### Methods

Adults aged 60-89 years were enrolled in Jiangsu, China, in 2015 and then followed prospectively for two years. Throughout the study period, participants were contacted weekly by telephone to identify acute respiratory illnesses (ARI). Home visits were arranged to collect a combined mid-turbinate and oropharyngeal swab  $\leq 7$  days of illness onset for influenza virus and RSV infections confirmed by rRT-PCR and document information on symptoms and illness duration and severity. We estimated the incidence of laboratory-confirmed influenza virus and RSV infections and compared characteristics and outcomes for those with or without laboratory-confirmed infections.

### Results

We enrolled 1,527 participants; <1% reported ever receiving influenza vaccination. From January 2016 to August 2017 (120,484 person-weeks), we identified 95 influenza and 22 RSV ARIs by rRT-PCR (Figure). In the first year (January to August, 2016), influenza ARI was identified in 0.4% (95% CI=0.1-0.7%) of participants or 1,065 cases/100,000 person-years, and 0.2% (95% CI=0.0-0.4%) had RSV ARI or 746 cases/100,000 person-years. In the second year (September, 2016 to August, 2017), 4.7% (95% CI=3.5-5.9%) had influenza ARI or 6,168 cases/100,000 person-years, and 0.8% (95% CI 0.3-1.3%) had RSV ARI or 1,088 cases/100,000 person-years. Most of the influenza (59%) and RSV (64%) cases were identified in December, January and February. Participants with rRT-PCR-confirmed influenza ARI compared to influenza-negative ARI had a statistically longer duration of illness (mean 12.4 vs. 10.8 days) and had higher percentages who received medication (83% vs. 71%) and sought medical care (82% vs. 64%) (p-values <.01). There were no statistically significant differences in illness characteristics of RSV-positive vs. RSV-negative ARIs.

### Conclusions

Both influenza and RSV were frequently detected in community-dwelling older adults in eastern China, although influenza ARI was more frequent.

*Keywords: Influenza; RSV; Incidence; Burden; Older adults*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11285

## INFLUENZA INFECTION IN HOSPITALIZED PATIENTS: GLOBAL INFLUENZA HOSPITAL SURVEILLANCE NETWORK (GIHSN) PROTOCOL, MOSCOW SITE, 2012-2018

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Introduction and objectives.

In Russia, from 2014 influenza vaccination was included in National calendar of immunization and recommended for groups of high risks morbidity and complications. In 2018-2019 season, the rate of vaccination was increasing up to 49% of population. N.F. Gamaleya NRCEM are involved in GIHSN from 2012 and monitors the influenza cases in hospitalized patients with acute respiratory influenza-like illness. The main goal of this study was to assess the efficacy of vaccination to predict hospitalization, severe cases of infection and deaths.

Materials and methods.

Due to Protocol of GIHSN, all patients were questioning, swabbing and testing using RT-PCR method on influenza viruses.

Results.

344 (4,2%) from 8219 hospitalized patients had vaccination in anamnesis and influenza virus infection was found in 96 (28%) of them. The frequency of influenza virus type/subtype was the following: A(H1N1)pdm09 – 14 (15%), A(H3N2) – 38 (40%), A untyped – 4 (4%), B/Victoria – 23 (24%), B/Yamagata – 5 (5%), B untyped – 12 (13%). Meantime in 2886 (37%) unvaccinated patients influenza infection was found as well: A (H1N1)pdm09 – 794 (28%), A (H3N2) – 1041 (36%), A untyped – 39 (1%), B untyped – 1012 (35%). Uncomplicated infection was found in 11% among vaccinated patients and in 21% - unvaccinated that, among them: bronchitis - 11% and 10% accordingly and pneumonia – in 1,5% and 2,0% accordingly. The number of days of hospitalization was found 3 days lower in group of vaccinated people. No one case of severe infection and death was found in vaccinated patients.

Conclusions.

The results of study found the rare number of hospitalizations in vaccinated patients and confirmed the efficacy of vaccine to predict the severe cases and lethal outcomes. Founding. This study was supported by Foundation of Influenza Epidemiology, France due to Protocol GIHSN.

*Keywords: influenza; flu; vaccinated people; surveillance; GIHSN*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11293

## The heterogeneity of influenza seasonality by subtype and lineage in China

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### Abstract

**Introduction:** A previous study identified variation in influenza seasonality patterns across China. Seasonality patterns of influenza A subtypes and influenza B lineages across China need to be well understood.

**Methods:** We compiled weekly influenza laboratory surveillance data and climate data from 30 Chinese provinces from October 2004 through May 2016. We used wavelet analysis to estimate periodicity of influenza subtype/lineage-specific epidemics in northern, mid-latitude and southernmost China and examined peak timing. Mixed-effects logistic regression model was used to evaluate the relationship between climatic factors and influenza subtype/lineage-specific epidemics.

**Results:** Epidemics of influenza A(H1N1)pdm09, B/Victoria and B/Yamagata only peaked in winter-spring in three Chinese regions while their epidemics were inversely associated with temperature in southern China. Epidemics of pre-2009 A(H1N1) and A(H3N2) peaked in winter and summer in mid-latitude and southernmost China with a twice-annual cycle in some years in mid-latitude China. There was a U-shaped association between relative or absolute humidity and epidemics of pre-2009 A(H1N1) and A(H3N2) in southern China

**Conclusions:** The heterogeneity of influenza subtype/lineage-specific seasonality could be related to a different role of climatic factors on transmission and survival of different influenza subtype/lineage. Influenza vaccination policies for mid-latitude and southernmost China should take into account the heterogeneity.

*Keywords: Influenza; Surveillance; Subtype; Lineage; Seasonality*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11316

## Understanding the social issues influencing risk perception of zoonotic influenza A virus transmission at swine exhibitions

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### Introduction

Most cases of variant influenza A virus (IAV) reported in the United States have been associated with youth swine exhibition. Given IAV ecology, each of these zoonotic transmissions could result in the next IAV pandemic. Active IAV surveillance in exhibition swine has provided information for the development of disease mitigation strategies. Although education about IAV transmission and mitigation strategies has been disseminated, youth swine exhibitors, their families, and livestock show organizers have been slow to adopt healthy habits aimed at reducing zoonotic disease transmission. While over 67% of swine exhibitors are in favor of proposed mitigation strategies, few exhibitors have implemented mitigation strategies specifically to reduce zoonotic IAV transmission. Understanding the economic and social issues molding perceptions of risk, leading to a lack of behavior change is imperative to guide future educational outreach.

### Methods

To understand the behaviors and perceptions of swine exhibitors, covert naturalistic observation, interview, and focus group qualitative research methods were used. Focus groups and interviews were conducted with show organizers, youth swine exhibitor's parents, swine breeders, and other individuals identified as influential in decision-making processes. Questions were formulated to gain insight into factors influencing the acceptance or denial of recommended mitigation strategies and to gauge the perceptions participants have toward the risks associated with IAVs.

### Results

Covert naturalistic observations were completed at 11 swine exhibitions during the summer of 2018. Observations revealed extensive, close contact between youth and pigs, without the use of precautions to prevent disease transmission. Additionally, opinions expressed by 27 individuals across three focus groups revealed a general lack of concern over zoonotic IAV transmission.

### Conclusions

Understanding exhibitors' perception of the recommendations and barriers to adoption is critical because exhibitor compliance with proposed mitigation strategies is key in decreasing IAV burden in swine, thus reducing IAV transmission risk from pigs to people.

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11340

## ESTIMATING THE AIRBORNE INFECTIOUS DOSE FROM INFLUENZA TRANSMISSION OBSERVED IN A HUMAN TRANSMISSION TRIAL WITH A CONTROLLED ENVIRONMENT

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**Introduction and Objectives:** Quantifying influenza transmission risk by contact, large droplet spray, and airborne (aerosol) transmission modes informs the strategic use of prevention strategies to reduce the burden of seasonal epidemics, and the threat of pandemics. We used data from the largest human influenza challenge-transmission trial (Evaluating Modes of Influenza Transmission, ClinicalTrials.gov number NCT01710111) to estimate the average rate of generation of Well's quantum of airborne infection (an airborne infectious dose 63% or ID<sub>63</sub>) by experimentally infected viral Donors, based on finding that aerosol transmission was likely to have been an important mode of transmission in this model.

**Methods:** We used continuous CO<sub>2</sub> measurements, the observed secondary attack rate (1/75), and the shared air equation to estimate the quantum generation rate  $q$  for experimental Donors infected with influenza (H3N2). We used measured viral RNA shed into fine particle aerosols ( $\leq 5\mu\text{m}$ ) from experimental Donors to estimate an upper bound for the number of RNA copies per quantum of infection (ID<sub>63</sub>).

**Results:** The average quantum generation rate for Donors was 0.11 per hour. Out of 42 infected Donors, 11 had detectable viral shedding into the fine particle aerosols with adjusted geometric mean  $4.7 \text{ E}+03$  and geometric standard deviation 6.0, range  $2.6 \text{ E}+02 - 1.6 \text{ E}+05$ , accounting for observations below detection limit and where no exhaled breath sample was collected. The exposure group where the single transmission event occurred had one of the three highest estimated viral aerosol exposures. Viral aerosol RNA copy number per quantum (ID<sub>63</sub>) was approximately  $1.5 \text{ E}+05$ .

**Conclusions:** We present a methodology for estimating influenza airborne infectious dose to facilitate direct prediction of SAR given measurements of the source strength of virus shed into exhaled breath and indoor air CO<sub>2</sub> concentration.

*Keywords: airborne transmission; quanta; aerosols; human transmission trial; indoor air*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11357

## COMPARISON OF SYMPTOMATOLOGY AND INFLUENZA A VIRAL SHEDDING IN RESPIRATORY DROPLETS PRODUCED BY EXPERIMENTALLY INFECTED VOLUNTEERS AND NATURALLY INFECTED COMMUNITY CASES

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**Introduction and Objectives:** We compared the influenza A viral aerosol shedding from volunteers nasally inoculated with A/Wisconsin/2005 (H3N2) and adults naturally infected with influenza A H3 recruited from a college community during 2012-13, selected for influenza-like illness with objectively measured fever or a positive Quidel QuickVue A&B test.

**Methods:** Crude differences in shedding risk and rate were previously reported at Options IX. We extend previous work by reporting temporal trends in symptoms and using propensity scores to control for differences in symptom presentation observed between experimentally and naturally infected groups.

**Results:** Among 39 experimentally infected H3 cases with qRT-PCR positive nasopharyngeal swabs, symptom scores peaked on day 3 post nasal inoculation; Among 83 naturally infected H3 cases, symptom scores were maximal on the first day post-onset of symptoms. On the day of peak aerosol shedding, median symptom scores for experimental infection were upper respiratory 4 (IQR 2, 5), lower respiratory 0 (0, 1), systemic 1 (0, 2), and cough 0 (0, 1), and for natural infections 7 (5, 9), 3 (2, 4), 6 (4, 8), and 2 (2, 3), respectively. As previously reported, 28% of experimental and 86% of natural cases shed into fine particle aerosols ( $p < 0.001$ ); the difference in median shedding was significant ( $p < 0.003$ ). To compare the aerosol shedders (11 experimental, 71 naturally infected) on their peak day of shedding, accounting for differences in illness severity, we computed 14 sets of propensity scores based on various combinations of symptom scores (upper, lower, systemic, total, cough), cough count during aerosol sampling, fever ( $> 37.9$  C), temperature, nasopharyngeal swab Ct value.

**Conclusion:** Using each set of propensity scores for matching, stratification, and inverse weighting, demonstrated the almost complete lack of overlap between groups (best model standardized group difference 86%) such that a propensity score adjusted shedding comparison could not be performed.

**Keywords:** aerosols, route of transmission, human challenge transmission trial, viral shedding, symptomatology



Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11717

## The Role of Latitudes in Mediating the Association between Influenza Activity and Meteorological Factors in 45 Japanese Prefectures

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**Introduction and Objectives:** Cold and dry conditions were well-documented as a major determinant of influenza seasonality in temperate countries but the association may not be consistent when the climate in temperate areas is closer to that in sub-tropical areas. We hypothesized latitudes may mediate the association between influenza activity and meteorological factors in 45 Japanese prefectures across latitudes.

**Methods:** We used the weekly incidence of influenza-like illness of 45 prefectures from 2000-2018 as a proxy of influenza activity in Japan, a temperate country lying off the east coast of Asia. Generalized-additive model was adopted to investigate associations between meteorological factors (average temperature, relative humidity, total rainfall, and actual vapour pressure, a proxy for absolute humidity) and the influenza incidence controlling for other covariates. Pearson correlation ( $r$ ) between latitude and the adjusted relative risk (ARR) of each meteorological factor was also assessed.

**Results:** Higher vapour pressure was significantly associated with a lower influenza risk but the ARR was strongly weakened along with the decrease of latitude ( $r=-0.60$  and  $p$ -value $<0.01$ ). Low temperature and low relative humidity were significantly associated with higher influenza risks in most prefectures but the correlations between their ARR and latitude were weaker when comparing with that from vapour pressure ( $r=0.18$  and  $p$ -value $=0.23$  for temperature;  $r=0.45$  and  $p$ -value $<0.01$  for relative humidity).

**Conclusion:** Even though the range of latitudes in Japan is small (26°N-43°N), the relationships between meteorological factors and influenza activity were mediated by the latitude. Our study echoed absolute humidity played a more important role in relating with the influenza risk, but we on the other hand showed its effect on influenza activity could be hampered in a low-latitude temperate region having a warmer climate. These findings thus offer a high-resolution characterization of the role of meteorological factors on influenza seasonality on top of the previous laboratory and epidemiological findings.

*Keywords: temperature; humidity; climate; seasonality; temperate; japan*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11723

## Does Routine Whole Genome Sequencing of Influenza Viruses for Surveillance Purposes Improve the Detection of Chains of Transmission in the Community? Experience from an outbreak of Influenza in a Welsh Prison Population during Late 2018/19 Season Circulation of H3N2 virus in Wales.

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Since 2017/18 whole genome sequencing (WGS) of influenza has been used routinely to augment the virological surveillance of circulating influenza in Wales, UK. Current turnaround times from sample collection to sequence upload to GISAID is less than two weeks. Viruses sequenced in Wales are named to reflect regional acquisition of infection rather than country designation, this allows for more accurate monitoring of introduction of the virus into new regions of Wales.

Emphasis is placed specifically on the haemagglutinin (HA) for characterisation and global circulation intelligence, this makes the remaining segment data largely redundant. This study aims to determine if transmission resolution can be enhanced using more of the available WGS data and whether an outbreak be clearly identified within countrywide data.

During March 2019, influenza A H3N2 began to circulate in Wales, cases were confirmed into June. During the peak of the late season in May, an outbreak of influenza was reported in a prison in SE Wales. All samples received from the outbreak were sequenced following our routine sequence and pipeline workflow. The outbreak was investigated fully to include onset dates of cases and vaccine status. Concurrent to this, samples from across Wales were also sequenced as part of the ongoing surveillance work and to determine clade circulation patterns.

Full haemagglutinin trees were drawn and it was apparent that by embedding the prison outbreak into the countrywide data the resolution wasn't clear enough to identify this defined cluster and it could only be found by nomenclature. By concatenating increasing numbers of segments the outbreak could be more clearly defined.

WGS for Influenza is becoming widely utilised in surveillance programmes, whilst HA provides the best data to determine clade and vaccine match it is clear that it alone cannot provide clear resolution for transmission events in country or monitor for introduction in new regions. Not all samples submitted for WGS have viral loads that enable full sequencing of all segments, a proportion will lose parts of the polymerase complex. However, by utilising more of the WGS data, transmission events can be determined quickly even within full countrywide data.

*Keywords: Outbreak, transmission, WGS, Surveillance, H3N2*

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11740

## The use of nebulisers should be reconsidered as an aerosol-generating procedure (AGP)

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**Background:** Currently, nebuliser use is not considered to be an aerosol-generating procedure. Yet, when such masks are used there are clearly visible plumes emanating from the mask side-vents.

**Methods:** We simulated a human patient with an influenza infection wearing a home nebuliser mask (Titan Portable Home Nebuliser, 0.2 ml/min fluid, 6-8 L/min airflow), using a heated manikin lying supine on a hospital bed in a full-scale isolation room mock-up. The manikin was modified to continuously exhale (at 10-11 L/min) air containing aerosols of live-attenuated influenza vaccine virus (LAIV, Fluenz Tetra, AstraZeneca) produced by a Collison nebuliser (10-11 L/min). Simultaneous air-sampling (with the room ventilation set at 12 ACH – air changes/hour), for 10 mins, using 3 SKC biosamplers (12 L/min, collecting into virus transport medium) from 3 different locations around the bed: 0.40 (head), 1.10 (abdomen), 1.70 (foot) metres from the manikin's nose (simulating healthcare worker positions during a ward round) was performed in triplicate, over two days.

**Results:** A mean airborne viral load was obtained using digital PCR quantitation of each SKC sample: Day 1: starting source LAIV viral load in Collison:  $5.22 \pm 0.41 \times 10^8$  copies/mL; head =  $7.43 \pm 0.32 \times 10^4$  copies/mL; abdomen =  $2.20 \pm 0.30 \times 10^4$  copies/mL; foot =  $1.48 \pm 0.14 \times 10^4$  copies/mL; Day 2: starting source:  $4.72 \pm 0.21 \times 10^8$  copies/mL; head =  $8.80 \pm 0.23 \times 10^4$  copies/mL; abdomen =  $2.23 \pm 0.60 \times 10^4$  copies/mL; foot =  $1.56 \pm 0.43 \times 10^4$  copies/mL.

**Conclusions:** These results showed that aerosols containing ~200-2000 influenza viruses/L air may be present around the patient (at head, abdomen and foot positions) after exhalation from the mouth at a concentration of ~700,000-7 million influenza viruses/L air, when nebulised at 6-8 L/min using a portable home nebuliser (as used by asthmatics), with a room ventilation of 12 ACH. Further experiments are ongoing in the presence of additional typical hospital mixed ventilation flows (at 0 and 6 ACH). Repeat studies at higher 10-15 L/min nebuliser flow-rates (as used in hospitals) will further assess the airborne infection risk from such devices. Virus viability studies for all collected air samples are also being conducted.

**Keywords:** influenza; airborne; transmission; nebuliser; infection control

Topic: Public Health: Epidemiology & Transmission  
Abstract No: 11775

## Live Poultry Exposure in Urban Bangladesh: evaluating poultry purchasing and contact patterns to identify avenues for avian influenza transmission at the human-poultry interface

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**Introduction:** Exposure to live poultry is an important risk factor for zoonotic transmission of avian influenza. Specific high-risk practices that have been identified include touching poultry, having poultry in the house, slaughtering/de-feathering poultry, as well as visiting live bird markets (LBMs). Avian influenza is endemic in Bangladesh, where greater than 90% of poultry and poultry products are marketed through LBMs, with the majority of these sold in an unprocessed form. However, patterns of poultry exposure in the general urban population have not been systematically evaluated in this setting. These are of particular importance given that they can inform prevention measures at key human-animal interfaces.

**Objective:** The proposed study aims to evaluate patterns of exposure to live poultry in the general urban population of Dhaka, Bangladesh to identify avenues for avian influenza transmission.

**Methods:** We are conducting a cross-sectional study nested within a unique cell-phone based disease surveillance platform. Random digit dialing is employed to recruit participants in the general urban population aged  $\geq 18$  years, and administer a questionnaire on self-reported poultry purchasing, contact and prevention behaviours. Descriptive statistics will be used to summarize live poultry exposure patterns separately for males and females. Spatial analyses will also be conducted to map and characterize the geographic distribution of LBM visits in relation to small-area population densities in Dhaka.

**Conclusion:** Results from the project pilot will be presented. The risk of avian influenza transmission from poultry exposure will be summarized and the significance of the findings in the context of a One Health framework will be further discussed. The proposed study is timely, and will support the creation of innovative evidence-based recommendations to reduce avian influenza exposure at the human-poultry interface in Bangladesh.

**Keywords:** avian influenza; Bangladesh; urban health; animal-human interface; transmission

Topic: Public Health: Epidemiology & Transmission  
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## LABORATORY-CONFIRMED INFLUENZA AMONG FAMILY CAREGIVERS IN DISTRICT HOSPITALS IN BANGLADESH 2015-2017

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**Introduction:** In Bangladesh hospitals, most of a patient's care is provided by family and friends, collectively referred to as family caregivers (FCGs). Illness combined with poor infection control among healthcare providers can place patients at increased risk for hospital-acquired infections. With an average of two FCGs per patient, FCGs represent the largest group of healthcare providers in Bangladesh hospitals. We seek to assess the potential for transmission of influenza by FCGs in Bangladeshi hospitals.

**Methods:** From December 2015–December 2017, we collected two nasopharyngeal and oropharyngeal swabs from each consenting FCG who reported symptoms of influenza-like illness (ILI) in four district hospitals in Bangladesh with ongoing influenza surveillance among patients. We tested one swab using a rapid point-of-care test (Sofia Influenza A+B FIA) at bedside and another swab by real-time RT-PCR for influenza in a reference laboratory. We compared results with national inpatient and outpatient influenza surveillance data.

**Result:** Among 389 participating FCGs, the median age was 30 years and 318 (82%) were female. One in six (16%) FCGs were positive for influenza by RT-PCR; of these, 10% were influenza A and 6% were influenza B. Among the influenza A strains, H1pdm09 and H3 were similarly common. Influenza among FCGs had similar trends and subtypes compared with Bangladesh national influenza surveillance.

**Conclusion:** FCGs are a potential source of influenza transmission in Bangladeshi hospitals. Although influenza trends and subtypes among FCGs reflect those of the community, infected FCGs' close contact with ill patients pose a risk for influenza transmission and subsequent serious complications to patients. Hospitals in Bangladesh should consider screening FCGs for ILI before permitting them to stay on inpatient wards and requiring FCGs with ILI to wear masks to prevent disease transmission.

*Keywords: Family Caregiver, Influenza, Bangladesh*

Topic: Public Health: Human Sero-Epidemiology Studies  
Abstract No: 10329

## Is IgM serological diagnosis suitable for detection of respiratory viruses in children and adults?

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**Introduction and Objectives:** IgM serological diagnosis is commonly used to detect infection with bacteria and viruses, but there are some limitations to this technique in detection of respiratory viruses. The objective of this study was to analyze the serological response to respiratory viral pathogens and to evaluate the diagnostic application of IgM serology in respiratory viral infections.

**Methods:** Serum specimens and throat swabs from patients with acute respiratory tract infections were collected between January 2015 and December 2017 in Guangzhou, China. IgM antibody titers and viral copy numbers of five common respiratory viruses were determined by indirect immunofluorescence assay (IFA) and quantitative real-time polymerase chain reaction (qPCR), respectively, including influenza A and B (FLU A, FLU B), respiratory syncytial virus (RSV), parainfluenza virus (PIV) and adenovirus (ADV).

**Results:** For the IgM antibody assays by IFA, among the total of 23310 specimens, 1.7% (402) were positive for PIV, 1.6% (364) were positive for FLU B, 0.4% (88) for ADV, 0.4% (90) for RSV, and 0.2% (52) for FLU A. Patients aged 5–19 years had the highest prevalence of FLU B ( $p < 0.05$ ) and PIV ( $p < 0.001$ ). ADV was mainly found in young children aged 0–4 years ( $p < 0.001$ ). Significant differences were found between the qPCR and IFA detection rates in FLU A patients ( $p < 0.05$ ). Two of seven adult patients infected with FLU B showed seroconversion, but those with FLU A infection ( $n = 17$ ) did not. For ADV, four coincident peaks of detection were found between IFA and qPCR results, while FLU B showed three coinciding peaks. As for PIV and RSV, two similar peaks were observed. However, only FLU A showed one similar peak between these assays over three years.

**Conclusion:** The data suggest that IgM antibody test by IFA may be applicable as an epidemiological reference in children for the respiratory viruses investigated except for FLU A.

**Keywords:** IgM serological diagnosis ; respiratory viruses ; children ; epidemiological reference ;

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## INFLUENZA D VIRUS: A POTENTIAL THREAT FOR HUMANS?

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### INTRODUCTION AND OBJECTIVES

Influenza D virus (IDV) is a novel influenza virus firstly isolated in swine in 2011 in Oklahoma. Several studies have isolated IDV in cattle from multiple geographic areas, suggesting cattle as a possible primary natural reservoir for the virus. To date, few studies have been performed on human samples and there is no conclusive evidence of IDV ability to infect humans.

This serological study aimed to assess the prevalence of antibodies against IDV in human population.

### METHODS

The IDV used in the serological analysis was Influenza D/bovine/Oklahoma/660/2013.

The human serum samples, collected in Italy between 2005 and 2017, were randomly selected from the laboratory internal serum bank and tested by haemagglutination inhibition assay (HI). HI positivity has been confirmed by virus neutralization assay (VN).

### RESULTS

Based on HI positivity (HI titers  $\geq 10$ ), a low prevalence (5%-10%) was observed between 2005 and 2007. There was a sharp increase since 2008 resulting in two main peaks in 2009-2010 and 2013-2014; a finding confirmed by statistical trend analysis (Fig. 1).

The same pattern and trends can be seen with higher HI titers of  $\geq 20$  and  $\geq 40$ .

### CONCLUSION

Prevalence of antibodies against IDV has increased in human population in Italy from 2005 to 2017. Low prevalence values between 2005 and 2007 suggest that IDV most probably has circulated before its detection in 2011, and maybe even before 2005.

In Italy IDV has been shown to circulate among swine and bovine herds. It is therefore possible that prevalence peaks in humans appear to follow infection epidemics in animals and not to persist in the population, resembling a spill-over event from an animal reservoir and showing that the virus may not circulate consistently in the human population. However, IDV showed the ability to elicit an immune response in humans.

*Keywords: Influenza D virus; seroepidemiology study; human population; Italy*

Topic: Public Health: Human Sero-Epidemiology Studies  
Abstract No: 11064

## Influenza trivalent vaccine induced immune response and waning immunity during the 2017/2018 influenza season

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### Introduction

The seasonal influenza vaccine is recommended for health professionals risk-groups. The annual vaccine campaign to the staff of the National Institute of Health-Portugal(INSA) provided a good opportunity to investigate the antibody response to the 2017/2018 trivalent influenza vaccine(TIV). The aim of the study was to measure the influenza vaccine induced immune response and the duration of the immunity in an adult population of healthy and active workers.

### Methods

During the 2017/2018 season a cohort of 74 INSA-staff members aged between 30-69, that voluntarily uptake the TIV, were recruited and followed (October/2017-June/2018). Three serum samples were collected [before vaccine,30-days,6-months after vaccination) and epidemiological data (age,sex,comorbidities and vaccination-history). Antibody titre for each influenza 2017/2018 TIV viruses<sup>1</sup> and new type/subtype variants<sup>2</sup>(annex-I) were assessed by hemagglutination inhibition assay (HAI). Seroprevalence rates (SPR) (titre $\geq$ 40), geometric mean of antibody titres (GMT), seroconversion rates (SCR) were calculated with 95%CI. Waning immunity was evaluated(GMT-fold).

### Results

The pre-vaccination SPR and GMT were higher for influenza A-subtypes than those to influenza-B. SCR and GMT, 30days after vaccination, were higher for influenza-A. SCR rates and GMT's for the vaccine viruses were higher for participants that hadn't been vaccinated in the two previous seasons ( $p \leq 0.005$ ). A statistically significant increase of GMT was observed(GMT-fold between 1,3 and 2,4;  $p \leq 0.005$ ) 30days after vaccination, for all tested viruses. Six months after vaccination the SPR and GMT decreased to/or under the pre-vaccination levels, except for the 2017/2018 predominant circulating B/Yamagata (B/Phuket/3073/2013-like). For this virus a 1,5 and 3,0 times increase of SPR and GMT was observed, respectively, at end of the season.

### Conclusions

TIV increased the SPR, GMT and SCR 30days after vaccination. SCR was statistically significant higher in non-previously vaccinated individuals. At the end of the season,SPR and GMT (for non-predominant circulating viruses)decreased to pre-vaccination levels supporting the need for annual vaccination.

*Keywords: TIV; immune-response; waning-immunity; cohort*



Topic: Public Health: Human Sero-Epidemiology Studies  
Abstract No: 11708

## HUMORAL IMMUNITY TO NATURAL INFECTION IN COLOMBIAN INDIGENOUS POPULATIONS

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Humoral immune responses to influenza infection or vaccination vary substantially among individuals. The factors responsible for this variability and how vaccination versus natural infection influence differ remain to be defined. In these studies, we evaluated longitudinal humoral responses to seasonal influenza virus strains in n = 385 rural Colombians as well as genetically unique indigenous populations. None of the indigenous and few of the rural Colombians have been vaccinated allowing us to monitor response to natural infection. The cohort was comprised of roughly equivalent numbers of males and females ranging from 2 to >70 years of age from different locations and genetic groups. Metadata was captured on health status, underlying conditions and contact with animals, including mammals and birds. We measured overall influenza antibodies by NP ELISA, hemagglutination inhibition (HAI) titers to human seasonal influenza A and B viruses, a panel of swine viruses and performed HAI and viral or HA-specific ELISA to avian HA strains as well as influenza B HA. Finally, we evaluated immune history in the cohort against human seasonal influenza A and B viruses. Preliminary studies suggest that humoral responses are influenced by genetics, age, sex, and contact with animals in a strain-dependent manner. Many people had antibodies to swine and avian viruses including H5, H7 and H9 AIV viruses. However, we were surprised to find that one of the indigenous tribes, Kogui population, had little to no antibody to influenza B viruses by either HAI and ELISA. Ongoing studies are evaluating individual serological responses over time, to different human and animal influenza strains and the underlying epidemiological factors driving the variability amongst the cohorts.

*Keywords: humoral immunity; indigenous populations*

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 10402

## POLICY IMPLEMENTATION TO IMPROVE SEASONAL INFLUENZA VACCINATION OF TARGET GROUPS IN SOUTH AFRICA, 2011–2018

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### *Introduction*

Seasonal influenza imposes significant public health and economic burden on national health systems. The South African Department of Health conducted national influenza vaccination campaigns since 2010, targeting people at increased risk for severe influenza and death. Influenza vaccine wastage was initially high; therefore, an influenza vaccine policy was developed and implemented to reduce vaccine wastage and improve utilisation.

### *Methods*

During 2010-2016, trivalent inactivated influenza vaccine was centrally procured and distributed to provinces for administration. Following development and implementation of the national influenza vaccine policy in 2017, provinces were responsible for vaccine procurement, administration and social mobilisation. Target groups were pregnant women, persons with chronic diseases, children aged 6 months to 4 years and adults aged 65 years and older. Target groups were revised by the National Advisory Group on Immunisation in 2017; children were removed while pregnant women and persons with HIV-infection were prioritised. We estimated vaccine coverage by dividing the number of doses administered to each target group by the target group population.

### *Results*

The number of vaccines procured in 2011-2016 ranged from 800,000 to 1,040,000; vaccine utilisation ranged from 81% to 91%. Vaccine coverage among target groups for 2011-2016 varied: pregnant women 11.5-15.7%; persons with chronic diseases 3.5-5.7%; children aged 6 months to 4 years 2.4-4.9% and adults aged 65 years and older 2.0-2.5%.

The number of vaccines procured in 2017 and 2018 were 822,205 and 933,235 with vaccine utilisation being 97% and 95%, respectively. Vaccine coverage among target groups for 2017-2018 remained low: pregnant women 12.3-13.3%; persons with chronic diseases 1.9-2.0%; adults aged 65 years and older 2.5-2.9%; and persons with HIV-infection 4.5-4.7%.

### *Conclusion*

Following influenza vaccine policy implementation, wastage reduced, provincial accountability increased, and active stakeholder collaboration including social mobilisation at community level improved. Vaccine coverage remains low but could increase with additional vaccine procurement.

*Keywords: influenza; vaccine; policy; utilization; coverage*

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 10478

## PRE-EMPTIVE SCHOOL CLOSURES (PSC) AS A PANDEMIC COUNTERMEASURE IN THE UNITED STATES: WHEN, WHERE, AND FOR HOW LONG?

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### Introduction and Objectives

In the United States, PSC may be recommended to mitigate severe, very severe, and extreme-severity pandemics. We explored options for PSC implementation, including timing, geographic scope, and duration.

### Methods

We used stochastic individual-based computer simulation models to evaluate likely effects of PSC in five pandemic severity scenarios: four based on historical pandemics (1918, 1957, 1968, and 2009) and one on a hypothetical H5N1-like pandemic. First, a single community model (~2000 people) was used to explore sensitivity of model outputs to key model parameters in six contact settings (households, household clusters, neighborhoods, communities, schools, and workplaces), expressed as setting-specific partial rank correlation coefficient (PRCC). Second, a regional model (~8.6 million people in the Chicago metropolitan area) was used to explore effects of different PSC timing, duration and geographic scope (school-by-school vs. region-wide PSC). Third, based on insights from these smaller-scale simulations, we designed the final simulation suite employing a model of the continental United States (~300 million people) for the severity scenarios most likely to merit PSC, including 1918-like and 1958-like pandemic). To halt simulations, all models assumed that a well-matched pandemic vaccine becomes available 6 months after initial U.S. case.

### Results

Single-community simulations suggest schools as the most impactful contact setting with regard to time-to-peak (PRCC=-0.55); for comparison, households were a distant second (PRCC=-0.27). In regional models, PSC delayed time-to-peak by 5-6 days per PSC-week in most scenarios. PSC lasting  $\geq 4$  weeks were most impactful in delaying time-to-peak; less-feasible school-by-school PSC appeared superior to region-wide PSC in reducing cumulative incidence. National-level simulations suggested county-wide PSC as superior to broader-scope PSC (Figure). Delayed triggering resulted in loss of PSC effects.

### Conclusion

Our models reaffirm PSC as an impactful pandemic countermeasure, and provide important, albeit largely qualitative, insights for optimal PSC implementation. Economic analyses should be performed before formulating specific policies.

*Keywords: schools; influenza; school closures; pandemics; non-pharmaceutical interventions*

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 10540

## Effectiveness of an Independent, Online Educational Program for Australian Healthcare Professionals on Seasonal Influenza Immunisation Strategies in Older Adults in 2018

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### Introduction:

To support the introduction of a new category of enhanced influenza vaccines in Australia, a high quality online accredited program was developed for Australian healthcare professionals (HCPs) by mdBriefCase™ and a panel of experts, supported by an independent grant from Seqirus. The format of the program followed a proven behavioural change process.

The objective of this survey was to evaluate the impact of the program on the knowledge and behaviour of participants pre- and post-education.

### Method:

Participants were asked attitudinal questions (on a scale of 1 to 5; 1 being low and 5 being high) prior to the program and two weeks after program completion.

### Results:

The changes in knowledge and attitudes were substantial (n=1,584 participants):

Pre-program, 56% of HCPs rated themselves highly (4/5 or 5/5) in their ability to differentiate between the types of vaccines used in patients ≥65 years of age vs 96% post-program.

63% of HCPs said they were confident (4/5 or 5/5) in helping older adults overcome influenza vaccine myths, fears or concerns, pre-program vs 97% post-program.

42% of HCPs rated themselves as confident (4/5 or 5/5) pre-program in explaining the unique challenges of influenza immunisation in older adults vs 96% post-program.

62% of HCPs said they were likely to select an age-specific (enhanced) vaccine for older adults, which increased to 96% post-program.

Finally, 34% of HCPs rated themselves as confident (4/5 or 5/5) pre-program, in describing the mechanisms by which newer generation influenza vaccines may help to improve immune responsiveness in older adults vs 93% post-program.

### Conclusion:

An online educational program which incorporated a proven behavioural change process successfully increased participants' knowledge and confidence in selecting an age-specific influenza vaccine for older adults.

*Keywords: continuing medical education; enhanced influenza vaccine; elderly; older adults; online; Australia; healthcare professionals; behavioural change*

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 10541

## Effectiveness of risk minimisation activities for Afluria Quad through an online survey - A quantitative research study

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### Introduction and Objectives:

Afluria<sup>®</sup> Quad is an inactivated quadrivalent influenza vaccine. In Australia, an additional risk minimisation measure was implemented through medical education program to mitigate its inadvertent use in persons less than 18 years of age. The program involved dissemination of a Dear Health Care Professional (DHCP) Letter, an Electrostatic Vaccine Refrigerator sticker and an Age Indication Card.

### Method:

The effectiveness of this program was evaluated through an online survey.

The survey (in ten fortnightly waves) was conducted amongst HCPs over two influenza seasons (2017 and 2018); where in Afluria<sup>®</sup> Quad was indicated for persons aged 18 years and over:

The survey focussed on:

1. Awareness of the age indication
2. Information sources from which they first learned about age indication
3. Other information sources which reinforced this learning

Respondents who did not know the correct age indication were asked about potential information sources that would be beneficial for use.

### Results:

Overall, the awareness of the correct age indication for Afluria<sup>®</sup> Quad due this medical education program increased significantly from 40% - 54% in March 2017 to 85%-97% in June 2018.

The two most common sources through which General Practitioners and Practice Nurses learned about the correct age indication were Age Indication Card and DHCP Letter, followed by Afluria<sup>®</sup> Quad Product Information. The Product Information was the most important source of information for Registered Pharmacists followed by other sources like Age Indication Card, Fridge Stickers and DHCP Letters.

### Conclusion:

The types of educational tools and timing of delivery of the program were highly effective in educating HCPs to use the product in a restricted population. It is important that such measures be timely and tailored to the specific issue that need to be addressed.

*Keywords: Risk Minimisation Measure, Influenza Vaccine, Afluria, Quatitative, Drug Safety*

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 10543

## Review of Control Measures Implemented for Reducing Transmission of Influenza A(H7N9) in China, 2013-2019

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**Introduction:** Since the first reported human infection of avian influenza A(H7N9) in 2013, China has experienced annual epidemics of varying severity. Since 2013, China implemented various measures to mitigate H7N9 outbreaks in humans and poultry.

**Methods:** We reviewed control measure policies, surveillance data, and field reports published by the National Health Commission, the Ministry of Agriculture (MOA) and local governments to describe the implementation of H7N9 prevention and control measures.

**Results:** We categorized the evolution of H7N9 prevention and control measures in three phases. In Phase 1 (03/2013–08/2014), prevention and control measures focused on case investigations, contact tracing, and temporary suspension of live poultry market (LPM) operations and poultry trade in affected areas. Because exposure to live poultry and/or LPM were the primary risk factors for human infection with H7N9, measures during Phase 2 (09/2014–08/2016) focused on reducing risk of exposure. These measures targeted strengthening LPM cleaning and sanitation procedures and enforcing temporary LPM closures in affected areas following reported human cases or virus detections through environmental surveillance. Heavily affected provinces adopted additional stringent measures including permanent LPM closures in urban areas, seasonal closures, and special area closures. During the 2016–2017 epidemic case counts peaked and geographic spread widened. Phase 3 (09/2016–08/2017) measures targeted live poultry trade and included prohibitions on inter-provincial live poultry transport and quarantine procedures. Following the detection of highly pathogenic H7N9, MOA implemented an annual nationwide H5/H7 bivalent vaccination campaign in commercial and backyard poultry starting September 2017. No H7N9 human cases or poultry outbreaks were reported from 06/2018–03/2019.

**Conclusions:** Multisectoral H7N9 control measures in China evolved in three phases. Further evaluation is needed to assess the effectiveness of different measures. Continued surveillance for avian influenza in human and animal health sectors is critical for detecting emerging trends in H7N9.

*Keywords: Control Measures; Influenza A(H7N9)*

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects

Abstract No: 10682

## A MULTIFACETED INTERVENTION TO OVERCOME THE FLU SEASON

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**Introduction & Objectives:** Each year the flu season poses a challenge for patient safety, when hospitals deal with high occupancy rates, and outbreaks of hospital-acquired influenza. We describe a multifaceted intervention to improve hospital preparedness for the flu season.

**Method:** Rapid diagnosis of FLUA/B/RSV was facilitated, allowing for results within 30 minutes, 7 days a week 8am to 5pm. Text messages alerting the physician of the test result were sent. Patients whose condition improved were discharged as soon as possible, even if they still had fever. The program was implemented during 2017/18 and 2018/19 flu seasons, with comparison to the 2016-17 season, and national data, including program costs.

**Results:** We compared 583 flu patients admitted to internal medicine departments in the 2017/18 season to 407 patients in those departments in the 2016/17 season. The time from admission to PCR results, decreased by 20 hrs. Discharge within 36 hours increased significantly from 16 to 36%, for 48 hours were 30 and 47% and for 72 hours – 54 and 67%, respectively. Mean occupancy rate decreased from 98 to 88% and was 26% lower than the national average. The proportion of antibiotic treatment before receiving the PCR result went down significantly: 69 to 55%. Hospital-acquired influenza decreased from 12.5 to 3.6%. No increase in hospital readmissions, repeated visits to the emergency department, ICU admission or 30-days mortality was observed. Preliminary results for 2018/19 show a similar decrease in hospital occupancy, increase in early diagnosis and discharge, and reduced length of stay, despite a more severe morbidity pattern in the current season.

**Conclusions:** A multifaceted intervention to improve hospital preparedness for the flu season led to earlier discharge of patients, decrease in occupancy rates, decreased use of antibiotics and a reduction in hospital-acquired influenza. This model can be copied to hospitals worldwide.

*Keywords: FLUA/B/RSV MULTIFACETED INTERVENTION FLU SEASON*

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 10741

## Effect of provider recommendations to combat low vaccination, low vaccine trust and low perceived influenza risk in US young adults

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**Introduction and Objectives:** Young adults in the US have low influenza vaccine coverage and seek healthcare only rarely. We identified barriers to, and promoters of, influenza vaccination among young US adults.

**Methods:** We administered a 91-question survey to a convenience sample of adults using Amazon Mechanical Turk. Individuals were eligible if they were 18-26 years old and lived in the US. We identified barriers to vaccination concerning access to healthcare, perceived risk of influenza, and trust of vaccines. We used log-binomial regression models to estimate the risk ratio of being vaccinated vs. not vaccinated against influenza, according to provider recommendation.

**Results:** There were 417 participants; 290 (69.7%) were female and the median (IQR) age was 24 (22 – 25). Only 232 (57.3%) had received an influenza vaccine in the past 3 years. A total of 291 (70.5%) reported they sought no healthcare from any health professional (healthcare access barrier). Approximately half of participants (n = 212, 51.0%) estimated their likelihood of getting influenza in the future as “somewhat low” or “very low” (perceived risk barrier). Finally, a total of 94 individuals (23.5%) responded “didn’t know”, “disagree”, or “strongly disagree” to the phrase “Vaccines recommended for use are safe” (vaccine trust barrier). Despite these significant barriers, a doctor recommendation of influenza vaccine was still associated with a significantly increased likelihood of influenza vaccination (RR: 2.91; 95% CI: 1.92 – 4.42) in a log-binomial regression model controlling for age, sex, and insurance status. This association remained in the subset of individuals with low vaccine trust (RR: 7.53, 95% CI: 1.11 – 51.21).

**Conclusions:** Young adults experience barriers to influenza vaccination including low trust in vaccines, low access to healthcare, and low perceived risk of influenza infection. However, a strong provider recommendation for influenza vaccine may be able to overcome these barriers.

*Keywords: vaccine hesitancy; young adult; risk perception*



Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 10815

## FEASIBILITY OF SOCIAL DISTANCING PRACTICES IN PRIMARY AND SECONDARY SCHOOLS IN THE UNITED STATES TO REDUCE INFLUENZA TRANSMISSION DURING A PANDEMIC

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**Introduction and Objectives.** There is substantive evidence that pre-emptive school closures can mitigate influenza pandemics, but if sustained for several weeks they entail substantial disruption for schools and wider society. We conducted inter-sectoral qualitative research to assess the feasibility of other social distancing practices for schools in the United States (US).

**Methods.** We conducted 36 focus groups with 158 participants including educators, school nurses, and state officials in 2017 from all ten US Health and Human Services regions. Dedoose<sup>®</sup> qualitative research software (SocioCultural Research Consultants, CA) was used to identify themes.

**Results.** Participants discussed 25 within-school practices that could be implemented as part of the school day and four practices that would require a reduced school schedule. Within-school practices perceived as most feasible (e.g., canceling field trips, canceling assemblies, and rearranging desks to increase physical distance between students) are unlikely to reduce within-school influenza transmission. For example, rearranging desks might be feasible, but educators emphasized that it would be difficult to limit students' movement in class. The reduced-schedule practice considered the most feasible was a shortened school week. Factors that increase the feasibility of shortening the school week included obtaining a waiver from the state so that district funding is not reduced, and proactively putting a distance learning plan in place to be able to continue instruction when students have to remain at home. Some historically or internationally used practices were identified as the least feasible (e.g. moving class outdoors, staggering class start and dismissal time, dismissing a single class or grade).

**Conclusion.** Shortening the school week appears as a promising alternative to prolonged pre-emptive school closure. Future research should evaluate the acceptability of shortening the school week for parents and students, and assess its effectiveness in reducing influenza transmission through mathematical modeling and epidemiologic studies.

*Keywords: social distancing, pandemic, school, education*

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 10821

## POTENTIAL DEMAND FOR FACEMASKS IN GENERAL POPULATION DURING A SEVERE INFLUENZA PANDEMIC, UNITED STATES

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**Introduction and Objectives.** During a response to a severe influenza pandemic, facemasks may be recommended for use by ill persons as source control measure to reduce the risk of infecting others. Additionally, facemasks may be recommended for personal protection to certain groups with special, high-risk circumstances (e.g., persons caring for ill household members at home, pregnant women and other persons at high risk for influenza complications).

**Methods.** To estimate potential demand for facemasks for use by the general population in a severe pandemic (excluding occupational needs of healthcare personnel), we developed a two-step multiplier model. First, using data from the US Census Bureau and published literature, we estimated the number of persons who may need facemasks during a pandemic. We then calculated the number of facemasks needed per person per week and factored in the duration of a pandemic wave, with an assumed influenza attack rate of 30% and illness duration of 2 weeks. We considered two facemask products: a disposable facemask similar to facemasks currently available (e.g., surgical disposable facemasks), and a hypothetical reusable facemask, with assumed per-person use of 1 day and 30 days, respectively.

**Results.** For a 10-week pandemic wave, the average estimated number of disposable facemasks needed for source control by ill persons is 1.4 billion, and for select high-risk well persons 10.4 billion (Figure). If a reusable facemask were to become available, the estimated facemask need for source control declines to 0.1 billion, and for select high-risk well persons to 0.4 billion.

**Conclusion.** If relying just on currently available disposable facemasks, the estimated facemask needs for the general public reach extreme ranges. The current commercial supply will likely not meet these projections. A reusable facemask would provide a more sustainable solution to meet general population needs in pandemic response.

*Keywords: facemasks; pandemic; preparedness*

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 11074

## PILOT EXPERIMENTS TO ESTIMATE RESPIRABLE AEROSOLS PRODUCED DURING POULTRY SLAUGHTERING AND DEFEATHERING

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### Introduction and objectives

Influenza viruses can be aerosolized during slaughter of infected chickens, which can increase the risk of zoonotic transmission. We developed interventions designed to decrease production of aerosols during these processes and conducted an experiment to monitor aerosol generation during slaughtering and defeathering to inform a protocol designed to evaluate these interventions.

### Methods

Inside a booth within a temperature-controlled room, we slaughtered 10 chickens by severing the cervical blood vessels and placing them in an open barrel to exsanguinate, and defeathered 10 chickens using a defeathering machine. Six PATS+ and one Sidepak aerosol monitors were used to measure concentrations of airborne particles <2.5 µm at baseline and during slaughtering and defeathering; we subtracted baseline measurements from experimental measurements to calculate average particle concentrations. The instruments were placed 148 cm above the floor during slaughtering and defeathering, corresponding to a worker's breathing level; 56 cm during slaughter, corresponding to the height of the mouth of the barrel; and 107 cm, during defeathering corresponding to the mouth of the machine.

### Results

During slaughter, the average particle concentrations at 148 cm were 75.8 µg/m<sup>3</sup> (SD 55.5) by the PATS+ instruments and 23.4 µg/m<sup>3</sup> (SD 10.3) by the Sidepak. At 56 cm, the average concentrations were 23.8 µg/m<sup>3</sup> (SD 20.7; PATS+) and 10.2 µg/m<sup>3</sup> (SD 9.3; Sidepak). During defeathering, the average concentrations at 148 cm were 19.9 µg/m<sup>3</sup> (SD 8.2; PATS+) and 9 µg/m<sup>3</sup> (SD 2.6; Sidepak). At 107 cm, the average concentrations were 16.1 µg/m<sup>3</sup> (SD 4.7; PATS+) and 9 µg/m<sup>3</sup> (SD 2.9; Sidepak).

### Conclusions

The experimental design allowed us to measure increases in aerosol concentrations during slaughtering and defeathering. This protocol can be used to test airborne particle generation during different slaughtering and defeathering techniques in order to identify procedures that can minimize workers' exposure to potentially hazardous aerosol particles.

*Keywords: Avian influenza, respirable aerosols, poultry slaughter, defeathering, particle*

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 11150

## A NATIONAL STRATEGY TO ADDRESS THE GAP IN RESPIRATORY PROTECTIVE DEVICES DURING AN INFLUENZA PANDEMIC

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**Intro:** In 2014, a US Department of Health and Human Services analysis estimated over 3 billion respiratory protective devices (RPDs) would be needed for healthcare workers (HCW) during a severe influenza pandemic. At the onset of a pandemic, in the absence of a vaccine and limited supply of antiviral drugs, providing RPDs to healthcare workers (HCW) will be critical to ensure protection and increase their willingness to provide care to patients.

**Method:** From 2017-2018, the CDC led an effort with USG partners to address this gap. This work assessed past research, current activities, and gaps to ensure RPD availability and use during a pandemic. The analysis also considered lessons learned from the 2009 H1N1 influenza pandemic and the 2014 Ebola response. Given the immense projected RPD gap, a layered strategy that considers shared responsibility across all levels of government and engagement with healthcare and manufacturers was developed.

**Results:** Four strategies were identified to improve RPD preparedness by increasing supply and decreasing demand of RPDs: 1) *Advance Research and Development of New Transformative Products* to develop new approaches and products. 2) *Advance Research to Optimize Use of Existing Products* and develop novel approaches using everyday materials. 3) *Develop and Improve Guidance, Standards and Tools* through research focused on transmission, effective use, and improving standards to products developed meet HCW needs. 4) *Establish Alternatives to Federal Stockpiling and Supply Chain Improvements* through decentralized stockpiles, advance supply chain visibility, improved ordering practices, and developing approaches to increase RPD surge.

**Conclusion:** Lessons from past responses, modeling, and assessments in preparedness gaps informed critical HCW protection needs during an influenza pandemic. This strategic approach outlines the core areas where innovation, guidance, and supply chain solutions may work in synchrony to optimize planning and response efforts.

*Keywords: Respirators; Pandemic; Preparedness; National Strategy; RPDs*

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects  
Abstract No: 11247

## Health promotion programme targeting early school-age children for influenza prevention in Hong Kong

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### Introduction

Young children are at particular high risk of influenza infections due to their suboptimal hygiene practices and relative lack of pre-existing immunity among them. Most available traditional health promotion (HP) materials are too complicated and incomprehensible to this age group.

### Methods

We developed an outreach HP programme, consisting of face-to-face interactive sessions for demonstrate the mode of influenza virus transmission, proper hand hygiene and face masks usage technique, cough and sneeze etiquette, in a format comprehensible to young children aged 5-8. In-house designed cartoon figures were used in story board, stickers, badges, and information leaflet to raise their interest. A handwashing song of the “Happy Birthday” melody, covering a 20-second duration as per the US CDC recommendation, was given updated lyrics to cover the 7 steps of proper hand hygiene.

### Results

The programme was delivered to 6,814 K3 to P2 students and 270 teachers in 53 schools, with the HP message also reaching >13,000 audiences indirectly through our informaiton leaflet and online platform. Pre-tests and post-tests evaluation revealed significantly improved knowledge, ranging from 1.13% to 10.59%, on symptoms of influenza infection, moments needing hand hygiene, preventive measures and correct cough etiquette. 80-90% of teachers agreed that the programme enhance the relevant preventive health concepts both for the children and themselves and suitable to long term implemented in schools. Perceived barriers for sustaining the programme included the lack of professional and technical support (53.3%), resources (52.2%), and time (46.7%).

### Conclusions

Our result highlighted the potential effectiveness of adopting an interesting and comprehensible approach in the HP effort to children of very young age. Significant improvement was achieved regarding conceptual and technical knowledge related to influenza prevention. More effort with explicit components to equip teachers and parents for sustaining the HP in the school and home environment is needed.

Topic: Public Health: Non-Pharmaceutical Interventions – Public health aspects

Abstract No: 11265

## Applying surface-remaining-disinfectant to better biosecurity in poultry facility

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**Introduction and objectives:** Poultry breeder farms and hatcheries play an important role in poultry production chains. Without thorough biosecurity, these facilities might contribute to the spread of pathogens which might cause significant economic losses in the poultry industries, via materials involving movement of eggs and day-old birds. There is a possibility of pathogen introduction by contaminated materials which move after cleaning and disinfection at first. In this study, we apply organosilane-based surface-remaining-disinfectant on poultry equipment and compare the efficacy of pathogen reduction rate between new and previous cleaning and disinfection process.

**Methods:** In this study, the level and duration of disinfection efficacy with organosilane-based surface-remaining-disinfectant was compared to that of untreated control surfaces in a same kind of goods and spaces after current cleaning and disinfection process. We applied influenza virus on the surface and waited for sufficient virus-disinfectant reaction time. After reaction time, we retreated the viruses and brought them into lab keeping refrigerate condition. Samples were serially 10-folds diluted. 0.1 ml of each diluent was inoculated into the allantoic cavity of 12-day-old embryonated chicken eggs. After the eggs were incubated for 72 h at 37 °C with daily monitoring of each embryo, allantoic fluid was harvested and tested for hemagglutinin (HA) activity. The virus amount of samples were indicated by EID<sub>50</sub>/ml calculated by Reed–Muench method.

**Results:** Our new disinfectant showed at least 100 fold virus reduction compared to previous cleaning and disinfection process. Its duration is also longer than conventional method. The reduction rate and duration depended on the cleaning cycle and the surface material.

**Conclusion:** Our new disinfectant-applied poultry equipment has better disinfection efficacy than previous disinfection method of poultry facility. This disinfectant showed disinfecting ability being in use and after standard cleansing process.

Topic: Public Health: Pandemic Preparedness  
Abstract No: 10322

## PANDEMIC RISKS OF SEVERAL ZONOTIC INFLUENZA VIRUSES ASSESSED USING A STANDARDIZED APPROACH

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### Introduction

Multiple incidences of human infection by zoonotic viruses highlighted the importance of monitoring the potential risks associated with the emerging influenza viruses to cause a pandemic. Influenza virus evolution is dynamic and constant, and understanding is still limited. Furthermore, the risk assessment associated with emerging influenza viruses is a complicated process. To better prepare for the next pandemic and to prioritize preparedness activities, it is important to conduct risk assessments timely, systematically and comparably. Since 2016 WHO developed and published a tool for influenza pandemic risk assessment (TIPRA), it has been used 7 times to assess 4 influenza viruses.

### Method

We assessed two components using a multi-attribute additive model: the pandemic likelihood (potential) of acquiring the capacity for sustained human to human transmission and the public health impact (morbidity and mortality) if human to human transmission started to occur. A broad spectrum of experts scored nine risk elements, including the properties of the virus (four elements), attributes in the human population (three elements) and virus ecology and epidemiology in non-human hosts (two elements), based on knowledge available about the virus at the time of assessment.

### Result

TIPRA was used in seven rounds of risk assessment exercises; HPAI H5N6 (2016 and 2018), H7N9 (2016 and 2017), LPAI H9N2 (2016 and 2019), and H1N1 virus possessing triple reassortment internal gene cassette (H1N1 TRIG) (2017). H1N1 TRIG showed the highest likelihood among the other viruses. H5N6 and H7N9 subtypes had a moderate-high impact though they had comparable likelihoods with the assessment year. During the risk assessment over the past two years, we identified challenges and areas for improvement in the current TIPRA guidance.

### Conclusion

Timely risk assessment using TIPRA to generate a risk map is a scientific approach to prioritize subtype/clade viruses for preparedness activities and relevant policy making.

*Keywords: influenza, zoonotic influenza, pandemic preparedness, risk assessment*

Topic: Public Health: Pandemic Preparedness  
Abstract No: 10323

## DEVELOPMENT OF THE TOOL FOR INFLUENZA PANDEMIC RISK ASSESSMENT (TIPRA)

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### Introduction

The threat to human and animal health propelled by globalization and industrialization has highlighted the importance of the human-animal-ecosystem interface in emerging zoonotic infectious diseases. Since the World Health Organization (WHO), World Organization for Animal Health (OIE), and Food and Agriculture Organization (FAO) established a framework of collaboration on animal influenza, the three parties have strengthened their long-standing partnership for global defense against zoonotic influenza.

### Method

To assess the pandemic potential of zoonotic influenza virus which started causing human infection(s), we took the approach to two critical aspects: 1) the likelihood of the virus acquiring the capacity for sustained human-to-human transmission and 2) the public health impact should the virus causes human-to-human transmission. Based on knowledge available at the time of risk assessment, nine risk elements covering properties of the virus, attributes in the human population, and virus ecology and epidemiology in non-human hosts, were scored. Overall likelihood and impact were calculated with a multi-attribute additive model.

### Result

TIPRA initiated seven rounds of the risk assessment for zoonotic influenza viruses; HPAI H5N6 and H7N9, LPAI H9N2, and H1N1 virus possessing triple reassortment internal gene cassette (H1N1 TRIG). At each risk assessment, challenges and room for improvement on the current TIPRA guidance were identified. We recognized that risk stratification guidance of each risk element should be more specific to minimize differences in interpretation among scorers. Influenza viruses which have not yet caused human infection should also be considered to assess.

### Conclusion

TIPRA is a powerful for pandemic risk assessment of zoonotic influenza viruses, especially the standardized and scientific approach make the results of assessment comparable. In addition, TIPRA can also identify priority knowledge gap/research priorities. TIPRA running needs global expertise and the latest possible information. Based on the use so far, we plan to update TIPRA in 2019.

*Keywords: influenza, zoonotic influenza, pandemic preparedness, risk assessment*



Topic: Public Health: Pandemic Preparedness  
Abstract No: 10333

## STRATEGIC INVESTMENT FOR GLOBAL INFLUENZA PREPAREDNESS

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### Introduction

Pandemic influenza preparedness is a priority for the World Health Organization (WHO) and countries around the world, including the United States. Pandemics of the last century justify the time and effort dedicated to preparedness; 2018 marked the centennial of the deadly 1918 “Spanish Flu,” where over 50 million people died worldwide. Global influenza monitoring, guidelines and norms setting have been functions of WHO for over 70 years, but limited influenza vaccine manufacturing capacity, particularly in low and middle income countries, remains a challenge.

### Method

In 2006, WHO and Member States created a unique program to address global pandemic preparedness and the inequitable access to influenza vaccines. Called the Global Action Plan for influenza vaccines (GAP), the 10 year program was designed to introduce influenza vaccine manufacturing and capacity in regions and countries where historically there had been none or minimal in the past. The plan addressed three primary goals: an increase in seasonal vaccine use, an increase in vaccine production capacity, and increased commitment to research and development.

Fourteen manufacturers in 14 different countries were selected as GAP grantees. These manufacturers were strategically spread out around the world, spanning the six WHO regions to have more equitable production and procurement of influenza vaccines.

### Result

GAP ended in 2016. Upon closure, impressive gains in pandemic preparedness have been realized. Among GAP grantees, potential pandemic influenza vaccine capacity has increased by 1.1 billion doses. Potential global capacity has increased from 500 million in 2006 to 6.4 billion doses in 2015.

### Conclusion

The past decade of GAP work has emphasized global cooperation and strategic investment to address global pandemic influenza preparedness. The global community now needs to review, evaluate, and decide on the path forward to ensure the influenza vaccine gains of the last century are continued.

*Keywords: Influenza; Global; Vaccine*

Topic: Public Health: Pandemic Preparedness

Abstract No: 10453

## INTERPANDEMIC EXPERIENCE WITH REACTIVE SCHOOL CLOSURES OVER SEVEN INFLUENZA SEASONS – UNITED STATES, 2011–2018

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### INTRODUCTION AND OBJECTIVES

In the United States, widespread disease during the fall wave of the 2009 influenza A (H1N1) pandemic led to 1,947 reactive school closures. However, little data are available on the interpandemic baseline of reactive closures associated with seasonal influenza activity.

### METHODS

From August 1, 2011, through May 18, 2018, we conducted systematic daily online searches to identify public announcements of unplanned school closures in the United States lasting  $\geq 1$  day, selecting those that mentioned influenza or influenza-like illness (ILI) as a reason for closure (ILI-SCs). We studied temporal patterns of ILI-SCs and compared them with reported outpatient ILI visits at the national levels. We calculated Spearman rank correlations to evaluate these relationships during influenza seasons.

### RESULTS

A total estimated 4,161 school closures were detected [109 (3%), 386 (9%), 11 (0%), 311 (7%), 43 (1%), 1,300 (31%), 2,001 (48%) during the 2011–12, 2012–13, 2013–14, 2014–15, 2015–16, 2016–17, and 2017–18 seasons, respectively] (Figure). Increased student absenteeism due to illness [316 (32.8%)] and rising numbers of ill students and staff [313 (32.5%)] were the most frequently cited reasons in ILI-SC announcements. Overall, ILI-SCs were moderately correlated with outpatient ILI visits ( $r_s=0.623$ ;  $p<0.001$ ); correlation was particularly strong in influenza A (H3N2)-dominant seasons of 2012–13, 2014–15, 2016–17, and 2017–18 [ $r_s=0.660$  ( $p<0.001$ ), 0.596 ( $p<0.001$ ), 0.833 ( $p<0.001$ ), and 0.836 ( $p<0.001$ ), respectively].

### CONCLUSION

ILI-related school closure occurrence patterns mirrored those of seasonal influenza activity, with the strongest correlations and greatest numbers of closures observed during seasons predominated by H3N2 strains. Two consecutive H3N2-dominant seasons, 2016–17 and 2017–18, together accounted for almost 80% of all ILI-SCs observed during the study period. Monitoring cause-specific student absenteeism may be helpful to proactively detect and respond to increased influenza activity in schools during severe influenza seasons and pandemics.

*Keywords: influenza, school closure, preparedness, nonpharmaceutical intervention*

Topic: Public Health: Pandemic Preparedness  
Abstract No: 10462

## Influenza vaccination introduction program for health care workers in Vietnam

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### Background

In 2017, the Vietnam Ministry of Health conducted a demonstration project to introduce seasonal influenza vaccination to health care workers (HCWs). A total of 11,000 doses of influenza vaccine, single-dose prefilled syringes, were provided free to HCWs at 29 selected hospitals, clinics, and research institutes in four provinces: Hanoi, Khanh Hoa, Dak Lak and Ho Chi Minh City.

### Methods

Before the campaign, a workshop was organized to discuss an implementation plan including technical requirements, cold chain, uptake reporting, and surveillance for adverse events following immunization (AEFIs). All sites distributed communication materials and encouraged their staff to register for vaccination. Following immunization sessions, sites sent reports on uptake and AEFI cases. Left-over vaccine was transferred to other sites to maximize vaccine use.

### Results

The average uptake was 57% for all HCWs, with 11 sites achieving 90% and above. These 11 sites were small with less than 500 staff, including 5 primary hospitals, 3 preventive medicine units, and 2 referral hospitals. Among the six biggest sites with over 1,000 staff, four sites had the lowest uptake (14%-47%). Most of the high-uptake sites were from the central to the south; only one site, a referral hospital, was from the north. After redistribution of left-over vaccine, only 130 vaccine doses (1.2%) were not used and destroyed. Based on factors that affected uptake, including registration levels, differing communication strategies, availability of vaccination, and commitment by health facility leaders, we recommended ways to increase HCW coverage; recommendations to improve AEFI reporting also were made.

### Conclusions

The project demonstrated that it was feasible to conduct influenza vaccination campaigns among HCWs in Vietnam. Improvements in promotion of registration, more intense pre-planning, especially at larger facilities, and wider, more consistent availability of communication materials will result in increased efficiency and coverage in this program's future expansion.

*Keywords: influenza, vaccination, health care workers*

Topic: Public Health: Pandemic Preparedness

Abstract No: 10666

## DETECTION OF INFLUENZA VIRUSES BY REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION: WHO EXTERNAL QUALITY ASSESSMENT PROGRAMME

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### Introduction

Detection and response to influenza epidemics and pandemics rely on timely and accurate identification of the virus. The WHO Influenza External Quality Assessment Programme (EQAP-PCR) was established in 2007 to monitor and guide the quality of laboratory influenza diagnosis using RT-PCR in the Global Influenza Surveillance and Response System (GISRS) and other reference influenza laboratories.

### Methods

Vacuum-dried inactivated influenza viruses have been dispatched to participating laboratories once or twice per year. The programme is coordinated by the World Health Organization (WHO) and implemented by the WHO H5 Reference Laboratory and National Influenza Centre at the Centre for Health Protection, Hong Kong Special Administrative Region of China, under ISO 17043 accreditation. In recent years, each panel contained 10 samples including 9 random samples of influenza viruses (seasonal: A and B; and zoonotic: H5, H7, H9) and a negative sample. Participation in the EQAP is voluntary. Since 2013, an optional panel for influenza antiviral susceptibility testing has been distributed, along with the PCR panel.

### Results

Between 2007 and 2018, a total of 17 panels were distributed. Between 54 and 174 laboratories participated in each panel from all WHO regions. In 2018, 174 laboratories from 136 countries participated in panel 17. Since 2007, correct identification of all viruses in a given panel increased from 67% to 87% of participating laboratories. Correct identification of influenza A/H5 rose from 77% to 93%. Correct identification of seasonal influenza viruses increased from 67% in 2007 to >97% in 2018. Participating laboratories reporting results for influenza B lineages increased from 60.3% in 2015 to 75.3% in 2018.

### Conclusion

The WHO EQAP-PCR played a key role in enhancing the quality of influenza virus detection in participating countries, thus enabling better preparedness for influenza epidemics and pandemics.

Topic: Public Health: Pandemic Preparedness  
Abstract No: 10700

## A Phase I Study to Assess Priming with Antigenically Mismatched Live Attenuated A/H7N3 Influenza Virus Vaccine followed by Inactivated A/H7N9 Influenza Virus Vaccine

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**Introduction:** In previous studies pandemic live attenuated vaccines (pLAIV) have demonstrated long-lived immune memory that can prime for subsequent responses to antigenically matched pandemic inactivated vaccines (pIIV). However, the potential benefits of antigenically mismatched pLAIV priming and pIIV boost are not known. This study tests the hypothesis that heterologous pLAIV-pIIV prime-boost with antigenically mismatched H7 viruses will elicit a broader antibody response than homologous prime-boost.

**Methods:** In a Phase I open-label study, healthy adults received 2 doses of intranasal A/chicken/British Columbia/CN-6/2004(H7N3) pLAIV at day 0 and 28 followed by antigenically mismatched unadjuvanted intramuscular A/Shanghai/2/2013(H7N9) pIIV at week 12. Sera from this study were tested concurrently with sera from a previous study in which subjects received two doses of A/Anhui/1/2013(H7N9) pLAIV followed by the A/Shanghai/2/2013(H7N9) pIIV at 12 weeks. Sera were tested by HAI and MN against the H7N3 and H7N9 vaccine viruses and against an A/Netherlands/219/2003 (H7N7) virus.

**Results:** Sixteen subjects were enrolled and received two doses of H7N3 pLAIV followed by a single dose of H7N9 pIIV. Reactogenicity was mild, with rhinorrhea occurring after pLAIV and injection site pain after pIIV. Four-fold or greater increases in HAI antibody after heterologous pIIV boost were seen in 50%, 44%, and 44% of subjects against H7N9, H7N3 and H7N7 viruses respectively, compared with 80%, 73%, and 73% of the subjects from the previous homologous prime-boost study. Among subjects who responded (Figure), homologous prime-boost generated higher titers to H7N9 (green, squares), but heterologous priming generated higher titers to H7N3 and H7N7 viruses (blue, diamonds).

**Conclusion:** In this small study, both heterologous and homologous pLAIV priming resulted in substantial responses to pIIV boost, with high titers of antibody against three antigenically diverse H7 viruses. The breadth of the antibody response elicited by the prime-boost strategy is remarkable and warrants further clinical development.

*Keywords: Pandemic Live Attenuated Vaccines, Prime-Boost*

Topic: Public Health: Pandemic Preparedness  
Abstract No: 10823

## Leveraging One Health: Protecting Delaware's Residents and Economy from Influenza

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A One Health approach to influenza detection and prevention acknowledges the interrelationship between humans, animals, and our shared environment. The One Health approach was established in 1967, when the Federal Agricultural Organization/World Health Organization committee on Zoonoses identified more than 150 diseases that affect humans. Today, nearly 75% of all emerging infectious diseases are Zoonotic. Establishing, maintaining, and promoting Delaware's "Partnership for One Health" is highly beneficial for sustaining and improving human and animal health. Animal health is important since Delaware's gross agricultural income exceeds one billion dollars per year. Broiler chickens dominate Delaware's agricultural industry and comprise more than 200 million birds. Poultry can be infected with Low or Highly Pathogenic Avian Influenza (AI), some strains of which may be transmissible to humans.

Preventing novel influenza among Delaware's residents is a preeminent public health concern and averting avian influenza (AI) in poultry is vital to the state's economy. Agricultural and public health scientists apply real-time Polymerase Chain Reaction (PCR) methods to detect novel influenza viruses. This approach exceeds surveillance goals designated by the Centers for Disease Control and Prevention (CDC). Comparable surveillance systems for AI in Delaware poultry are in place as determined by industry, state, and federal veterinarians.

The Partnership for One Health Delaware convenes on a quarterly basis to plan, exercise, and review Zoonotic threats while capitalizing on Delaware's geographic location, small size, and shared goals of protecting public health, food security, and economic stability. When a Delaware resident presented with influenza symptoms after visiting a Maryland fair, the causative pathogen was quickly diagnosed as H3N2 swine variant, triggering investigation into a fair not previously implicated.

Delaware's Partnership for One Health is a reliable, integrative, and transparent, model for protecting humans and animals from influenza virus. It serves as an exemplary model for use by other jurisdictions.

Topic: Public Health: Pandemic Preparedness  
Abstract No: 10845

## Assessing severity in the unusual 2017/18 Influenza epidemic in Germany using PISA indicators

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### Introduction and Objectives

Pandemic Influenza Severity Assessment (PISA) was developed after pandemic 2009 to allow for a weekly assessment of seasonal influenza epidemic or pandemic severity. PISA can use several data sources where data is rapidly available. In Germany, PISA was piloted during season 2017/18.

### Methods

We evaluated PISA indicators transmissibility, seriousness and impact using a new visualisation dashboard that allowed integration of several parameters per PISA indicator and enabled easy comparison of age groups. We used data from outpatient sentinel on acute respiratory infections for transmissibility. We assessed indicators seriousness and impact by several parameters using data from inpatient sentinel on severe acute respiratory infections (SARI) as well as data on notified laboratory confirmed influenza cases. PISA parameters can reach levels from low to extra-ordinary. We calculated parameter thresholds separately for age groups using data from past seasons. Thresholds were then applied to current data during season 2017/18.

### Results

The 2017/18 influenza epidemic lasted 15 weeks. Overall transmission reached extra-ordinary level during 2 weeks of the epidemic. All age groups except the 0-to-4-year-olds had extra-ordinary transmission for at least one week. Overall seriousness varied between levels low (inpatient sentinel data, cumulative death-to-hospitalization ratio of SARI), moderate (inpatient sentinel data, cumulative ICU-to-hospitalization ratio of SARI) and high (notification data, cumulative death-to-hospitalization ratio on influenza cases). Levels of seriousness in age groups were inconsistent with overall levels. Both impact parameters were extra-ordinary for several weeks overall and in all age groups.

### Conclusions

The visualisation tool was very useful to pilot PISA. Transmissibility indicator was plausible. Seriousness parameters should be assessed per age group, not overall. For younger age groups, ICU-to-hospitalization ratio is more meaningful than death-to-hospitalization ratio. Impact parameters should be adjusted.

*Keywords: risk assessment; transmissibility; seriousness of disease; impact; PISA-Tool*

Topic: Public Health: Pandemic Preparedness  
Abstract No: 10852

## Strengthening One Health approach in tackling Avian Influenza cases: a lesson learnt from Indonesia

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The zoonotic cases of animal influenza viruses have been recognised as a global public health threat since the emergence of Avian Influenza (AI) cases H5N1 in 1997. Up to date, WHO recorded 850 AI H5N1 human cases with 449 deaths globally. Moreover, other zoonotic influenza A cases as H9N2, H6N1, H5N6, H7N2, H7N7, H10N7 and H10N8 have also been recently documented.

Indonesia is endemic for AI H5N1 in the poultry sector and up until 2018, 200 human cases with 84% fatality have been reported from 15 provinces. The recent AI surveillance in the poultry in Indonesia detected two clades of H5N1 circulating (2.3.2.1 and 2.1.3). The latest cases of human AI H5N1 have also shown a connection with the virus circulated in the poultry.

Responding to the challenges, Indonesia has conducted efforts to control AI by implementing the national strategic plan through strengthening surveillance, laboratory diagnosis, case management and referral system, risk communication, and pandemic preparedness. Comprehensive analysis and timely sharing information on epidemiology and virology among human and animal health sectors for risk assessment are crucial for early detection and prompt response to zoonotic Emerging Infectious Diseases as part of securing public health.

*Keywords: avian influenza, zoonotic, Indonesia*



Topic: Public Health: Pandemic Preparedness  
Abstract No: 10908

## A Multi-pronged Strategy to Increase Influenza Vaccination Uptake by an Acute Tertiary Care Hospital in Singapore

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**Background:** Influenza infection increases hospitalisation and mortality risk among high-risk groups, including elderly patients and those with chronic disease. While vaccination is well-known to be an important preventive measure, adult influenza vaccination rates in Singapore are low, at an estimated 17.0%. Tan Tock Seng Hospital (TTSH), an acute care tertiary hospital in Singapore, has adopted a multi-pronged approach to increase vaccination uptake rates to safeguard population health.

**Methods:** Descriptive analysis of TTSH's inpatient and outpatient influenza vaccination programmes, the hospital's staff vaccination programme, and a community-based programme in the Central Region of Singapore reaching out to needy seniors, from years 2016-2018.

**Results:** The hospital's vaccination strategy focused on protecting the most vulnerable groups in each setting (aged  $\geq 65$  yrs or with chronic diseases), as well as all hospital staff. Principal considerations included the need to reduce financial barriers, reduce inconvenience, ensure sufficient clinical support, adopt a multi-disciplinary approach including nurses and pharmacists, and integrate built-in workflows into clinical care. For years 2016, 2017 and 2018 respectively, the numbers of influenza vaccinations given out by each programme were as follows: inpatient pre-discharge programme – 3014, 3347, and 3846; outpatient programmes (in 3 specialist clinics) – 4,389, 2820, and 4333; staff vaccination – 6612, 6449, and 7402 (vaccination rates of 78.5%, 77.4%, and 83.3% respectively;  $p < 0.001$ ); community-based programme – 2976, 3181, and 3080.

**Conclusion:** The hospital's multi-pronged approach has facilitated influenza vaccination among high-risk patients and staff, with a general increase in numbers over the years. Additional resources to expand existing programmes, measures to further reduce barriers to vaccination, and public education to overcome vaccine hesitancy are vital to improve vaccination rates.

Topic: Public Health: Pandemic Preparedness  
Abstract No: 10914

## Severe viral pneumonia surveillance to identify novel influenza viruses including influenza A/H7N9, Northern region of Vietnam, 2013 – 2018

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SVP surveillance system in Northern region in Viet Nam was established to indentify influenza A/H5N1 firstly. In the priod of 6 years (2013-2018), there were 656 SVP cases collected and investigated. One third (35% [228/656]) tested positive for influenza A or B. Influenza A/H1N1pdm09 accounted for 65% (149/228), A/H3N2 25% (56/228), and influenza B 10% (22/228) of influenza positive SVPs. No A/H7N9, A/H5N6, A/H5N1 or other avian influenza viruses were detected.

*Keywords: SVP surveillance, A/H1N1 pandemic09, Viet Nam, Northern Region, human*

Topic: Public Health: Pandemic Preparedness

Abstract No: 10929

## Prioritizing influenza vaccine allocation during a pandemic – a review of pandemic plans

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**Introduction:** Vaccination is key to controlling and preventing further transmission, morbidity and mortality during an influenza pandemic. However, vaccine development can take many months, and once available supply will be insufficient to vaccinate an entire population, therefore requiring a staged allocation of pandemic vaccine. The World Health Organization (WHO) recommends that nations establish goals and priorities for pandemic influenza vaccine use and priority groups for vaccination.

**Methods:** We reviewed current national pandemic plans of Organisation for Economic Co-operation and Development (OECD) countries, including Australia, the United States, Canada, New Zealand and the United Kingdom. In addition, semi-structured interviews were held with representatives from these five countries, the WHO and the European Centres for Disease Control to lend clarity to the decisions made around vaccine prioritisation in pandemic plans.

**Results:** Only 12 of 34 possibly relevant pandemic plans could be retrieved, and only six had been published or updated (in English) since 2009. Updated plans recommended a flexible vaccine prioritisation strategy that responds to the epidemiology of the pandemic; this was also a key point raised during interviews. Pandemic plans tended to prioritise individuals at high risk of death and complications from influenza infection, including Indigenous populations, pregnant women, age-defined risk groups (e.g. children), and individuals working in high-risk settings (e.g. health care workers and essential service providers). The importance of clear communication was emphasized to ensure acceptability of any prioritisation plan among the populace.

**Conclusions:** Given that our search identified just six updated pandemic plans, we urge other countries with updated plans to make them publicly accessible. Increased availability of pandemic plans may encourage those countries which have not updated their plans to do so. In particular, it would enable plans to be updated in line with emerging consensus about the need for flexible prioritisation strategies.

**Keywords:** *pandemic planning; vaccine; prioritization*

Topic: Public Health: Pandemic Preparedness  
Abstract No: 10998

## IMPLEMENTATION OF THE PANDEMIC INFLUENZA PREPAREDNESS (PIP) FRAMEWORK PARTNERSHIP CONTRIBUTION, 2018

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**Introduction:** The Pandemic Influenza Preparedness (PIP) Framework is an innovative international instrument to improve pandemic influenza preparedness for an effective and equitable future pandemic response. Under the Framework, manufacturers who use the Global Influenza Surveillance and Response System (GISRS) provide to the World Health Organization (WHO) an annual Partnership Contribution (PC) that WHO uses to strengthen country and global readiness for the next influenza pandemic. Progress made in 2018 is presented.

**Methods:** PC funds focus on six areas: laboratory and surveillance; burden of disease; regulatory capacity building; risk communications and community engagement; planning for pandemic product deployment; and influenza pandemic preparedness planning. For 2018-2019, activities are implemented globally, regionally and in 72 PC recipient countries under a six-year plan. Indicators are used to monitor progress, and 2018 data were analyzed descriptively.

**Results:** In 2018, progress was made on 15 of 17 indicators, where four met or exceeded the biennial target. Achievements included an increase in the proportion of countries sharing epidemiological and virological data with WHO (20% and 4% increase respectively), and with timely sharing of influenza viruses with GISRS (20% increase). Two countries increased their regulatory capacity maturity levels, and four exercised their pandemic plans.

**Conclusion:** Thanks to the commitment of countries and leveraging partner investments, country pandemic influenza preparedness improved using PC funds in 2018. Each gain is an important building block for a timely and appropriate response at the time of the next pandemic. Sharing influenza specimens and surveillance data contributes to better risk assessment and response measures. Robust regulatory oversight will translate to more timely approval and quality monitoring of pandemic influenza products. Countries planning and exercising their response measures will enhance operational readiness to implement control measures. Global health security will be enhanced and more lives will be saved.

*Keywords: pandemic, World Health Organization, PIP Framework, preparedness, planning, policy*

Topic: Public Health: Pandemic Preparedness  
Abstract No: 11028

## ETHICS FRAMEWORK FOR PRIORITISING SCARCE PANDEMIC INFLUENZA VACCINE

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### Introduction

The WHO urges all countries to have a pandemic influenza plan in place and suggests that planners must consider ethical issues raised by responses to a pandemic. The literature offers a number of frameworks for rationing pandemic vaccines but they tend to focus on generalities and are insufficiently sensitive to context. We build upon the findings from our critical interpretative review of the literature and propose a new framework for prioritising pandemic vaccines.

### Methods

Critical interpretative review of the ethics literature on the topic of who to prioritise in a pandemic, followed by normative analysis.

### Results

We suggest that due to the unpredictability of virus characteristics, uncertainties in vaccine development, efficacy and effectiveness of the vaccine, as well as the potential differing impact on different population groups, no single answer as to how to guide priorities for pandemic vaccines can be produced in advance. Instead we propose a staged ethics framework as follows: Step 1: define a set of procedures that must necessarily underpin resource allocation in pandemic situations as a foundational requirement (this can be proposed and discussed in advance). Step 2: define a clear aim or set of aims for the pandemic vaccination programme (this can be debated in advance, but can only be finalised once the nature of the virus and vaccine are known). Step 3: propose a flexible and dynamic set of practical questions to guide decision making about priorities.

### Conclusion

Some priorities may be determined in advance of a pandemic, e.g. where there are important and ongoing commitments to certain groups. Others will be decided in view of the particular pandemic situation. Despite uncertainty many activities to support pandemic planning, including community consultation, can be put in place in advance.

*Keywords: Pandemic; influenza; vaccination; ethics; rationing*

Topic: Public Health: Pandemic Preparedness  
Abstract No: 11032

## ACCURATE INFLUENZA CLASSIFICATION FROM SHORT-READ DATA IMPROVES ROBUSTNESS OF BIOINFORMATICS PIPELINES FOR ROUTINE SURVEILLANCE AND PANDEMIC PREPAREDNESS

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### Background

Whole-genome sequencing (WGS) has begun to emerge as a useful tool in the study and surveillance of influenza. However, continual genetic drift and noise in short read data can present challenges for existing bioinformatics approaches, including both reference-based mapping and *de novo* assembly. Furthermore, for adequate pandemic preparedness, pipelines must be robust to sequences originating from avians or swine. We aimed to demonstrate that accurate pre-classification can minimize data loss and erroneous sequence reconstruction.

### Methods

We developed an algorithm for classification of influenza short reads using de Bruijn graphs. Simulated reads from publicly available influenza gene sequences, and 257 real WGS datasets, were used to assess performance. Classification was assessed by comparison of the percentage identity of retrievals to sampled sequences for simulations, and comparison with *de novo* assemblies for real data. Mapping recovery was assessed by comparing the number of reads mapping to a single or multiple references versus a single reference chosen by our algorithm.

### Results

For short-read datasets simulated from influenza hemagglutinin (HA) sequences of non-human origin, read recovery after single-reference mapping with a human reference was often lower than 10% across several different simulations. With pre-classification, recovery was over 99.7%. In real data benchmarking, classifications had a mean of >99.8% identity to assembled contigs, and identified possible misassemblies. This resulted in an increase in the number of mapped reads by 6.8% on average, up to a maximum of 13.3% for routine samples.

### Conclusion

Conventional mapping approaches can be insufficient with a sub-optimal reference strain. Furthermore, misassembly is possible with *de novo* approaches. Accurate pre-classification from short-read data allows detection of highly similar strains, including those of non-human origin, maximizes read recovery in mapping, and provides a means for validating *de novo* assemblies.

*Keywords: Bioinformatics, whole-genome sequencing, classification, pandemic preparedness*

Topic: Public Health: Pandemic Preparedness  
Abstract No: 11137

## deltaFLU TECHNOLOGY FOR GLOBAL PANDEMIC PREPAREDNESS

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Pandemic influenza outbreaks are predictably unpredictable. The global death toll for the most recent influenza pandemic in 2009 is estimated to have exceeded 284,000 lives. The best option to mitigate the health and societal consequences of a pandemic is to have access to an efficacious vaccine in a timely manner – currently difficult with the state-of-the-art vaccine (egg-based) manufacturing technology and ineffective vaccines. deltaFLU is an advanced, interferon-inducing influenza vaccine that provides broad protection against unmatched strains, indicating its promise as a universal influenza vaccine. deltaFLU is the only vaccine in development with the mode of attenuation and mechanism of action based on deletion of the influenza NS1 gene. Lacking NS1, deltaFLU strains rapidly induce interferon, a key component of the immune response to viral infection. Administered as a nasal spray, deltaFLU elicits a robust interferon response in the nasal passages through the first line of defence at the point of entry against infection. The self-adjuvanting vaccine also creates a second line of defence by activating a broadly protective systemic immune response. Development of new strains with enhanced growth properties using our innovative research and development provides opportunities for enhancing the efficiency and economics.

Scalable and cost-effective strategy enables product commercialization. The VIVALDI and ESCO ASTER strategic partnership addresses biomanufacturing processes involving proprietary, animal-free component cell culture media, novel Tide Motion™ packed-bed bioreactors designed to achieve high-density VERO cell cultures resulting in high viral titers. Coupled with innovative downstream purification strategy that results in higher recoveries and shorter processing times. In general, an increase in viral titer production can significantly lower cost-of-goods and increase capacity of vaccine manufacturing platforms, making such vaccines accessible and affordable to the world population.

*Keywords: deltaflu;tidemotion;self-adjuvanting vaccine;biomanufacturing process*

Topic: Public Health: Pandemic Preparedness

Abstract No: 11147

## MEDFINDER: A SYSTEM TO HELP FIND ANTIVIRAL DRUGS DURING A PANDEMIC

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**Intro:** Antiviral drugs (AVDs) are the only drugs available to treat influenza. Ensuring people know where to get these medications during an influenza pandemic is key for early treatment. No real-time method exists for patients and providers to know which pharmacies have AVDs to fill prescriptions. Situational awareness on AVD supply and shortages is also needed by public health decision makers to identify when additional AVD may be needed and inform release of stockpiles. MedFinder address these needs.

**Method:** MedFinder is a free website for the public to locate nearby pharmacies where AVDs may be available. Mapped results display, indicating <24hour, 24-48hour, or >48hour supply of AVDs. CDC and Boston Children's Hospital conducted 4 pilots (2016-2018) with pharmacies to test MedFinder. A mathematical algorithm was created to estimate a stores' ability to fill an AVD prescription based on inventory on-hand; these data were reported daily by pharmacies to MedFinder. Pharmacy reports were validated through calls to randomly selected stores. A dashboard synthesizing store-level drug availability was also developed and tested.

**Results:** 12,712 pharmacies nationwide participated in pilots during the 2016-18 flu seasons to test accuracy of store-level reporting into MedFinder. Supply levels were reported for AVDs (oseltamivir brand/generic, zanamivir, baloxavir) and a control drug with predictable supply (simvastatin). Reported supply was checked and found >90% accuracy in stores reporting 24-48hour supply, or >48hour supply. Stores with supply <24hours showed more conservative reporting with 26% of stores reporting that they did not have AVD on-hand when they did.

**Conclusion:** Findings support MedFinder as a reliable tool to help patients find which pharmacies have antiviral medications, improving timely access to critical influenza medications during an influenza season or a pandemic. Currently, +85% of the US population lives within a 5 mile radius of a pharmacy reporting into MedFinder.

*Keywords: antivirals; pandemic; preparedness; early treatment*



Topic: Public Health: Pandemic Preparedness  
 Abstract No: 11321

## WHO PANDEMIC INFLUENZA SPECIAL STUDIES: PREPARING FOR THE NEW PANDEMIC

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**Introduction:** Epidemiologic, virologic, and clinical data are needed at various stages of an influenza pandemic to rapidly assess the characteristics of the novel virus and to optimize public health response. Targeted research studies will supplement surveillance systems to answer specific questions and will need to be designed to be resource- and time- efficient. The World Health Organization (WHO) has established a Pandemic Influenza Special studies (PSS) network, which aims to enhance preparedness and coordination efforts.

**Methods:** Building on several global consortia, the PSS network includes representative influenza experts from all 6 WHO Regions. PSS aims to identify, harmonize and prioritize the special studies needed at each stage of a pandemic response, while focusing on the early stages. PSS will provide study protocols and tools relevant for pandemic influenza risk assessment, modelling and evaluation of response measures. PSS will also assemble a network of sites, geographically distributed and operationally ready, which can be primed for a range of special pandemic studies. During the next pandemic, research processes and data sharing will be streamlined through the PSS network.

**Results:** The PSS now includes 15 experts covering major influenza transmission zones. Actions taken include: the development of an inventory of publicly available study protocols, the identification of 13 priority questions to be asked and responded to during the early phase of a pandemic, and 6 additional questions for the later phases. A two years' work plan was also developed.

**Conclusion:** Data from PSS will be used to estimate key transmissibility and severity parameters needed to predict the course of the pandemic, aid identification of population groups in need of targeted interventions, and support global assessment of country-level impact. The outputs of the network will enhance country and global readiness for the next influenza pandemic with the goal of saving lives.

*Keywords: Influenza, research, pandemic, preparedness, response, epidemiology, surveillance, severity*

Topic: Public Health: Pandemic Preparedness  
Abstract No: 11343

## HHS BIOMEDICAL ADVANCED RESEARCH AND DEVELOPMENT AUTHORITY INTERNATIONAL INFLUENZA VACCINE MANUFACTURING CAPACITY BUILDING PROGRAM

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### Introduction

The BARDA International Influenza Vaccine Manufacturing Capacity Building Program was established in 2006 with the objective to improve global pandemic preparedness by assisting developing/under-resourced countries to build and operate in-country vaccine manufacturing facilities. BARDA utilizes an integrated approach based on public-private partnerships to:

1. Expand global vaccine manufacturing capacity.
2. Provide in-country technical implementation assistance.
3. Make available technology for scalable manufacturing capacity.

### Methods

BARDA, in partnership with the World Health Organization through its Global Action Plan for Influenza Vaccines, supported the establishment of influenza vaccine manufacturing facilities for production of influenza vaccines. Fourteen manufacturers in thirteen countries were awarded technical and financial support contracts to establish influenza vaccine manufacturing capacity. BARDA supported manufacturing workforce training and on-site follow-up technical support. BARDA partnered with PATH to provide targeted clinical trial and manufacturing technical support to manufacturers achieve product licensure. BARDA partnered with IDRI to establish a vaccine adjuvant hub to make available clinically tested vaccine adjuvant to manufacturers.

### Results

1. Over 250 technical staff from participating developing countries attended comprehensive vaccine manufacturing training programs at BARDA-supported academic institutions in North Carolina and Utah.
2. Four seasonal influenza vaccines (with one receiving WHO pre-qualification) and nine pandemic vaccines (with two receiving WHO pre-qualification) were licensed or submitted BLAs in six developing countries
3. High leverage factor: every \$1 in BARDA funding has leveraged \$17 in local funding

### Conclusion

Global capacity to produce pandemic influenza vaccine by manufacturers in participating developing countries is projected to increase from less than 1 million doses in 2005 to up to 1.1 billion doses of vaccine to respond to a pandemic by the end of 2019. Sustainable capability in developing countries to produce influenza vaccine will improve global pandemic influenza mitigation and promote international health security.

*Keywords: pandemic influenza vaccine, manufacturing facilities, low and middle income countries, access and equity*

Topic: Public Health: Pandemic Preparedness

Abstract No: 11358

**INTEGRATED INFLUENZA SURVEILLANCE SYSTEM, REAL-TIME  
EPIDEMIOLOGICAL AND GLYCAN-ARRAY DATA ANALYSES,  
SEROLOGICAL SURVEILLANCE AMONG HIGH-RISK POPULATIONS  
PLUS MACHINE-LEARNING APPROACHES CAN MINIMIZE PANDEMIC  
THREAT – TAIWAN’S EXPERIENCES**

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Past influenza pandemics had novel viruses originated from animal hosts acquiring fast-spread capability in human populations. However, decision-makers lack a system providing sufficient information to give early warnings. We hereby established an integrated surveillance system through transmission chains involving different host species (wild birds, poultry, livestock, and high risk animal-handling workers) plus immediate glycan-array of novel viruses and seroepidemiological data analyses among high-risk populations.

We found that human influenza H3 viruses from those with animal exposures had higher animal-specific amino acids percentages. The pdmH1N1/09-hemagglutinin (HA)-E374K virus variants with higher transmissibility increased through the 2009 pandemic, particularly in areas with high-population densities in two metropolises of Taiwan, regardless of different intervention strategies. Moreover, schoolchildren’ mass immunization was most effective to interrupt viral transmission whereas decreasing social distance before large-gatherings minimized further spread. As most of avian influenza viruses (AIVs) are not easy to infect humans, serological surveillance of AIV, (such as H5 and H6 subtypes infection) in high risk populations of animal-related workers become crucially important to verify possible adaptation of novel influenza viruses from animals to human populations.

To identify high-risk populations for pandemic flu, machine learning approaches including decision tree (DT) and association rule mining (ARM) algorithms were applied to hospital-admitted cases of influenza-like illness. Different one or multiple comorbidities were identified for clinical intervention and public health planning to minimize ILI-associated deaths during the 2009 pandemic.

In conclusion, early detection of novel influenza virus through integrated surveillance, timely viral sequence analyses on amino acids with inter-species transmission capability, increasing viral transmissibility, pathogenicity and virulence, presence of patterns of  $\alpha$ -2, 6 sialic acid in HA using glycan array, serological surveillance, and machine-learning approaches on big data analyses, all together can reduce the possibility of viral adaptation to different hosts and minimize health threat of influenza pandemics through international collaboration in future years.

*Keywords: Pandemic Influenza Preparedness in Taiwan, Integrated Surveillance, Public Health Policy, Emergency Health Planning, Taiwan, Risk Assessment, Glycan array, Machine learning, Seroepidemiology, Global Health*

Topic: Public Health: Pandemic Preparedness  
Abstract No: 11721

## **Progress toward sustainable influenza vaccination in the Lao Peoples' Democratic Republic, 2012-2018**

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Despite global recommendations for influenza vaccination of high-risk, target populations, few low and middle-income countries have national influenza vaccination programs. Among 2012-2017, Lao PDR planned and conducted the series of activities to develop its national influenza vaccine program as a part of its overall national immunization program. In this paper, we review the underlying strategic planning for this process, and outline the sequence of activities, research studies, partnerships and policy decisions that were required to build Laos' influenza vaccine program. The successful development and sustainability of the program in Laos offers lessons for other low and middle-income countries interested in initiating or expanding influenza immunization.

*Keywords: Influenza; Vaccine; Policy; Laos; Vaccination Program;*

Topic: Public Health: Pandemic Preparedness

Abstract No: 11758

## DETECTION AND CHARACTERIZATION OF SWINE-ORIGIN INFLUENZA A(H1N1)PDM09 VIRUSES IN HUMANS FOLLOWING ZONOTIC TRANSMISSION

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### Introduction

Human-to-swine transmission of seasonal influenza viruses has led to sustained circulation of human-like viruses in swine. While these viruses evolve and become adapted in swine, nascent reverse zoonoses can result in virus detections that are difficult to distinguish as 'swine-origin' or 'human-origin' due to the genetic similarity of viruses circulating in both hosts. Herein, we report the identification of two zoonotic infections with A(H1N1)pdm09 viruses derived from swine hosts in the USA.

### Methods

Real-time RT-PCR testing of specimens collected from two patients from Iowa and Michigan during 2017 and 2019, respectively, indicated infection with seasonal influenza A(H1N1)pdm09 viruses. Viruses were further characterized using universal influenza A multisegment reverse transcription-PCR genome amplification, followed by Illumina MiSeq sequencing and phylogenetic analysis. Virus isolation and hemagglutination-inhibition testing were also performed. A k-mer based algorithm was tested to differentiate between swine- and human-associated influenza A(H1N1)pdm09 virus sequence fragments.

### Results

Sequence analyses suggested the human infections were swine-origin influenza A(H1N1) variant viruses most likely acquired following exposure to infected swine. Phylogenetic analyses revealed that, while the HA and NA genes were closely related to circulating human strains, these viruses were reassortants containing internal protein coding genes derived from triple reassortant internal genes (TRIG) and other swine-origin gene lineages. Results indicated that single nucleotide polymorphisms in sequence fragments can differentiate the species-origin of closely related influenza A(H1N1)pdm09 viruses. Antigenic characterization demonstrated that current seasonal vaccines induced neutralizing responses in ferrets and human population immunity is expected to be high.

### Conclusion

Although the swine-origin of these viruses was resolved by sequence comparisons with available swine viruses and k-mer analyses, future cases may not be as readily discernable. Rapid identification and characterization of zoonotic viruses with HA and NA genes closely related to circulating human strains is important for identifying novel virus infections especially in persons with influenza-like illness and history of swine exposure.

*Keywords: swine influenza virus, zoonotic infection, pandemic preparedness*

Topic: Public Health: Pandemic Preparedness  
Abstract No: 11790

## Review of the National Pandemic Flu Service treatment algorithm on community cases: Results from the Flu Watch Study

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**Introduction and Objectives:** During the 2009 pandemic the UK's National Pandemic Flu Service (NPFS) operated an internet and telephone-based service that assessed respiratory illnesses and authorised antiviral prescriptions to those meeting specific case definitions. To inform the current review of the NPFS treatment algorithm we assessed the 2009 algorithm on contemporaneous UK community cases, many of whom did not consult the NPFS.

**Methods:** Flu Watch was a UK community cohort study of influenza (2006-2011). Participants were prospectively followed up with weekly surveys on symptoms, health-seeking behaviour and treatment of respiratory illnesses. They were also asked to submit self-administered nasal swabs for PCR analysis on day two of any illness. During NPFS operation, we calculated 1) the proportion of illnesses meeting the NPFS influenza-like-illness (ILI) case definition within seven days (and were thus eligible for antivirals), 2) the proportion of illnesses meeting the case definition among participants with chronic illness and 3) the sensitivity, specificity, positive and negative predictive values of the case definition among illnesses with PCR data. Calculations were performed on all illnesses and separately on the subset that consulted the NPFS.

**Results:** After excluding illnesses which would have been diverted to other services, there were 1867 illnesses (67% with PCR data), of which 2% had an NPFS consultation, 10% had chronic illness, and 13% met the NPFS ILI case definition (14% among cases with chronic illness). The case definition had 52% sensitivity, 87% specificity, 19% positive and 97% negative predictive values. Among the 35 NPFS consultations, 71% met the case definition which had 50% sensitivity, 25% specificity, a 17% positive- and 63% negative predictive values.

**Conclusion:** Given the vast number of illnesses which could potentially consult a future NPFS, and the generally low sensitivity and specificity of symptom-based case definitions, it is worth considering having two NPFS ILI case definitions: a more inclusive and sensitive case definition for individuals at high risk of severe disease and a more restrictive and specific case definition for the low risk (majority) group. This would simultaneously target antiviral treatment to those who would most benefit from it whilst also preserving antiviral stockpiles.

*Keywords: antivirals, community studies, pandemic, case definitions*

Topic: Public Health: Pharmaco-economics, Cost-Effectiveness Studies  
Abstract No: 10388

## THE COST PER EPISODE OF INFLUENZA-LIKE ILLNESS IN INFANTS AND CHILDREN IN THAILAND, 2011-2016

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**Introduction:** Maternal influenza vaccination during pregnancy may provide protection to infants and children aged <1 year. To inform vaccination policy, we assessed costs of outpatient laboratory-confirmed influenza, and influenza-like illness (ILI), in children aged <5 years at a Bangkok, Thailand tertiary care hospital.

**Methods:** We combined the results from two prospective cohorts, conducted in children aged 0-5 years, and <6 months, respectively. Both cohorts included weekly surveillance for outpatient ILI and RT-PCR confirmation of influenza viruses. ILI episodes were independent if separated by  $\geq 14$  days. Illness-associated costs (medical, travel, reported income loss, and opportunity loss) were log-transformed, adjusted to 2016 values, and compared across age and risk group (healthy vs. those existing conditions ['high-risk']) using Student's T-test/ANOVA.

**Results:** Among 856 children, 144 (7%) of 2,133 ILI episodes were laboratory-confirmed as influenza. The number of ILI cases (% positive for influenza) were 445 (5%); 781 (7%); and 907 (8%) in <1 year; 1 to <2 year; and 2-5 year age groups, respectively. High-risk children accounted for 872 episodes (5%, or 41, positive for influenza). The distribution of costs attributed to laboratory-confirmed influenza vs. non-influenza ILI, respectively, were: healthcare related direct costs (56% vs. 80%,  $p=0.38$ ); non-healthcare related costs (16% vs. 9%,  $p=0.81$ ); and indirect costs (28% vs. 11%,  $p=0.09$ ). The median cost per episode of laboratory-confirmed influenza ( $n=144$ ) and non-influenza ILI ( $n=1,989$ ) were US\$22 each (IQR, 12-41 and 14-38, respectively). The cost of laboratory-confirmed influenza did not differ by age or risk group. However, the ILI cost per episode was greater in children <1 year (US\$27; IQR 16-42,  $p<0.05$ ) and in high-risk children (US\$24; IQR, 13-48) than in healthy children (US\$21; IQR, 12-38,  $p<0.01$ ).

**Conclusions:** These findings support efforts to evaluate the cost effectiveness of influenza vaccination programs, and highlight the cost of ILI in very young and high-risk children.

Topic: Public Health: Pharmaco-economics, Cost-Effectiveness Studies  
Abstract No: 10405

## THE ECONOMIC ADVANTAGES OF A CELL-BASED QUADRIVALENT INFLUENZA VACCINE IN THE ADULT POPULATION IN EUROPE. THE COST-EFFECTIVENESS EVIDENCE IN UNITED KINGDOM AND SPAIN

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### Objective

The European Medicine Agency recently approved the first quadrivalent cell-based vaccine (QIVc). There is emerging data to suggest the cell-based (QIVc) may have advantages over egg-based quadrivalent vaccines (QIVe). Therefore, modelling the incremental cost-effective ratios (ICER) between QIVc and the current available vaccines in UK and Spain using different models appropriate for the country policy is relevant for the public health policies.

### Methods

The analyses were conducted for the 18-64yrs population in UK and 9-64yrs in Spain. Both assessments were evaluated from the national health system (NHS) and social perspectives with an annual time horizon. For the UK a dynamic transmission model was used and for the Spanish case a decision tree model. Costs and clinical benefits were modelled for the QIVc against the current vaccination (QIVe and Trivalent Inactivated Vaccine(TIV)) strategies. Effectiveness and epidemiological data were obtained from UK and Spanish literature, respectively. Local costs were obtained from national sources. Probabilistic sensitivity analyses were conducted in both models.

### Results

In UK, using QIVc instead of QIVe in 18-64yrs subjects, QIVc can reduce on average 158,300 symptomatic flu cases, 18,300 outpatient visits, 410 hospitalizations and 66 deaths. In Spain, replacing TIV with QIVc, in 9-64yrs population, QIVc would avoid 23,846 symptomatic flu cases, 9,547 outpatient visits, 218 hospitalizations and 14 deaths. Likewise, replacing QIVe; QIVc would avoid 14,991 symptomatic flu cases, 6,013 GP visits, 114 hospitalizations and 7 deaths. From the NHS perspective, QIVc showed to be cost-effective below local published ICER thresholds (€683 in UK and €19,914 vs TIV and €8,672 vs QIVe in Spain) and from social perspective QIVc will result cost-saving in both countries.

### Conclusions

The inclusion of the QIVc in UK and Spain will be cost effective, reduce the disease burden, and improve the efficiency within these national health systems.

*Keywords: Cell-based vaccine, Influenza, Cost-Effectiveness, United Kingdom, Spain*



Topic: Public Health: Pharmaco-economics, Cost-Effectiveness Studies  
Abstract No: 10421

## Optimal design of population-level financial incentives of influenza vaccination for elderly

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**Introduction and Objectives:** How monetary incentive affect influenza vaccination uptake rate and assessment of optimal subsidy through a randomized control experiment.

**Methods:** 4,000 people aged 65 and above were randomly assigned to 4 treatment groups (1,000 each), each offered a monetary incentive (in shopping vouchers) if they chose to participate. The baseline group was to complete a questionnaire for SGD 10, while the other three groups were to complete the questionnaire and be vaccinated against influenza for incentives of SGD 10, 20 or 30.

**Results:** Increasing the monetary incentive from SGD 10 to SGD 20 increased participation rates from 4.5% to 7.5% ( $p < 0.001$ ). Further increasing the incentive from SGD 20 to SGD 30 increased the participation rate to 9.2% ( $p > 0.05$ ). The group of non-working elderly were more sensitive to changes in incentives than those who are working. The effects of increasing incentives on influenza vaccination rate differed by ethnicity, socio-economic status, household size and a measure of social resilience. However, there were no evidence of differential effects by age group, gender, or education. The cost of the programme per completed vaccination under SGD 20 incentive is SGD 36.80, which was smallest among the three intervention arms. For a hypothetical population-level financial incentive programme to promote influenza vaccination among the elderly, accounting for transmission dynamics, the incentive minimising the incremental cost effectiveness ratio ranges from SGD 10 to SGD 20.

**Conclusion:** Appropriate monetary incentives can boost the influenza vaccination rate. Given the average cost to the consumer of influenza vaccination at SGD 32, increasing monetary incentive from SGD 10 to SGD 20 can improve the influenza vaccination uptake rate, but further increasing the monetary incentive to SGD 30 may not raise the uptake rate. A partial subsidy may therefore be considered to improve vaccination coverage in this high-risk group.

Topic: Public Health: Pharmaco-economics, Cost-Effectiveness Studies  
 Abstract No: 10500

## Economic assessment of a high dose versus an adjuvanted influenza vaccine: an evaluation of hospitalization costs based on a cohort study

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### Introduction and Objectives

Adults 65 years and older are at greater risk for complications following influenza infection. Two influenza vaccines are licensed in the U.S. exclusively for seniors: a trivalent inactivated high dose (HD) influenza vaccine (Fluzone® High-Dose, Sanofi Pasteur) and a trivalent inactivated adjuvanted (aTIV) influenza vaccine (Fluad®, Seqirus). We recently estimated a relative effectiveness (rVE) of HD vs aTIV of 12% (95% confidence interval: 3.3% – 20%) for influenza related hospitalizations using a retrospective study design. Here we report the effect both vaccines had on the hospitalization costs.

### Methods

We used claims data from UnitedHealth Group to compare costs of hospitalization for respiratory disease between recipients of HD and aTIV during two influenza seasons: 2016/17 and 2017/18. Subjects were 65 years and older at time of vaccination. Hospitalization rates were adjusted for demographics, comorbid conditions, previous influenza vaccination and geography. Vaccine costs were obtained from the Medicare pricing schedule. Our economic assessment includes index hospitalizations and readmissions.

### Results

We analyzed 1,900,920 HD and 223,793 aTIV recipients. Average vaccine prices were \$46.23 and \$48.26 for HD and aTIV, respectively. The hospitalization rates for respiratory disease in HD and aTIV recipients were 187 (95% confidence interval: 184 – 189) and 212 (195 – 232) per 10,000 persons-years, respectively. Attributing the average cost per hospitalization of \$11,523 (\$11,278 – \$11,768) to the difference in hospitalization rates, we estimate net savings of HD to be \$31 (\$10 – \$55) per recipient. We cannot fully adjust by insurance plan type; readmission differences may not be vaccine preventable.

### Conclusion

In this retrospective observational study, we found that HD was associated with lower hospitalization costs compared to aTIV in UnitedHealth vaccine recipients in A/H3N2 predominant seasons. Reduced hospitalizations affect healthcare utilization overall, and therefore other costly health outcomes.

This study was funded by Sanofi Pasteur

*Keywords: high-dose influenza vaccine; adjuvanted influenza vaccine; economic analysis*

Topic: Public Health: Pharmaco-economics, Cost-Effectiveness Studies  
Abstract No: 10684

## The Economic Burden of Influenza in the United States since 2009

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**Introduction:** Since 2009, seasonal influenza in the United States has resulted in 140,000–960,000 hospitalizations and 12,000–79,000 deaths annually, contributing to direct healthcare costs and indirect costs due to lost productivity or premature death.

**Methods:** We developed a probabilistic model to estimate the economic burden of the 2009 pandemic and the 2010–11 through 2017–18 influenza seasons for four illness outcomes (non-medically attended, medically-attended, hospitalization, and death). We estimated direct and indirect costs by five age groups (0–4, 5–17, 18–49, 50–64, and ≥65 years old) and presence of an underlying high-risk condition. We based epidemiologic inputs on existing CDC surveillance data and burden estimates and derived costs from health insurance claims and published data on average wages and lost productivity.

**Results:** Since 2009, influenza has resulted in over \$375 billion in total costs while the annual economic burden ranged from \$15.0 billion during the low-severity 2011–12 season to \$56.2 billion during the 2009 pandemic and \$63.8 billion during the high-severity 2017–18 season. Persons aged ≥65 accounted for 6.6% of the total economic burden during the 2009 pandemic but up to 52% during the high-severity 2014–15 season. Over the same period, lost productivity due to death contributed 61–79% of the indirect cost and hospitalizations contributed 51–70% of the direct cost; direct costs contributed 25–38% of the total economic burden. Influenza illness was associated with up to 92 million productive days lost during the 2009 pandemic, and influenza-associated hospitalizations occupied up to 3.4 million hospital days during 2017–18.

**Conclusion:** Influenza has resulted in a substantial combined and annual economic burden since 2009, including up to \$63.8 billion (0.3% of the 2018 U.S. gross domestic product) during a high-severity season. Further efforts to reduce the burden of influenza are needed.

Topic: Public Health: Pharmaco-economics, Cost-Effectiveness Studies  
 Abstract No: 10790

## Influenza Vaccine Programs with the Cell-Based Quadrivalent Influenza Vaccine are Highly Effective in Canada

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### Introduction

The cell-culture based quadrivalent influenza vaccine (QIVc) production platform aims to address the mutagenesis of influenza viruses that are propagated in eggs (QIV) and the associated decreased vaccine effectiveness. Recent studies have demonstrated the better relative effectiveness of QIVc versus standard, egg-based vaccines. The current analysis evaluates the public health impact of its introduction in Canada.

### Methods

An SEIR dynamic-transmission model was created for the Canadian population and adapted to the influenza strains A/H1N1, A/H3N2, B/Yamagata, B/Victoria for the 2011-2018 seasons. Strain-specific circulation was obtained from FLUNET. Influenza incidence estimates were calibrated using published mortality data. QIVc was assumed to be better matched against circulating A/H3N2 strains. Frequency of egg-based genetic changes were estimated from a published review of Crick Institute reports. Estimates of age-specific vaccine efficacy were derived from the published literature and calibrated for the specific age group (4-17, 18-64, and ≥65 years of age).

### Results

Results of the model are presented below. Fewer counts of all outcomes evaluated were observed in influenza program scenarios where QIVc was included compared to standard, egg-based QIV. The greatest benefit was observed when QIVc was implemented in all individuals ≥5 years of age.

Outcomes	Influenza Vaccine Program Scenarios					
	QIV for all	QIV: 6 months-4years	QIV: 6 months-17 years	Marginal analyses		
		QIVc: ≥ 5 years	QIVc: ≥ 18yr	Scenario 1 vs. Baseline	Scenario 1 vs. Baseline	Scenario 1 vs. Scenario 2
	[Baseline]	Scenario 1	Scenario 2	Scenario 1 vs. Baseline	Scenario 1 vs. Baseline	Scenario 1 vs. Scenario 2
Cases	2,525,610	1,800,280	1,996,140	-725,330	-529,470	-195,860
GP visits	349,240	252,710	279,600	-96,530	-69,640	-26,890
ED visits	87,310	63,180	69,900	-24,130	-17,410	-6,720
Hospitalisations	21,590	14,920	16,460	-6,670	-5,130	-1,540
ICU Hospitalisations	4,240	2,950	3,250	-1,290	-990	-300
Deaths	4,070	2,400	2,650	-1,670	-1,420	-250

### Conclusion

The greatest public health impact is obtained by the introduction of QIVc in influenza vaccine programs in Canada.

*Keywords: influenza; vaccine; dynamic transmission model; epidemiology; health economics*

Topic: Public Health: Pharmaco-economics, Cost-Effectiveness Studies  
Abstract No: 10988

## EVALUATING THE POTENTIAL HEALTH AND ECONOMIC IMPACT OF FREE INFLUENZA VACCINATION IN THE ELDERLY IN MAINLAND CHINA

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**Introduction and Objectives:** WHO recommends annual seasonal influenza vaccination for the elderly, with an increased risk of hospitalization or death if infected. China is amongst the ageing societies, with around 15% of total population aged 60 years old and over. In mainland China, over 80% influenza-related excess mortality occurs in the elderly. However, influenza vaccines are used in the private sector and paid by vaccines. Uptake of self-paid influenza vaccine is extremely low in the elderly, only 7.4% even in large cities. Influenza vaccine is in the priority list under consideration to be fully funded by government. This study aims to evaluate the health and economic impact of free influenza vaccination in the elderly, which could indeed help policy-making.

**Methods:** A decision tree model using China-specific data was developed to answer the questions whether the potential free influenza vaccination for nearly a quarter of the world's older persons is a good value for money from the societal perspective. Cost were presented in 2013 US\$.

**Results:** For the base case scenario (assumed an uptake rate of 40%), 77.3 million old persons are expected to be vaccinated annually. Vaccination is estimated to cost US\$ 574 (95%CI 551-599) million, but expected to prevent 190,000 (112,000-273,000) symptomatic influenza cases, and 45,000 (17,000-88,000) influenza-related deaths due to Respiratory and Cardiovascular Diseases. A total of 409,000 (153,000-796,000) QALYs are estimated to be gained. The incremental cost-effectiveness ratio (ICER) is US\$ 1 480 (574-3 423) per QALY gained. Vaccination programme in the old adults is cost-effective at a threshold of US\$ 4,550 per QALY gained. The probability sensitivity analysis shows almost 100% of Monte Carlo samples are considered cost-effective.

**Conclusion:** The findings revealed that providing free influenza vaccination for the elderly by government could vastly decreased the disease burden, and the investment represents a "good buy".

*Keywords: Influenza; free vaccination; elderly; cost-effectiveness; China*

Topic: Public Health: Pharmaco-economics, Cost-Effectiveness Studies  
Abstract No: 11296

## CHALLENGES ASSOCIATED WITH THE ASSESSMENT OF THE COST-EFFECTIVENESS OF QUADRIVALENT INFLUENZA VACCINE (QIV) IN EUROPE

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**INTRODUCTION AND OBJECTIVES:** The cost-effectiveness of the quadrivalent seasonal influenza vaccine over trivalent one was assessed for five European countries using a static cost-utility model. Thanks to national surveillance networks, most of the country-specific epidemiological data were available but some of them conveyed strong uncertainties. The objective was to highlight the challenges associated with data availability and robustness for the analyses in European countries.

**METHODS:** The input collections performed to conduct analyses for Germany, Italy, Slovakia, Poland and Portugal were reviewed, focusing on the epidemiological parameters. The data availability and robustness between the countries were assessed. The most recurrent issues were identified and discussed.

**RESULTS:** Sources of uncertainty identified in the model were the inputs related to influenza burden: excess outpatient visit, hospitalisation and mortality rates. Ideally, these inputs were provided by official report published by national public health agencies. For excess hospitalisations or mortality, however, data for several countries or age groups, were not available and estimates from other countries had to be used. Moreover, some data were likely underestimated. For example, while national sources did not report any influenza-related deaths in Germany for the season 2013/2014 and in Poland in 2014/2015, published data obtained from the FluMoMo model estimated that the excess mortality in European countries for these seasons was 30 and 185 per 100,000, respectively. Similarly, the excess hospitalisation rates reported by the national sources in Germany were very low (peak at 138/100,000) compared with the ones reported in the literature for the same seasons (peak at 603/100,000).

**CONCLUSION:** Data from national sources should be preferred when available, however local estimates should be cross-checked and extensively tested in sensitivity analyses to assess and control the uncertainty around them. Despite a potential underestimation of its actual benefit, QIV appeared to be a cost-effective strategy in the countries studied.

*Keywords: quadrivalent, influenza, cost-effectiveness, Europe*

Topic: Public Health: Pharmaco-economics, Cost-Effectiveness Studies  
Abstract No: 11303

## CHALLENGES ASSOCIATED WITH THE ASSESSMENT OF THE PUBLIC HEALTH IMPACT OF A QUADRIVALENT INFLUENZA VACCINE (QIV) IN LATIN AMERICA

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**INTRODUCTION AND OBJECTIVES:** A model was developed to estimate the potential benefit that QIV would have had if it had been used instead of the trivalent vaccine during recent influenza seasons. To estimate the impact of QIV in terms of prevention of influenza and its consequences, the model required influenza-related outcomes observed in prior seasons. The objective was to assess the availability and the robustness of data to inform this model in Latin America.

**METHODS:** The input collections performed to conduct analyses for Mexico, Peru and Costa-Rica were reviewed, focusing on parameters on the burden of the disease. Data availability and robustness were assessed. The most recurrent issues were identified and discussed.

**RESULTS:** In Mexico, no epidemiological data needed for the model were publicly available, therefore the excess rates were obtained by adjusting data from the US found in the literature. For the countries where data were available, very different excess outcome rates attributable to influenza (outpatient visit, hospitalisation, death) were found. For example, in 2016 the excess general practitioner consultation rate reported by the Ministry of Health was 5,769 per 100,000 in Peru compared to 20 per 100,000 in Costa-Rica. To a lesser extent, heterogeneity was also observed in excess hospitalisation rates (e.g. 21 vs. 3 per 100,000 in Peru and Costa-Rica respectively in 2016) and death rates (e.g. 13 vs. 2 per 100,000 in 2016). This variability in the reporting exists also in the scientific literature. In Costa-Rica for example, estimates published appeared to be much higher than the ones obtained from national sources.

**CONCLUSION:** While the coverage rate for influenza vaccines is increasing in this region, issues and heterogeneity in the data calls for a harmonised and transparent approach for collecting reliable influenza epidemiological inputs across Latin America to support robust assessment of the vaccines benefit.

*Keywords: quadrivalent, influenza, public health, LATAM*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 10325

## SURVEILLANCE OF SEVERE INFLUENZA IN THE CZECH REPUBLIC DURING 2015-16, 2016-17 AND 2017-18 INFLUENZA SEASONS

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### Introduction and Objectives

Influenza infection varies from mild to severe and life-threatening. Severe influenza (SARI) is defined as laboratory confirmed acute respiratory infection that requires hospitalization at intensive care unit. The objective of our study was to analyse SARI cases during influenza seasons 2015-16, 2016-17 and 2017-18 in the Czech Republic (CZ). Due to unpredictable influenza B lineages circulation we also investigated circulation of influenza viruses in order to evaluate the importance of a quadrivalent influenza vaccine usage.

### Methods

The epidemiological and virological surveillance system of influenza in CZ is active through the year and uses EU case definition for influenza. SARI surveillance has been established as national surveillance in all 14 regions from all hospital's ICUs. Case-based data were analysed.

### Results

248, 337 and 667 SARI cases (of which 85, 115 and 261 deaths) were reported during 2015-16, 2016-17 and 2017-18 seasons in CZ. Mean age of SARI patient was 56.2 years (age range 0-91) during 2015-16 season, 69.2 years (0-96) during 2016-17 season and 61.3 years (0-97) during 2017-18 season. Most patients had at least one risk factor for severe influenza infection. Influenza B was positive in 7.7% (19/248), 4.2% (14/337) and 56.8% (379/667) of cases during individual seasons.

Among children and adolescents up to 18 years, 10 SARI cases (1 death), 12 SARI cases (0 death) and 43 SARI cases (5 deaths) were reported during the mentioned seasons.

### Conclusion

Influenza epidemics differ in duration, magnitude and the circulating A subtypes/B lineages. The severity of some seasonal epidemic is comparable with the pandemic in 2009-10. Quadrivalent influenza vaccine should be used in order to address the uncertainties of influenza B strain circulation, and to offer direct protection against co-circulating two B lineages simultaneously.

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*Keywords: influenza; severe influenza; SARI; influenza vaccine; surveillance*



Topic: Public Health: Surveillance & Forecasting  
Abstract No: 10328

## The application of CUSUM model and Serfling regression model in early detecting of onset of seasonal influenza

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**Objectives:** To evaluate the efficiency of Cumulative Sum (CUSUM) model and Serfling regression model for detecting the onset of the epidemic of seasonal influenza.

**Methods:** The surveillance data of influenza like illness (ILI) surveillance and virological surveillance in Beijing, China from 2013 to 2018 were analyzed to detect the onset weeks of seasonal influenza epidemic, using CUSUM model and Serfling regression model. The results at different combinations of parameter values were compared with the gold standard based on virological data. The performances of the two models were evaluated based on the indicators comprising sensitivity, specificity, Youden's index and lead time.

**Results:** At different scenarios of CUSUM model, the sensitivities ranged from 48.78-53.66%, the specificities ranged from 77.53%-86.52%, and Youden's indexes ranged from 0.33-0.50. With regards to the performance of Serfling regression model, the sensitivities ranged from 40.24-56.10%, the specificities ranged from 76.40-96.63%, and Youden's indexes ranged from 0.33-0.50. Both CUSUM model and Serfling regression model triggered the signal within three weeks of the epidemic of seasonal influenza.

**Conclusions:** CUSUM model and Serfling regression model were suitable for detecting the onset of seasonal influenza epidemic.

**Keywords:** seasonal influenza , early detecion , CUSUM , Serfling

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 10379

## Influenza Surveillance Enhancements: Improving influenza surveillance to answer questions about disease impact

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**Introduction:** During the 2018-2019 influenza season, the Influenza Division of the Centers for Disease Control and Prevention (CDC), in collaboration with the Council of State and Territorial Epidemiologists (CSTE), supported the enhancement of influenza surveillance at state and local health departments. Three priority surveillance gaps were targeted:

1. Determining the proportion of outpatient visits for influenza-like illness (ILI) that are due to influenza virus infection;
2. Assessing the effect of virus characteristics on influenza disease severity;
3. Estimating population based rates of outpatient ILI.

**Methods:** Seventeen state and local jurisdictions were chosen from those applying for funds available from CSTE. Sites worked with outpatient providers to systematically collect respiratory specimens from patients presenting with ILI, determined whether specimens tested at the PHL were from outpatients or hospitalized patients, and estimated the population served by outpatient providers reporting ILI data. Modifications were made to existing data reporting and analysis mechanisms to handle the enhancements and enable reporting of these data to CDC.

**Results:** As of March 1, 2019, ten jurisdictions enrolled 45 providers that systematically collected 1,530 specimens from ILI patients and reported ILI data weekly to CDC. Fourteen jurisdictions identified and reported to CDC the level of care (outpatient/hospitalized) for patients associated with 5,025 specimens tested at public health laboratories. Nine jurisdictions estimated the population served by 124 outpatient providers thus enabling calculation of ILI rates when previously only the percent of patients presenting with ILI could be determined.

**Conclusion:** State and local health departments and CDC successfully modified their respective data collection, reporting and analysis systems to accommodate data elements beyond those historically collected as part of the national U.S. Influenza Surveillance System. These additional data enhanced our ability to interpret data from existing surveillance components during the season and will be used to improve future disease burden estimates.

*Keywords: influenza, surveillance, ILI, level-of-care*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 10442

## Evaluation of the application of Moving Epidemic Method on making influenza epidemic thresholds in the 7 climate zones in China mainland

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**Introduction** In this study, we planned to make full use of historical influenza surveillance data and apply some methods to improve the application of this method to calculate influenza epidemic threshold in China mainland. We want to provide a practical and feasible method for the establishment of influenza epidemic thresholds in China.

**Methods** The positive rate of influenza virus was obtained from the National Influenza Surveillance Network System from 2010/2011 to 2017/2018. We divided the 31 provinces into 7 climatic zones according to previous literatures. 3 of the 7 zones have two epidemic waves in some flu season and these two-waves-seasons were divided into two parts for analysis.

**Results** We used Moving Epidemic Method to calculate influenza epidemic thresholds and intensity thresholds for the 7 climate zones in China. The average sensitivity was 86.16%, the average specificity was 94.92%, the average positive predictive value was 89.87%, the average negative predictive value was 92.96%.

**Conclusion** Overall, the method performs well, much better than the previous study. Comparison with the climate zones with one single epidemic wave, the model performs less well in climate zones with two epidemic waves, but still within acceptable range.

*Keywords: Influenza; Moving Epidemic Method; Epidemic thresholds; Intensity thresholds*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 10457

## Characterization of Influenza B Victoria Lineage viruses in recent two seasons in South Korea

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Influenza B virus has induced influenza epidemics affecting disease burden like type A viruses and finally resulted in application of quadrivalent influenza vaccine composing both Yamagata and Victoria lineages. National Influenza Center in South Korea found variant in Victoria lineage viruses through influenza laboratory surveillance and want to report their characterization in this study.

The respiratory specimens collected from Korea Influenza and Respiratory viruses Laboratory Surveillance System in 2017-2018 and 2018-2019 seasons. Real-time RT-PCR was applied to detect influenza B virus and differentiate lineages. HA genetic sequences of influenza B viruses were used for phylogenetic analysis. Three types of ferret antisera to Victoria lineage viruses were used for antigenic characterization

Influenza B virus was prevalent in 2017-2018 season in South Korea and the most lineage (98%) was Yamagata. Interestingly, the other lineage was Victoria hovering three deletion (3del) in 162-164 amino acid of Hemagglutinin (HA). The Victoria lineage was antigenetically distinct from B/Brisbane/60/2008 selected as a vaccine strain in 2017-2018 season. In the next season (2018-2019), Influenza B virus detection rate was quite low during the influenza epidemic. However the detection rate began to increase since the first detection in 52th week 2018 and the better part of influenza B virus detected till 9 week 2019 belonged to Victoria lineage (88.8%). The Victoria lineage viruses appeared in three sub-lineages such as three deletion (3del) in 162-164 amino acid, two deletion (2del) in 162-163 amino acid and no deletion of HA. Almost half of Victoria lineage viruses were 3del variants and their antigenicity was distinct from B/Colorado/06/2017 updated vaccine strain in 2018-2019 season.

It is inevitable to detect variant of influenza B Victoria lineage virus as well as analyze antigenic characterization for the optimal selection of influenza vaccine component.

This study was supported by intramural funds (4851-304) of KCDC.

Topic: Public Health: Surveillance & Forecasting

Abstract No: 10496

## Current practices for Respiratory Syncytial Virus surveillance across the EU/EEA Member States, 2017

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**Introduction:** Respiratory syncytial virus (RSV) is a major contributor to lower respiratory tract infections worldwide. Currently, several new RSV vaccine candidates are in development. Following vaccine introduction, a reliable RSV surveillance system should enable monitoring of vaccination impact. Data on RSV disease burden within the EU are generally sparse and no harmonised case definition, reporting or guidelines for RSV typing are in place. The aim of this study was to gather knowledge on current practices regarding national RSV surveillance in the EU/EEA Member States.

**Methods:** National Coordinators and National Focal Points for Influenza from the EU/EEA Member States (n=31) were invited to participate in an online survey in August-September 2017. The questionnaire covered questions on both epidemiological and laboratory aspects of RSV surveillance.

**Results:** All EU/EEA countries, except for Liechtenstein, replied to the survey (30/31). Eighteen countries (60%) reported to have a sentinel surveillance system, 26 countries (87%) a non-sentinel surveillance system and three countries (10%) reported to have neither of those. RSV surveillance was mostly part of influenza surveillance. A wide range of diagnostic assays was used for the detection of RSV within the reporting countries.

**Conclusion:** The results of this survey demonstrate that the majority of EU/EEA countries have some surveillance for RSV in place. National RSV surveillance is crucial for monitoring epidemiological trends and to guide national decision making for vaccination programmes. In order to improve RSV surveillance, its goals should be well-established and agreed as different requirements might apply across the EU/EEA.

Topic: Public Health: Surveillance & Forecasting

Abstract No: 10501

## Seasonal influenza activity in the World Health Organization European Region, 2010 to 2018

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**Background:** Influenza virus infections are common and lead to substantial morbidity and mortality worldwide. In the WHO European Region 50 countries and regions comprising about 900 million inhabitants routinely collect and report influenza surveillance data to the European Surveillance System. We characterized the first eight influenza epidemics following the 2009 influenza pandemic by comparing the distribution of viruses by outpatient and hospital setting and described epidemics temporally and geographically across the Region.

**Methods:** We retrospectively analysed laboratory-confirmed influenza detections by PCR in ambulatory patients from sentinel sites. Data were aggregated by country and season (weeks 40 to 20) for seasons 2010-2011 to 2017-2018. We explored geographical spread using correlation coefficients.

**Results:** There was variation in the regional influenza epidemics during the study period. Influenza A virus subtypes alternated in dominance, except for 2013-2014 during which both co-circulated, and in only one season (2017-2018) was B virus dominant. The median start week for epidemics in the Region was week 50, the time to the peak ranged between four and 13 weeks and the duration of the epidemic ranged between 19 and 25 weeks. There was evidence of a west to east spread across the Region during epidemics in 2010-2011 ( $r=0.365$ ;  $p=0.019$ ), 2012-2013 ( $r=0.484$ ;  $p=0.001$ ), 2014-2015 ( $r=0.423$ ;  $p=0.006$ ) and 2017-2018 ( $r=0.566$ ;  $p<0.001$ ) seasons. Variation in virus distribution and timing existed within countries across seasons and across countries for a given season.

**Conclusions:** Data for these eight years demonstrate that substantial diversity exists between influenza epidemics in the Region. These data can inform public health experts at national and international level when planning response strategies and risk communications. Aggregated, regional surveillance data in early affected countries might give early warning to other countries of circulating viruses and intensity.

*Keywords: Influenza, Surveillance, Europe, Central Asia*

Topic: Public Health: Surveillance & Forecasting

Abstract No: 10552

## USE OF WHOLE GENOME SEQUENCING TO IMPROVE THE INFLUENZA SURVEILLANCE IN BELGIUM DURING THE 2016-2017 PILOT SEASON

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Monitoring and detecting sequence variations in influenza viruses is critical to public health epidemiology, pandemic preparedness, and prevention and treatment of human influenza. Whole genome sequencing (WGS) can be a valuable alternative to more traditional approaches, such as RT-qPCR and Sanger sequencing, offering the possibility to explore the epidemiological effects of intra- and inter-season evolutionary dynamics and within subtype reassortments of circulating influenza viruses.

To obtain whole genome influenza sequences, a published multiplex RT-PCR protocol was improved to generate amplicons of the 8 influenza segments from swab samples that were subsequently sequenced using the Illumina MiSeq. In total, 253 whole genomes of A(H3N2) viruses were obtained that were detected in mild, moderate and severe cases during the 2016-2017 influenza season in Belgium. Patient information, including location, age, gender, date of sampling, disease severity and vaccine status, were integrated into the WGS data analysis. The WGS data were used to genetically characterize the different circulating strains, and to identify genetic drift variants, mutations and reassortments compared to the A/Hong Kong/4801/2014 vaccine strain. Integrating WGS data with patient vaccination history and disease outcome, will facilitate correlating (minor) genetic variants with vaccine effectiveness or disease severity. WGS data allowed to construct phylogenetic trees based on all segments in contrast to current methods based typically on only the HA segment. Therefore, it allows for a more robust inference of reassortments and clusters of strains associated with disease severity and/or vaccination history. Our study illustrates that WGS combined with clinical and epidemiological data is feasible to strengthen the laboratory surveillance of influenza virus.

*Keywords: Whole Genome Sequencing; Influenza; Surveillance; A(H3N2)*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 10562

## BioFire Syndromic Trends: A worldwide, online network tracking respiratory disease in real-time

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**Introduction and Objectives:** Real-time data collection of respiratory disease is important for identifying pathogen prevalence, facilitating early outbreak detection, determining clinically relevant codetections, and observing disease seasonality. Healthcare professionals use tools such as FluView and FluNet to help identify local pathogen circulation; however, these tools are typically restricted to tracking a limited set of pathogens and lack real-time reporting and comprehensive codetection identification.

Here we describe BioFire<sup>®</sup> Syndromic Trends (Trends) – a cloud-based, research epidemiology system that receives real-time data from BioFire<sup>®</sup> FilmArray<sup>®</sup> Respiratory Panel (RP) tests run in the United States (US), Latin America (LATAM), Europe (EU), and South Africa (SA). The BioFire RP Panel offers detection of up to 22 viral and bacterial targets.

**Methods:** The Trends database received over 736,869 de-identified BioFire RP Panel tests from 82 laboratories across the world – 56 US labs, 19 LATAM labs, 3 EU labs, and 4 SA labs. We calculated pathogen detection and co-detection rates of each region and then plotted sum positivity to observe regional and seasonal trends.

**Results:** The overall calculated positivity rates of BioFire RP Panel tests varied between the US (46%), LATAM (64%), EU (50%), and SA (71%). We additionally observed varying co-detection rates between these regions. Of all positive detections during this time, rates of co-detection were 8% (US), 14% (LATAM), 6% (EU), and 23% (SA). The data furthermore displays worldwide seasonality for several pathogens.

**Conclusion:** Trends shows great promise in deciphering common respiratory infections and identifying global differences in disease patterns over time. One limitation of this dataset is the small number of non-US clinical laboratories connected to Trends, and we are unsure if observed differences are due to geographic location or imbalanced distribution of test data. We will perform further study as our global network grows.

*Keywords: Respiratory, infection, epidemiology, disease surveillance*



Topic: Public Health: Surveillance & Forecasting

Abstract No: 10679

**FIRST INTERNATIONAL WORKSHOP ON PREPARATION OF INFLUENZA CANDIDATE  
VACCINE VIRUSES BY REASSORTMENT**

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**Introduction:** A workshop was held on generation of Influenza virus reassortants in September 2018 in Atlanta, USA. Participants included representatives from all reassorting laboratories who make reassortant candidate vaccine viruses generally available to Influenza vaccine manufacturers, either directly or through WHO Collaborating centres for Influenza, as well as representatives from influenza vaccine manufacturers.

**Method:** The reassorting laboratories were surveyed on topics that included reassorting protocols, use of high growth donors, use of polyclonal or monoclonal antisera, gene composition, serological sub-typing, assessment of potential manufacturing yield and selection of viruses to prioritise for reassortment.

**Result:** This presentation will review the detailed findings of this survey and the outcomes of the workshop

**Conclusion:** Positive outcomes were enhanced communication between stakeholders and sharing of new technology, and establishment of a co-ordinated approach to activities globally.

*Keywords: vaccine; reassortant; workshop*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 10690

## IMPACT OF SEASONAL INFLUENZA ON POLYCLINIC ATTENDANCES IN SINGAPORE, 2012-2017

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**Introduction:** The burden of influenza on primary healthcare services is not well-established in tropical countries where there are no clearly defined influenza seasons. We aimed to estimate the association between influenza infection activity and polyclinic attendances in the Singapore population. We also aimed to use this association to approximate the number of polyclinic attendances attributable to influenza.

**Methods:** We used generalized additive time series models to estimate the association between the proportion of respiratory tests positive for influenza infection in Singapore reported to the World Health Organization every week, and the number of polyclinic attendances in Singapore for acute upper respiratory tract infections (URTIs), for a total of 6 years from 2012 to 2017. Other available febrile illnesses that could result in influenza-like symptoms were included in the analysis.

**Results:** Influenza, dengue fever and chickenpox (varicella) were positively associated with acute URTI polyclinic attendances. The estimated polyclinic attendance rates attributable to influenza, dengue fever and chickenpox were 785.4, 354.9 and 1657.4 per 100,000 population per year, respectively.

**Conclusion:** Influenza poses a considerable burden on primary healthcare services in Singapore. However, a substantial proportion of polyclinic attendances due to other infections such as dengue fever and chickenpox may be misclassified as upper respiratory tract infections.

*Keywords: influenza; Singapore; dengue; chickenpox; polyclinic*

Topic: Public Health: Surveillance & Forecasting

Abstract No: 10714

## Phylogenetic Diversity of Swine H1N2 Influenza A Virus in Korea

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Influenza A virus can infect a broad range of hosts including avian, humans and swine. Swine is known to play the role of 'mixing vessel' for the generation of a novel reassortant, which may acquire transmissibility to humans as the case of swine origin pandemic H1N1 virus of 2009. For this reason, constant surveillance of swine influenza virus is warranted. In Korea, H1N1, H1N2 and H3N2 swine influenza A viruses have been reported for the past several years. We have been conducting swine influenza surveillance in Korea. Several swine influenza viruses were isolated by inoculating the collected nasopharyngeal samples of swine in eggs followed by plaque purifying in MDCK cells. Viral RNAs extracted from the isolated viruses were subjected to a whole genome sequencing using the Next Generation Sequencing. The sequences were then phylogenetically analyzed. Here we report that four isolates were novel reassortants of North American triple reassortant *swine* lineage, pandemic H1N1/2009 lineage, Eurasian-avian like swine lineage and twenty isolates were double reassortants of North American triple reassortant *swine* lineage, pandemic H1N1/2009 lineage. These data suggest that frequently occur reassortment event of swine influenza A virus in Korea, which implies the importance of sustained surveillance against swine influenza A virus.

*Keywords: Influenza , H1N2 , Swine , Korea*

Topic: Public Health: Surveillance & Forecasting

Abstract No: 10721

## A COMPARISON OF INFLUENZA A(H3N2) AND A(H1N1)PDM09-ASSOCIATED PEDIATRIC DEATHS IN THE UNITED STATES, 2010-2011 TO 2017-2018 SEASON

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**Introduction and Objectives:** Influenza-associated pediatric death is a nationally notifiable disease; allowing for the characterization of severe influenza-associated disease and identification of at-risk groups for targeting prevention and treatment strategies.

**Methods:** The study compared demographic characteristics, clinical course of illness, underlying medical conditions and vaccination status among influenza A(H3N2) and influenza A(H1N1)pdm09-associated deaths among children reported to the Centers for Disease Control and Prevention.

**Results:** Of the 625 pediatric deaths associated with laboratory-confirmed influenza A virus infections and reported to CDC from October 3, 2010, to September 29, 2018, information on subtype was available on 390 (62%) deaths. Of these, 161 (41%) were associated with influenza A(H1N1)pdm09 viruses and 229 (59%) were associated with influenza A(H3N2) viruses. Children with influenza A(H1N1)pdm09 viruses were more likely to develop pneumonia ( $p=0.03$ ), acute respiratory distress syndrome ( $p=0.002$ ), and to have been born prematurely (<37 weeks gestation) ( $p=0.04$ ) compared to children with influenza A(H3N2) viruses. Children with influenza A(H3N2) viruses were significantly more likely to have *Staphylococcus aureus* identified from a sterile-site bacterial culture than were those with influenza A(H1N1)pdm09 viruses ( $p=0.02$ ).

**Conclusions:** Influenza-associated pediatric deaths are reported each year in the United States. Information on influenza subtype may not impact clinical decision-making, however, clinical presentation for severe cases can vary by virus type/subtype. These findings underscore the importance of annual seasonal vaccination for all children  $\geq 6$  months of age and treatment as soon as possible with influenza antiviral medications of children with severe illness requiring hospitalization or at high risk for influenza complications.

**Keywords:** influenza, surveillance, pediatric death

Topic: Public Health: Surveillance & Forecasting  
 Abstract No: 10722

## Genetic and pathogenic characteristics of a novel reassortant, highly pathogenic avian influenza (HPAI) H5N6 virus isolated in Korea, 2017

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### 1. Introduction

Since the first H5N8 (clade 2.3.4.4) avian influenza A virus was reported in Korean poultry in 2014, it has been continued to subsequently spread to worldwide and created novel H5Nx subtypes including H5N6 subtype. In late November 2017, novel influenza A(H5N6) viruses were the first isolated from migratory birds in South Korea following a reported die-off of domestic poultry. In this study, we reported here the genetic characterization of the novel influenza A(H5N6) virus and their pathogenic potential in chickens and ducks.

### 2. Method

To identify the pathogenicity in poultry species, the H5N6 virus was inoculated to chickens and ducks and viral titers were measured in trachea and cloacal swabs. Further, we also evaluated its mortality and multi-organ detection in infected birds.

### 3. Result

Genetic characterization revealed that the H5N6 virus was the reassortant between the HPAI H5N8 strain from previous Korea and the N6 gene from a LPAI H3N6 virus from the Netherlands in the Eurasian gene pool of avian influenza viruses. In animal infection studies, the IVPI of the H5N6 was recorded to be 2.76 in chickens and it showed the highly pathogenic in both chickens and ducks. When SPF chickens were infected with  $10^6$  EID<sub>50</sub>/mL dose of H5N6 virus, all chickens died on the 4dpi and viruses were detected in all organs. However, the ducks all survived and viruses were detected in all organs except the brain on the 3 and 5 dpi.

### 4. Conclusion

With the rapid spread of novel HPAI H5Nx (clade 2.3.4.4) viruses worldwide, the continuous monitoring of avian influenza viruses in wild migratory birds are needed to understand their rapid evolution patterns, including virulence and pathogenicity studies using laboratory animals.

*Keywords: HPAI (Highly Pathogenic Avian Influenza virus), reassortment, H5N6, Surveillance, Pathogenesis*

Topic: Public Health: Surveillance & Forecasting  
 Abstract No: 10744

## INFLUENZA SURVEILLANCE IN 18 AFRICAN COUNTRIES WHO ARE MEMBERS OF THE ANISE NETWORK, 2011-2017.

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**Background:** Ministries of health and partner organizations have provided financial and technical assistance to strengthen influenza surveillance throughout Africa. We sought to quantify influenza surveillance gains during 2011-2017.

**Methods:** We surveyed representatives from countries who are members of the African Network for **Influenza Surveillance** and **Epidemiology** (ANISE). We used a standardized survey to collect data about surveillance protocols, operational costs, and the number of samples tested and positive for influenza by age group. Data were summarized using descriptive statistics.

**Results:** Eighteen (75%) of 24 ANISE members participated in the survey. Cost for influenza surveillance ranged between \$10,000 and \$1,200,000 annually. Surveillance was co-funded by the Centers for Disease Control and Prevention (94%), The US Health and Human Services Office of the Assistant Secretary for Preparedness and Response (17%), Ministries of Health (24%), and the World Health Organization (22%). All countries conducted sentinel surveillance. Countries scored an average of 95% on their quality assurance panels and tested a median of 1,373 (IQR: 745 – 1,975) specimens through rRT-PCR per year of which 14% tested positive for influenza (range 7%-41%). All countries implemented influenza A subtyping and nine (50%) gained the ability to lineage-type influenza B. Between 2011 and 2017, the number of severe acute respiratory infections samples tested for influenza increased from 9,339 to 12,398. Testing remained level for influenza-like illness (14,584 and 14,477 samples respectively). There were eight (44%) National Influenza Centers in 2011; three additional laboratories gained NIC status during 2011-2017. Most of the samples collected were from children aged <5 years (53%; 49,738/94,681 from SARI and 61%; 53,837/88,000 from ILI); few were from persons aged ≥65 years (1%; 1,276/94,681 from SARI and 3%; 2,521/88,000 from ILI).

**Conclusions:** Additional countries gained NIC status, used B lineage assays, and tested more SARI cases. Surveillance systems relied mostly on external funds.

**Keywords:** Influenza Surveillance, Africa, ANISE, Sentinel Surveillance, Influenza

Topic: Public Health: Surveillance & Forecasting

Abstract No: 10768

## Evolution-informed ahead-of-season influenza incidence forecasting for the US

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Inter-pandemic or seasonal influenza exacts an enormous annual burden in terms of global human health and economic impact to society. Incidence prediction ahead of season remains a challenge largely because of the virus' antigenic evolution. We present here a forecasting framework that incorporates evolutionary change into an epidemiological model, and remains sufficiently parsimonious for parameter estimation based on retrospective surveillance. Based on the real forecasts for the last 3 seasons in the US, we demonstrate the feasibility of prediction ahead of season based on our framework, which includes both single subtype models and multi-subtype models

*Keywords: seasonal influenza, US, forecasting, evolution, model*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 10776

## INFLUENZA A(H3N2) ANTIGENIC PROFILE IN RUSSIA IN 2017-2018 AND 2018-2019 EPIDEMIC SEASONS

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**INTRODUCTION:** Influenza A (H3N2) viruses demonstrate the highest level of evolutionary variability compared to other influenza subtypes. Antigenic analysis of influenza viruses is critical for optimal selection of candidate vaccine strains for the upcoming epidemic season.

**METHODS:** Virus isolation, identification, antigenic analysis in HI and microneutralization assays (MN) assay using a panel of post-infection ferret and rat antisera, antigenic cartography

**RESULTS:** In epidemic season 2017-2018 influenza A(H3N2) was responsible for 36% PCR-positive cases. Influenza epidemic started much later than in previous seasons and was classified as the epidemic of moderate intensity. In epidemic season 2018-2019 A(H1N1)pdm09 subtype dominated ( 63.25%) while A(H3N2) comprised 36.75%.

All strains isolated in 2017-2019 were titrated with human RBCs in the presence of 20nM Oseltamivir carboxylate. The majority of isolated strains (44.6% in 2017–2018 and 43.5% in 2018-2019) had the NA-induced agglutination of erythrocytes.

Antigenic analysis has shown that all strains were recognized by the antisera raised against A/Singapore/INFIMH-16-0019/2016 (MDCK-Siat1). Isolates of 2017-2018 epidemic season were well recognized by the antiserum raised against A/Hong Kong/4801/14. However a part of the strains from 2018-2019 were poorly recognized by this antiserum (1/8-1/16 of homologous titre). Most of the strains 2018-2019 in Russia were antigenically similar to the reference virus A/La Rioja/2202/2017 (genetic clade 3C.2a1b). None of the 3C.3a clade (reference virus A/Switzerland/9715293/13) were detected in the study period in Russia.

**CONCLUSION:** In the last epidemic season an apparent change of major antigenic group of A(H3N2) in Russia was registered. We also confirmed the necessity of application in the antigenic analysis of H3N2 of the complex of new methods: isolation in Siat-1 cells, titration in the presence of oseltamivir carboxylate and application of MN assay along with HI assay.

**Keywords:** Influenza A(H3N2); antigenic cartography; Russia; influenza surveillance; antigenic profile



Topic: Public Health: Surveillance & Forecasting

Abstract No: 10783

## MAGNITUDE OF RESPIRATORY VIRUSES AMONG ARI AND SARI CASES: MULTI-SITE STUDY FROM INDIA

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**Background:** As part of Global Health Security Agenda (GHS), we set up a surveillance network of six sites, representing different geographic and climatic areas of India, to detect respiratory viruses among acute respiratory illness cases.

**Methods:** Between September 2016-December 2018, each week, sites enrolled 5-10 cases of all ages of Acute respiratory infection (ARI) from outpatients and Severe acute respiratory infection (SARI) from inpatients at sentinel hospitals using standard case definitions. Nasal and/or throat specimens were tested for 12 respiratory viruses by duplex RT-PCR. Phylogenetic analysis of influenza and RSV was studied. Antiviral susceptibility of influenza viruses was assessed genetically and phenotypically.

**Results:** Among 16,372 cases (8,965 ARI and 7,407 SARI) enrolled, 34% were <5 years. We detected respiratory viruses in 5,460 (33.3%) (Table 1) with co-detections in 458 (2.9%) specimens. Influenza viruses were the most frequently detected viruses among aged ≥5 years in all sites (9.3-21.8%) and RSV was most frequently detected viruses among <5 years (1.1 - 26.5%) in all sites except Kolkata. Both lineages of influenza B circulated, B/Victoria (95%) was predominant in 2016-17 and B/Yamagata (74%) predominated in 2017-18; no B/Victoria had an amino acid deletion variance.

Phylogenetic analysis of G gene of RSV showed circulation of the ON1 genotype with 72 nucleotide duplication. Phylogenetic analysis of hemagglutinin gene of influenza A(H1N1)pdm09 revealed that 2016 and 2017 strains were similar to vaccine strain A/Michigan/45/2015 while 2018 strains were similar to vaccine strain A/Brisbane/02/2018, representing Clade 6B.1 group. Among 1127 specimens with influenza A(H1N1)pdm09 virus, only five viruses had a H275Y substitution and increased IC<sub>50</sub> value indicating moderately resistance to oseltamivir.

**Conclusion:** We found influenza as the most commonly detected virus among ARI and SARI cases with majority being susceptible to oseltamivir. Both type B lineages were in circulation during the study period highlighting the importance of quadrivalent influenza vaccine.

**Keywords:** India; SARI; ARI; Influenza; RSV; ON1 genotype; Clade 6B.1; Type B, Quadrivalent Vaccine,

Topic: Public Health: Surveillance & Forecasting

Abstract No: 10802

## GENETIC AND ANTIGENIC CHARACTERISTICS OF CIRCULATING INFLUENZA A VIRUSES, WHO EUROPEAN REGION, 2018/19

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**Introduction/Objectives:** The 2018/19 influenza season in Europe was dominated by A viruses. Mid-season vaccine effectiveness (VE) estimates from six European countries ranged from 41-75% against A(H1N1)pdm09 and -39-24% against A(H3N2) for all age groups. We analysed circulating A viruses reported by National Influenza Centres (NICs) to The European Surveillance System to compare them with the 2018/19 vaccine components.

**Methods:** Virus characterisation data reported by the NICs of the WHO European Region from week 40/2018 to week 4/2019 were used to analyse circulating influenza viruses genetically (19 countries) and antigenically (15 countries). Phylogenetic analysis was performed on available haemagglutinin (HA) sequences to compare them to the 2018/19 vaccine virus sequences.

**Results:** The majority (460/505) of A(H1)pdm09 viruses for which HA sequences were reported fell in subclade 6B.1A and carried amino acid substitution S183P in HA. Of the 352 A(H3) sequences, 309 fell in clade 3C.2a1, 200 of which in subclade 3C.2a1b, 24 in 3C.2a2 and 82 in 3C.3a. The HA genes of circulating influenza A viruses are genetically diverse from the current vaccine virus components and accumulate amino-acid substitutions on antigenic sites. Available antigenic data showed no evidence of antigenic changes of A(H1N1)pdm09 6B.1A from the vaccine virus A/Michigan/45/2015. 3C.2a A(H3N2) viruses were similar to the cell-based A/Singapore/INFIMH-16-0019/2016, while 3C.3a viruses were antigenically distinct. 3C.3a viruses were detected in some European countries (mainly in Austria, France, Netherlands, Spain) and their proportion among A(H3N2) viruses increased from 0 to 23%.

**Conclusions:** We observed a genetic variety of 6B.1A subclade A(H1N1)pdm09 and 3C.2a subclade A(H3N2) viruses that are antigenically similar to the respective 2018/19 vaccine components. Antigenically distinct A(H3N2) viruses of the 3C.3a clade were detected in parts of the Region. Simultaneous circulation of genetically and antigenically diverse strains of A(H3N2) viruses may partly explain significantly lower vaccine effectiveness estimates than for the A(H1N1)pdm09 reported this season.

**Keywords:** Influenza; Europe; genetic; sequencing; vaccine

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 10885

## Significant detection of influenza C viruses among patients with Influenza-like Illness from October 2018 to February 2019, Republic of Korea.

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### Introduction and Objectives

Influenza C virus (ICV) generally causes mild upper respiratory illness in young adults or children. Immune response is maintained for a long time because mutation rate is low. Therefore, ICV detection has been largely neglected, compared to influenza A and B viruses, and is not routinely recommended for in clinical practices. Recently several studies have indicated that ICV causes severe acute respiratory illness and pneumonia. We studied to detect ICV and evaluate their clinical significance among patients with influenza-like illness (ILI).

### Methods

From October 2018 to February 2019, 226 patients with ILI were enrolled through Hospital-based Influenza Morbidity and Mortality (HIMM) surveillance system. PCR methods were used to test nasopharyngeal samples for influenza viruses including influenza A, B, and C viruses. We analyzed the clinical manifestations of patients with ICV infection.

### Results and Conclusion

Among 226 study subjects, 76 cases (33.63%) of influenza A virus, 1 case (0.44%) of influenza B virus, and 11 cases (4.87%) of influenza C virus were detected. Also, we confirmed 4 cases of co-infection. Especially, infection with ICV was found in 3 cases (1.92%) in adults and 8 cases (11.43%) in children. The common symptoms of case with ICV infection were fever and cough. Interestingly, sputum was more common in cases with co-infections than influenza virus alone (influenza A, 46.48%; influenza C, 28.59%; influenza A and C, 100%). In conclusion, ICV was significantly circulated during winter and confirmed in all age in Republic of Korea. Interestingly, ICV infections were more common in children (11.43%) than in adults (4.87%). we need to further investigate the prevalence of ICV over the extended period and elucidate the its disease burden in different age groups.

*Keywords: Influenza C virus; RT-PCR.*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 10930

## GUIDING COUNTRIES ON THRESHOLD SETTING FOR SURVEILLANCE DATA

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### Introduction and Objectives

Influenza surveillance data are collected to monitor and trigger public health action. Thresholds based on historic data help to interpret the data and put them in to the historic context for countries. The Moving Epidemic Method (MEM) has been used by a number of countries to define the start of the season, but for some countries these methods remain challenging. We analyzed the data to understand how threshold setting guidance could be improved based on sample testing practices within different countries.

### Methods

Using data from FLUID and FLUNET from 2011 onwards, we classified epidemics as periods when the historical rate was consecutively higher than the median rate for at least five weeks. For each country with available data, we optimised the epidemic threshold, and the number of consecutive weeks over the threshold needed to declare an epidemic, using all possible thresholds. For each combination of parameters, sensitivity and specificity was calculated. After this, countries were categorized according to the number of samples tested by week. For each category, we analysed the combination of parameters needed to optimize sensitivity and specificity.

### Results

We analysed data from 75 different countries to determine the ideal combination of parameters to maximise specificity, while retaining as much sensitivity as possible. We found that, in countries testing less than 100 samples per week, 53% of countries could achieve specificity higher than 75% when crossing the threshold for two weeks. Countries that had more than 150 samples tested per week reached acceptable sensitivity and specificity values with a single week crossing the threshold.

### Conclusion

In countries performing surveillance with influenza testing data, different threshold setting methods are needed, largely depending on the amount of sampling occurring per week and country seasonality. This analysis will be used to establish generalised threshold setting guidance.

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 10968

## EVALUATION OF A HIGH-THROUGHPUT NUCLEIC ACID EXTRACTION PLATFORM TO IMPROVE INFLUENZA SURVEILLANCE TESTING WORKFLOW AND EFFICIENCY

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**Introduction and objectives:** Influenza surveillance testing is critical to capture a comprehensive view of the antigenic, genetic and antiviral properties of both actively circulating and emerging strains of the virus worldwide. The result of this testing aids in the creation of vaccines, treatment options, and for the preparation of the emergence of new influenza strains with pandemic potential. In order to provide these surveillance data there is a need for rapid, accurate and reliable testing methodologies. This study aims at evaluating a new fully-automated nucleic acid extraction platform to improve efficiency of testing.

**Methods:** The bioMérieux EMAG® provides a high-throughput and high quality extraction process that when used in conjunction with the CDC Influenza RT-PCR panel, allows for rapid and accurate influenza typing results. A comparison study was performed using 53 Influenza samples (33 positive and 20 negative) previously extracted using the Roche MagNa Pure Compact extraction method and then re-extracted with the bioMérieux EMAG®. Additionally, 45 ESwab®, 18 bronchial wash, and 7 nasal wash samples previously tested were also evaluated.

**Results:** Overall we found 97% accuracy, sensitivity and specificity when comparing the results of the 123 previously tested Influenza samples, ESwab, bronchial wash and nasal wash samples. For 2018-2019, we have tested 1154 specimens in 5 months as compared to 2017-2018 where we tested 565 specimens.

**Conclusion:** The introduction of a high-throughput extraction platform using the EMAG® increased our capacity to report influenza screening and subtyping results in real-time during the busy influenza season. This instrument is a good solution for laboratories with high volume of samples that require extraction since it needs little hands-on time. These data in turn have provided increased submission to surveillance data that informs public health decision making, vaccine development, and overall preparation for detecting and responding to potential emerging new influenza strains.

*Keywords: Influenza test 2018-2019*

Topic: Public Health: Surveillance & Forecasting

Abstract No: 10970

## Over-representation of influenza A(H1N1)pdm09 and influenza B viruses among influenza-associated pediatric deaths

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### Introduction

Laboratory confirmed influenza-associated deaths in children <18 years of age is a nationally notifiable condition in the United States. Since 2009, virologic surveillance testing for influenza viruses in all state public health laboratories (PHLs) is performed using a polymerase chain reaction (pcr) assay developed by the Centers for Disease Control and Prevention (CDC) and clinical use of molecular assays has increased. We sought to describe the relative contribution of different virus (sub)types among pediatric deaths compared to national virologic surveillance.

### Method

We examined influenza-associated pediatric deaths confirmed by a molecular assay and virologic surveillance data from 2010-11 through 2017-18. A Chi-square test with Yates' correction was used to compare the relative contribution of influenza virus (sub)type among pediatric deaths and influenza virus positives for the same age group reported through national surveillance.

### Result

From 2010-11 through 2017-18, 981 laboratory confirmed influenza-associated pediatric deaths were reported to CDC; 690 were confirmed using pcr. Four deaths due to influenza coinfections were deleted from the analysis. For the same timeframe, 79,253 influenza positives were reported through surveillance among children <18 years. The proportion of infections due to influenza B viruses was higher among influenza-associated pediatric deaths than surveillance samples in total and during all eight seasons examined. The difference was statistically significant overall and for two seasons. The proportion of influenza A viruses that were A(H1N1)pdm09 was higher among pediatric deaths overall and in 7 of 8 seasons. The difference was statistically significant overall and during four seasons.

### Conclusion

Our analysis shows that influenza B and A(H1N1)pdm09 viruses are over represented among pediatric deaths relative to cases reported through surveillance. Although influenza A(H3N2) viruses are associated with higher levels of mortality for the US population overall, influenza B and H1N1pdm09 viruses are a significant cause of influenza-associated death in children.

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11010

**Measuring influenza and RSV hospitalizations: a novel methodology developed for the Burden of Influenza and RSV Disease (BIRD) consortium using administrative clinical records and the Global Burden of Disease study**

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Introduction and Objectives

Influenza and RSV infections are a major cause of morbidity and mortality worldwide. It is of interest to measure the rates of hospitalizations related to these conditions globally, but relatively few countries have administrative data readily available to measure inpatient admissions associated with influenza and RSV. In this study, we propose a novel method of measuring hospitalization rates due to influenza and RSV.

Methods

We utilized and refined methods developed for the Global Burden of Disease (GBD) study, which is a comprehensive assessment of global morbidity and mortality. We reviewed administrative clinical sources in the GBD clinical informatics database. We identified sources that included multiple diagnosis fields (i.e. primary and secondary diagnoses) for a given admission. We measured the rates of influenza or RSV diagnosis codes among admissions for acute respiratory infections to determine the fraction of admissions among each age, sex, and location that received an influenza or RSV diagnosis during the admission. These admission fractions were combined with estimates from a literature review to inform a meta-analysis. We applied these admission fractions to sources which lacked multiple diagnosis codes in order to estimate the rate of hospitalizations in each country of interest.

Results

Globally, we reviewed records from 2.6 billion admissions from 50 different countries around the world. Among these, six countries had diagnosis fields that were used for measuring admission fractions. As an example result, we estimated 4,300 influenza- or RSV-related admissions in Nepal in 2010.

Conclusion

This study describes a strategy for utilizing clinical administrative data to measure influenza and RSV admissions. Ongoing data collection, methods refinement, and results estimation are underway as part of the Burden of Influenza and RSV Disease (BIRD) study.

Funding

The study received funding support from the Foundation for Influenza Epidemiology, partly supported by Sanofi Pasteur.

Topic: Public Health: Surveillance & Forecasting  
 Abstract No: 11011

**USE OF AN INFLUENZA-LIKE ILLNESS SCHOOL ABSENTEEISM MONITORING SYSTEM TO IDENTIFY SEASONAL INFLUENZA OUTBREAKS IN THE COMMUNITY: EXPERIENCE FROM AN ONGOING STUDY IN WISCONSIN, UNITED STATES**

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**Introduction and Objectives:** Schools are purported to be primary venues of influenza transmission and amplification with secondary spread to communities. We assessed K—12 student absenteeism monitoring as a means for early detection of influenza activity in the community.

**Methods:** Since 2014, we have been conducting a prospective observational study of all-cause (a-TOT), illness-associated (a-I), and influenza-like illness-associated (a-ILI) absenteeism within the Oregon School District, Oregon, WI (OSD: enrollment = 3,900 students). Absenteeism reporting was facilitated by automated processes within OSD's electronic student information system. Students were screened for ILI, and visited at home, where pharyngeal specimens were collected for influenza RT-PCR and multipathogen testing. Surveillance of medically attended laboratory-confirmed influenza (MAI) occurred in five primary care clinics in and adjoining OSD as part of the Wisconsin Influenza Incidence Surveillance Project using the same laboratory testing. Poisson general additive log linear regression models of daily counts of absenteeism and MAI were compared using correlation analysis. Results for 2017--2019 will be added prior to the presentation.

**Results:** (From 1/06/2015—6/08/2017, influenza A and B were detected in 249 and 93 of 1514 visited students, respectively. Of MAI patients, 630 had influenza A and 246 had influenza B. Over the first 3 years, a-I was significantly correlated with MAI in the community ( $r = 0.472$ ;  $P < 0.001$ ) with a 15-day lead time. a-ILI was significantly correlated with MAI in the community ( $r = 0.480$ ;  $P < 0.001$ ) with a 1-day lead time. a-TOT performed poorly ( $r = 0.278$ ;  $P < 0.001$ ), following MAI by 9 days (Figure 1).

**Conclusion:** Surveillance using cause-specific absenteeism was feasible to implement in OSD and performed well over a 3-year period marked by diverse presentations of seasonal influenza. Monitoring a-I and a-ILI can detect influenza outbreaks in the community, providing early warning in time for community mitigation efforts for seasonal and pandemic influenza.

*Keywords: influenza; surveillance; outbreak; absenteeism monitoring; school-based*



Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11014

## ADEQUACY OF STUDY PARTICIPANT- VS. RESEARCH STAFF- COLLECTED RESPIRATORY SPECIMENS FOR DETECTION OF INFLUENZA VIRUSES BY RT-PCR

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**INTRODUCTION AND OBJECTIVES:** We evaluated adequacy of study participant-collected nasal swabs—defined as collection by one adult participant from him/herself, from another adult household member, or from a child in the household—compared to samples collected by trained staff in a large, community-based study.

**METHODS:** The Oregon Child Absenteeism due to Respiratory Disease Study (ORCHARDS) is a community-based study evaluating relationships between school absenteeism and influenza activity. Study staff visited households of children with influenza-like illnesses and collected an anterior nasal specimen along with a pharyngeal specimen. In each household, one parent was trained by ORCHARDS staff to collect specimens from him/herself and other family members using a flocked mid-turbinate swab (Year 1) or an anterior nasal foam-tipped applicator (Year 2). Specimens were tested for influenza A and B virus and human RNaseP (RP). The RP crossing threshold (Ct) value for each specimen was evaluated and determined adequate if Ct<38.

**RESULTS:** Overall, 4,352 swabs were collected from 2,796 new and repeat participants. In Year 1, staff collected 376 specimens from school children (4 to 18 years) while participants collected 1,424 specimens. In Year 2, staff collected 424 specimens while participants collected 2,128 specimens. Overall adequacy was 99.9% for staff collection and 96.3% for participant collection. Participant-collected mid-turbinate swabs had adequacies of 95.2%, while anterior nasal swabs had adequacies of 97.2% (figure). In Year 1, mean RP Ct for staff-collected NP/OP was 28.6 compared to 31.2 (participant-collected from all ages) and 31.2 (participant-collected from school-aged children). In Year 2, mean RP Ct for staff-collected NP/OP was 27.3 compared to 28.5 (all ages) and 28.2 (school-aged children).

**CONCLUSIONS:** Adequacy of participant-collected and staff-collected nasal specimens was comparable. This finding warrants further field evaluation of at-home sample collection for rRT-PCR testing to improve influenza activity detection while limiting patient exposure in clinical settings.

*Keywords: self-swab; influenza; nasal swab; comparison; community-based*

Topic: Public Health: Surveillance & Forecasting

Abstract No: 11043

## INFLUENZA SENTINEL SURVEILLANCE IN UKRAINE AND CHARACTERISTICS OF ISOLATES DURING THE SEASONS 2017/18 AND 2018/19

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**Introduction.** Since 2008 the influenza sentinel surveillance system was existed in Ukraine. This system thanks to CDC Cooperative agreement has been functioning stable and demonstrating high quality results.

**Methods.** The clinical and epidemiological information was collected from 18 adult and pediatric clinics in four cities which were located in different geographical regions (Kyiv, Khmelnytsky, Dnipro and Odesa). The physicians used the pre 2011 WHO Regional Office for Europe case definition for ILI and SARI. The samples from patients with influenza-like illness (ILI) and severe acute respiratory infections (SARI) were collected during 2017/18 and 2018/19 influenza seasons. All specimens were tested for influenza A and B by RT-PCR. Then the influenza positive specimens have been used for virus isolation on MDCK and MDCK-SIAT cells. The epidemiological and virological information collect during the whole year.

**Results.** Both influenza seasons 2017/18 and 2018/19 were low in Ukraine. The first one was associated with predominant circulation of influenza B viruses, especially – with B/Victoria-lineage virus (74% from all B viruses) and the season 2018/19 – with A(H3N2) viruses (75 % from all A viruses) with less participation of A(H1N1)pdm (25 %) viruses.

The dominant virus B in 2017/18 season was representing by old strain - B/Brisbane/60/2008. And the population of A(H3N2) viruses of 2018/19 season was located within the 3C.2a dominant genetic subcluster and all viruses were belongad from the vaccine strain A/Singapore/INFIMH-16-0019/2016. In general, during the 2018/19 season, 2 genetic branches of influenza A (H3N2) viruses circulated: 3s2.A1b/135K (which carried the substitution of T128A and T135K (antigenic site A); and 3s2.A1b/131K (with T131K mutation).

**Conclusions.** There was proved the both last influenza seasons (2017/18-2018/19) there were only partial coincidence of influenza viruses, which caused the influenza epidemic in Ukraine and in the countries of North Hemisphere.

**Keywords:** SENTINEL SURVEILLANCE; INFLUENZA EPIDEMY; SARI; ILI

Topic: Public Health: Surveillance & Forecasting

Abstract No: 11054

## DEVELOPMENTS OF THE GLOBAL INFLUENZA HOSPITAL SURVEILLANCE NETWORK TO SUPPORT BETTER MONITORING OF INFLUENZA VIRUS GENETIC EVOLUTIONS: THE GIHSN- SevVIR NETWORK.

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### Introduction

After seven seasons of active influenza surveillance, the Global Influenza Hospital Surveillance Network (GIHSN) is leveraging capacities to link clinical and virological data. The main objective is to analyze and monitor Influenza viruses' characteristics from hospitalized cases, and to provide this information to WHO for vaccine strain composition decisions.

### Methods

During the 2018-2019 season, a coordinated approach was developed by the French National Reference Laboratory in Lyon. GIHSN surveillance sites and associated laboratories were mapped for their sequencing capacities. A standardized method was proposed using Whole Genome Sequencing and the sites were invited either to share information from sequenced strains or send material for sequencing in Lyon. This sequencing data was linked to detailed epidemiological and rich clinical information on hospitalized patients collected by GIHSN.

### Results

All eighteen countries participating in GIHSN have laboratory capacity for influenza typing and subtyping. Sixteen laboratories participated in the sequencing data survey, ten (including nine national reference laboratories) perform strain sequencing and share their sequence data with WHO's GISRS network via the GISAID platform. Three laboratories (Valencia, St. Petersburg, Lyon) shared reports with the WHO ahead of the February Vaccine composition meeting. Specific GIHSN preliminary results reported about 25 A(H3N2) and 37 A(H1N1)pdm09 sequences from viruses detected by these three laboratories. Eighteen A(H3N2) belonged to clade 3C.2a1b while only seven viruses were from clade 3C.3a, and 35/37 A(H1N1)pdm09 had the S183P substitution as described in the A/Brisbane/2/2018 reference strain.

### Conclusion

The development of a coordinated approach to link detailed clinical and virological information is key to improve interpretation of Influenza strain circulation and associated clinical characteristics of patients. The first months of the GIHSN sequencing development are promising and additional information will be obtained in April. This new set of data will support the WHO vaccine composition meeting.

*Keywords: Hospital surveillance, clinical impact, full genome sequencing, genotyping,*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11062

## A COMPARISON OF INFLUENZA-ASSOCIATED PEDIATRIC DEATHS IN CHILDREN 0-4 YEARS AND 5-17 YEARS IN THE UNITED STATES, 2010-2011 TO 2017-2018 SEASON

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**Introduction and Objectives:** Influenza-associated pediatric deaths occur each year. Children younger than 5 years of age and children with underlying medical conditions of any age are at high risk of influenza-related complications.

**Methods:** Data collected from the Influenza-Associated Pediatric Mortality Surveillance System was used to compare demographic characteristics, clinical course of illness, underlying medical conditions, vaccination status and influenza antiviral use between children aged 0-4 years and 5-17 years. All deaths reported to the Centers for Disease Control and Prevention between October 3, 2010, to September 29, 2018 were included in the analysis.

**Results:** Of the 961 pediatric deaths associated with laboratory-confirmed influenza virus reported to CDC, 410 (43%) occurred in children aged 0-4 years and 551 (57%) occurred in children aged 0-17 years. Among these pediatric deaths, children aged 5-17 years were more likely than children aged 0-4 years to develop pneumonia (rate ratio (RR)=1.3, 95% CI: 1.1-1.4), have *Staphylococcus aureus* (RR=1.6; CI=1.3-2.0) or be prescribed influenza antivirals after illness onset (RR=1.3; CI=1.1-1.5). Children aged 0-4 years were more likely to have influenza A virus infection (RR=1.3; CI=1.2-1.4), die at home or in the emergency department (RR=1.5; CI=1.3-1.8), have a viral respiratory co-infection (RR=2.0; CI=1.3-3.0), develop bronchiolitis (RR=2.8; CI=1.3-6.0), have underlying cardiac or congenital heart disease (RR=1.9; CI=1.3-2.9), or been born prematurely (RR=3.1; CI=1.9-4.9).

**Conclusion:** Influenza can be fatal in children. Among pediatric deaths, children 0-4 years of age were 2 times more likely to report another viral respiratory co-infection and/or bronchiolitis. Furthermore, very young children were 1.5 times more likely to die prior to hospital admission. Annual influenza vaccination is the best defense against influenza infection. However, treatment as soon as possible with influenza antiviral medications is recommended for patients with severe illness, who require hospitalization, or are at high risk for influenza complications.

*Keywords: influenza, surveillance, pediatric deaths*

Topic: Public Health: Surveillance & Forecasting

Abstract No: 11066

## IN SILICO ANTIGENIC VARIANTS PREDICTION FOR INFLUENZA VIRUS USING CONVOLUTIONAL NEURAL NETWORK

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### Introduction and Objectives

The rapid evolution of influenza viruses alters their antigenic properties and leads to annual need in updating vaccine compositions. The gold-standard method for antigenic characterization of influenza viruses is hemagglutination inhibition (HI) assay. The HI assay has several drawbacks: it is time-consuming and rather expensive. Also, direct influenza sequencing from clinical specimens generates enormous amounts of sequence data lacking experimental antigenic data. Therefore it is tempting to design a mathematical model providing a high precision forecast of antigenic properties by sequence. In this paper, we propose such a model based on Convolutional Neural Network (CNN) and proof its performance by numerical evaluation.

### Methods

CNN is a deep learning algorithm which is applied to model a classification or regression task. Knowing the relationship between antigenicity and amino acids properties, e.g., hydrophobicity, in order to train CNN using HA1, the protein amino acid sequence is converted to numerical using AAindex database and then normalized. Putting together HA1 sequences of both test and reference strains of each entry from HI assay table, a multi-channel pseudo image is created which is fed into CNN with the logarithm of associated HI titer (as its label).

### Results

We carried out numerical experiments to show that chosen CNN architecture (AlexNet) is suitable for this task. The implementation of this system on a real database of seasonal influenza virus subtype H1N1 (1998-2009) in Caffe framework has shown its performance in predicting the HI titer value. The proposed system achieved the accuracy of similar models of antigenic variant prediction and attained mean absolute error of 0.8 and 0.92 antigenic unit for yearly modeling and prediction, respectively.

Conclusion. Our model is able to predict the result of the HI assay based on the part of the hemagglutinin protein (HA1) sequence and amino acid physicochemical properties.

*Keywords: Antigenicity, Antigenic Variants, Convolutional Neural Network, Deep Learning, H1N1*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11080

## Community-Based Early Warning System (CEWS) Using Mobile Phone SMS Service to Report Illnesses and Deaths

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### Introduction and Objectives

Hospital-based surveillances (HBS) potentially miss events and outbreaks in communities where medical services are only sought for severe illnesses. We implemented a community-based early warning system (CEWS) to collect information on reported illness and death from respiratory illness (fever with cough/breathing difficulty) and encephalitis (fever with altered mental status/convulsion/unconsciousness) by using mobile phone short message service (SMS) and determined its functionality and feasibility.

### Methods

The study was conducted in 53 Bangladeshi villages from August 2017 to December 2018. We enrolled and trained 89 participants including drug sellers, community healthcare providers (CHCPs), homeopaths, village representatives and union parishad (lowest local government administrative office) staff to cover a catchment population of 67,000. They sent SMS on a daily (illness) and weekly (death) basis for 12 months. A total of 28,416 SMSs were projected. We compared the CEWS data with the active HBS data in the same population during the same period. We collected feedback on feasibility of CEWS using interviews, group discussions and surveys.

### Results

We received 43% of the projected SMS. CHCPs were the most consistent (53%; 1,152/2,190) in reporting illness and union parishad staff (58%; 30/52) in reporting death. The system reported 2,899 cases and two deaths with respiratory symptoms, and no encephalitis cases or deaths. Eleven respiratory cases and two encephalitis cases were identified by HBS. The major barriers identified were forgetfulness (62%; 50/81) and technical problems with mobile phone (30%; 24/81) while the most frequently (86%; 70/81) mentioned motivation was the wellbeing of the country. The most frequently mentioned suggestions were increasing reminder calls (62%; 36/58) and involving local government authorities (41%; 24/58).

### Conclusion

The CEWS was functional and feasible to detect respiratory illness and death, but not functional for encephalitis. Future iterations should consider involving local government authorities and emphasize on reminder calls.

*Keywords: community-based, early warning system, mobile phone, respiratory illness, encephalitis*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11093

## Forecasting influenza incidence as an ordinal variable using machine learning method: Booted Regression Tree

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Diverse characteristics of influenza among different countries pose challenges for operational influenza forecast. A wide variety of machine learning methods which are suited to forecast have been developed, but to our best knowledge, these methods are less frequently used in influenza forecast than statistical models. In this study, we aim to apply a relatively new machine learning method, Boosted Regression Tree(BRT), to country-level data for prediction purpose and validate machine learning methods can have better predictive performance.

We developed and validated a BRT model by comparing model prediction results to historical average model. In the historical average model, predictions of the incidence of one week in any year are given by the average of the corresponding week in other years. We obtained 68 WHO countries influenza-like-illness data which records weekly incidence between year 2010 and 2018, and applied data to models by country. Relevant biological and geographic predictors including weekly incidence of prior week and of prior two-week, prevalent strains, and latitude of the country were as covariates in BRT model to improve predictive performance. To evaluate model performance, the proportion of correct predictions for each country given by two models were compared.

In all countries, BRT model showed higher proportions of correct prediction results than historical average model. In particular, the accuracy proportions give be BRT model in 56 countries were over 50%, the highest accuracy proportion can be up to 97%. But historical average model only predicted more than 50% accuracy in 31 countries.

These results highlight the accuracy of machine learning methods in forecasting influenza incidence. In particular, BRT model is flexible to be extended by including more potential covariates to capture complicated patterns to help prediction process. Therefore, BRT may improve forecast of influenza weekly incidence to indicate timing of influenza seasons and seasonal sizes accurately.

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11096

## ANALYSIS OF GENETIC VARIABILITY OF RESPIRATORY SYNCYTIAL VIRUSES GROUP A IN SAINT-PETERSBURG IN 2016-2018 YEARS

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**Introduction:** In 2016 the WHO announced the RSV Pilot in order to test the possibility of utilizing Global Influenza Surveillance and Response System platform for RSV surveillance. To date 14 countries are participating in this project, including Russian Federation.

The aim of this study is to investigate the circulation patterns of the RSVA in Saint-Petersburg in 2016-2018 epidemic seasons.

**Methods:** RNA extraction, real-time PCR, RT-PCR of G gene, Sanger sequencing, phylogenetic analysis

**Results:** In 2016-2017 epidemic season 3880 nasopharyngeal swabs from hospitalized patients were tested for influenza, hRSV and other respiratory viruses by rRT-PCR. 335 RSV-positive specimens were identified. The occurrence of HRSV in the various age groups was as follows: 28.4 % for infants under 5 months, 57.6 % for 0.5-4 years, 11.3% for 5-64 years, none for people older than 65 years and 2.7 % - no information about age. HRSV activity starts at approximately the week 36 and ends at week 21, the peak was at the week 4.

In 20 specimens the G-gene was sequenced. All sequenced viruses belong to GA2 (ON1) genetic group.

In 2017-2018 epidemic season 536 RSV-positive specimens were identified from 4781 nasopharyngeal swabs. The occurrence of HRSV in the various age groups was as follows: 32.5 % - under 5 months, 54.5% - 0.5-4 years, 11% - 5-64 years, 2.2% - older than 65 years. HRSV activity starts at approximately the week 47 and ends at week 20, the peak was at the week 9.

In 18 specimens the G-gene was sequenced. All sequenced viruses belong to GA2 (ON1) genetic group.

**Conclusion:** HRSV is the most common viral pathogen causing lower respiratory tract infections among infants and young children. HRSV surveillance could help us understand the evolutionary variability of HRSV and its interactions with other respiratory pathogens.

**Keywords:** *HRSV; respiratory pathogens; sequencing*



Topic: Public Health: Surveillance & Forecasting

Abstract No: 11124

## **Establishment of Influenza Surveillance System and Epidemiology of Influenza subtypes in Bangkok Private Hospitals, September 1, 2016 – December 31, 2017.**

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Bangkok is a capital city of Thailand with 10 million population plus over 30 international visitors annually. Understanding epidemiology of influenza and circulating virus subtypes benefits influenza prevention and control program. Therefore, an integrated surveillance system at private hospitals was established under the support of Thailand Ministry of Public Health and U.S. CDC Collaboration to monitor trend and proportion of influenza virus subtypes among influenza-like illness (ILI) patients seeking medical care at private hospitals in Bangkok. Three steps in the development included 1) conduct feasibility study to assess the hospital capacity and leaderships commitment at 12 sampled hospitals located in 6 geographical zones of Bangkok, 2) implement surveillance protocol and collect nasopharyngeal/throat swabs from hospitalized patients diagnosed with ILI, severe acute respiratory infection (SARI) or community acquired pneumonia (CAP) and test for influenza virus subtypes by PCR technique at Thai National Institute of Health and Chulalongkorn University, 3) provide technical support to hospital staff for strengthening data analysis.

The result found that 9/12 hospitals (75%) joined sentinel surveillance network for monitoring influenza virus subtypes. The enrolled patients were female (50.3%), median of age was 44 years (range 0-94 years) and lived in Bangkok (80.5%). CAP accounted for 36.5%, 81.6% had no influenza vaccination in the past 12 months. Of those 693 specimens tested, 23.6% were positive for influenza virus subtypes: Flu A-H1pdm09 36.0%, Flu A-H3 35.2%, Flu B 26.1%. The surveillance data and epidemiological information have been shared through monthly report and annual meeting of stakeholders.

This project has increased the representativeness of the influenza virus subtypes surveillance system in Thailand by including previously non-participating hospitals in the capital city. The system should be further strengthened and explored to identify high risk population and factors associated with severe influenza in order to guide intervention and policy formation.

*Keywords: Influenza Surveillance*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11180

## CHARACTERISATION OF INFLUENZA VIRUSES CIRCULATING IN AUSTRALIA DURING A HIGH INTER-SEASONAL PERIOD IN 2018-9

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### Introduction

Influenza causes seasonal epidemics in temperate regions of Australia, with peak activity occurring in winter-spring seasons (July-September). Here we analyse influenza viruses that have been circulating in unusually high numbers Australia-wide during the most recent inter-seasonal period (Dec 2018- April 2019)

### Methods

We analysed influenza positive samples sent to the WHO Collaborating Centre for Influenza (WHO CC) and notifications of laboratory-confirmed influenza from the Australian National Notifiable Diseases Surveillance System (NNDSS).

### Results

During the influenza inter-seasonal period (December 2018-April 2019), the WHO CC received 1890 samples compared to the combined inter-seasonal periods of the previous **three** years, of 1669 samples. This data was reflected in the NNDSS reporting of laboratory-confirmed cases of influenza, with 35,257 (to 15 April 2019) cases reported in the current inter-seasonal period, compared to 49,275 for the previous three years combined. The current inter-seasonal period has been dominated by the A(H1N1)pdm virus, with 63% of viruses analysed here belonging to this subtype, followed by A(H3N2) (31%), and influenza B (5%). Antigenically, the A(H1N1)pdm viruses were similar to the virus included in the 2019 southern hemisphere vaccine though there was considerable genetic heterogeneity. The A(H3N2) viruses also fell into several distinct genetic clades, with the predominant subclade being 3C2a1b with a 131K substitution in the HA, similar to A(H3) viruses circulating in Japan and China during this period.

### Conclusion

Summer influenza activity in Australia has been at record levels in 2018-19, with both A(H3N2) and A(H1N1)pdm09 viruses co-circulating. The causes of this dramatic increase in influenza activity are unclear at this stage but may be related to a late epidemic of H1N1pdm09 in Northern Australia in 2018 combined with a late and extended influenza 2018-9 season in Asia and elsewhere, that may have resulted in A(H3N2)-infected tourists returning to Australia.

*Keywords: inter-seasonal epidemic, A(H1N1)pdm, Australia,*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11191

## INFLUENZA EPIDEMIC THRESHOLD USING THE MOVING EPIDEMIC METHOD IN TUNISIA, 2009-2018

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### Introduction and objectives

Annual seasonal epidemics continue to have a significant impact. Detecting the start of these epidemics and assessing their intensity are crucial to implement preventive and control measures at a timely and appropriate manner. In Tunisia, the epidemic threshold adopted until now is 10% and was based on combination of criteria. Our study's objective was to determine the epidemic threshold of influenza season in Tunisia using the Moving Epidemic Method.

### Methods

We used the R package of the MEM Moving Epidemic Method (MEM) to calculate the epidemic and the different intensity thresholds based on historical ILI surveillance data of the past nine influenza seasons (2009–10 to 2017–18). Data used were the weekly ILI proportions over all outpatient acute consultations. Thresholds were determined using two models; one including and the other excluding the 2009-10 pandemic season.

### Results

When including the pandemic season, epidemic threshold was 8.99%. When we excluded 2009-10 season, epidemic threshold decreased to 6.25%. The different levels of intensity also decreased to 9.74%, 12.05% and 13.27% respectively for low, medium and high levels. The model's specificity decreased from 87% to 69% when excluding the pandemic season, but sensitivity increased from 39% to 85%.

### Conclusion

This is the first study of epidemic threshold of influenza in Tunisia using the MEM. We excluded the pandemic season as it affected the calculations. The epidemic threshold was 6.25% and the model's sensitivity and specificity were high. Next steps consist in implementing its use for public health purposes

*Keywords: Influenza, surveillance, epidemic, Tunisia*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11203

## MONITORING H5, H7, H9 AVIAN INFLUENZA VIRUS ACTIVITY AT LIVE POULTRY MARKETS VIA ENVIRONMENTAL SAMPLES

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### Introduction

Real-time monitoring of avian influenza virus (AIV) activities at live poultry markets (LPMs) via routine surveillance is essential for pandemic preparedness. We accessed the association between AIV prevalence in chickens and environmental detection rate.

### Method

From December 2015-July 2018, longitudinal surveillance was performed monthly at one wholesale and one retail LPM in Guangzhou. Paired chicken oropharyngeal and cloacal swabs, environmental samples and air samples were collected. Viral RNA was extracted to detect AIV M gene and H5, H7, and H9 hemagglutinin genes.

### Results

A total of 9259 samples were collected over the study period. Monthly AIV detection rates were significantly higher at the retail LPM than that at the wholesale LPM in poultry swabs (Mann-Whitney test,  $p < 0.001$ ), air samples ( $p = 0.02$ ) and environmental swabs ( $p < 0.001$ ). H9 viral RNA was detected frequently from chicken and environmental samples all year long, while H5 and H7 were detected mainly in winter and spring-winter at LPMs respectively. Among different environmental samples collected from poultry holding (fecal droppings, drinking water and diet), slaughtering (defeathering machines), or selling (chopping boards and display tables) areas, H5 and H7 viral RNA were most frequently detected from chopping boards (median monthly positive rate at 33%, 95% CI=0-50%) and drinking water (median monthly positive rate at 3.3%, 95% CI=0.0-15.4%) sampled at the retail market, respectively. On the other hand, H9 viral RNA was detected frequently from the poultry holding, slaughtering, and selling areas. Spearman correlation analyses showed that H5, H7, H9 detection frequencies in fecal droppings correlated the best with H5, H7, H9 prevalence detected in poultry swabs at the wholesale and retail markets.

### Conclusion

Our results suggest that different environmental samples can be applied to sensitively detect H5, H7, or H9 viral RNAs. Fecal dropping may be applied to monitor viral prevalence in poultry at the LPMs.

*Keywords: Avian influenza virus; Live poultry market; Live bird market; Environmental samples*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11205

## RAPID TESTING IN SECONDARY CARE AND INFLUENZA SURVEILLANCE IN ENGLAND: ANY IMPACT?

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**Introduction:** The use of rapid testing for influenza diagnosis is becoming increasingly popular. Used at the point of care (POC) or impact (POI), these tests are most commonly nucleic acid based and detect influenza A and B viruses; though not all distinguish between subtypes. The UK Severe Influenza Surveillance System collects surveillance data on laboratory confirmed influenza admissions to secondary care in England.

This study set out to understand how rapid testing might influence the availability of subtyping data collected on influenza cases admitted to secondary care in England.

**Methods:** At the end of the 2017/18 influenza season a questionnaire was sent to all intensive care units (ICU) in England, with a follow-up survey underway at the end of 2018/19 season, to evaluate the use of rapid testing.

**Results:** In 2017/18 the survey was sent to 144 NHS trusts. Of responding trusts, 42% (13/31) used rapid tests either POC or POI. POI tests comprised the majority (11/13). 30% of trusts reported planning to increase the use of rapid testing in the 2018/19 season.

Data from past influenza seasons shows varying proportions of ICU admissions without subtyping information. In recent influenza A/H1N1pdm09 dominant seasons the proportion of ICU admissions with unknown subtype was 44% in 2013/14, 42% in 2015/16 and 63% in 2018/19. In A/H3N2 and B co-dominant seasons it was 55% in 2014/15 and 35% in 2017/18 and A/H3N2 dominant seasons it was 56% in 2016/17.

**Conclusion:** Subtyping information for surveillance remains unknown on a large proportion of severe cases in England. The use of rapid tests may be contributing since further sampling or additional work on samples may be required to ascertain subtyping information. Given the clear clinical advantages of rapid tests, further work must be done to accommodate their usage to prevent disruption to data flows for surveillance.

*Keywords: Rapid testing; influenza surveillance*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11208

## Using smartphone application-based platform to improve the surveillance performance for influenza-like-illness in Hong Kong

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### Introduction

Children at schools are susceptible to the constant risk of institutional outbreaks of infectious diseases. There is no prospective surveillance system for the routine and continuous monitoring of communicable disease activity in local schools. This project explored the feasibility of employing a smartphone application platform for CD surveillance in a school setting.

### Methods

A surveillance system was developed riding on a smartphone application platform of an electronic school administration system, for the regular and prospective reporting of the nature, cause, and symptom details of sickness absence by parents. Additional backend systems for automatic data transfer, cleaning, aggregation, and analysis, and feedback of the surveillance intelligence were developed. The performance of this surveillance system was evaluated according to international guidelines.

### Results

Our system covered a total of 7,711 students in 13 participating schools, and captured 95412 person-days of absence over the study period from 11/2016-06/2018. The temporal pattern of ILI activity was much better delineated by the school absence rate than the reference gold standard of sentinel GP ILI proportion x influenza virus isolate rate. Epidemic peaks shown by the new data preceded the peaks shown by traditional data the by 2-3 weeks. Rescaling of all-cause absent rate by the percentage of sick leave caused by URTI improved the performance of sensitivity, specificity, and PPV. Most teachers and parents found the surveillance system stable, simple and easy to use, and is useful for monitoring absenteeism and influenza activity among students.

### Conclusions

Smartphone application-based surveillance system represents a suitable and feasible approach for prospective disease surveillance. The system is stable and of good acceptability, and achieved an improvement of both data specificity and timeliness at the same time. The reduced workload implication would help to avoid the problem of surveillance fatigue, and contribute to better data accuracy and system sustainability.

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11220

## LEVERAGING GISRS FOR RSV SURVEILLANCE – OPPORTUNITIES AND CHALLENGES

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### Introduction and Objectives

Respiratory syncytial virus (RSV) associated acute lower respiratory infection is a common cause for hospitalization in children globally. The lack of a uniform surveillance case definition poses a challenge. Global standards for RSV surveillance will provide evidence to inform immunization policy when RSV vaccines become available. The WHO piloted a strategy to test the feasibility to leverage the capacities of the Global Influenza Surveillance and Response system (GISRS) to better understand RSV epidemiology and virology globally.

### Methods

The RSV surveillance strategy was piloted at sentinel hospitals and outpatient clinics in 14 countries from all six WHO regions. Patients across all age groups were tested for RSV by RT PCR, all year-round using an extended severe acute respiratory infection (SARI) and acute respiratory infection (ARI) case definitions that did not require fever, in hospital and primary care settings respectively.

### Results

Among 21,221 patients tested for RSV between January 2017 and September 2018, 73% were sourced from hospital admissions. Among hospitalized RSV positive patients, 50% were less than 6 months and 88% less than 2 years age. RSV percent positivity was 37% and 25% in children less than 6 months, and 6 months to 2 years respectively. Fever reduced the odds ratio (0.74; 95% CI: 0.63 – 0.86) for detecting RSV. For infants less than 6 months, 29% of RSV cases would be missed if fever were to be included in the case definition. No significant adverse effects were noted on influenza surveillance activities.

### Conclusion

Several challenges were addressed related to case definitions and the need to focus surveillance on young children. Including fever in a case definition lowers its sensitivity to detect RSV in young children. Countries should consider ways to leverage GISRS to implement RSV surveillance with an augmented case definition among children less than 2 years.

*Keywords: respiratory syncytial virus; case definition; epidemiology*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11229

## IMPLEMENTING A NATIONAL, CLOSE TO REAL-TIME, SURVEILLANCE SYSTEM USING WHOLE GENOME SEQUENCING ENABLES THE IDENTIFICATION OF IN-SEASON REGIONAL GENETIC VARIATION OF INFLUENZA

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**Introduction:** In 2018 the Welsh Assembly Government made funds available for the development of a national influenza surveillance system using next generation sequencing. This required extensive bioinformatics pipeline development, which was completed in time for the 2018-2019 influenza season in Wales. Our objective in developing the system was to provide a complete (wetlab and bioinformatics) sample-to-results solution that can be easily deployed elsewhere.

**Methods:** We perform Illumina whole genome sequencing on samples that have been flagged as influenza positive using our frontline tests. The sequence data is then automatically picked up from the sequencing instrument and analysed by our reproducible (using Nextflow and Singularity, running locally or on the cloud) bioinformatics pipeline that performs QC, removes non-influenza reads and assembles each segment de novo. The generated segments are then analysed using phylogenetics and population genetics to characterise and cluster samples.

**Results:** Using the end-to-end process, over the 18/19 season, we have consistently processed routine samples submitted from across Wales rapidly (less than 7 days from receipt of sample). The bioinformatics pipeline is assembly-based and avoids errors associated with mapping (e.g. from reference selection) and is fully automated, removing potential human error. In total in the 18/19 season we performed whole genome sequencing on 224 isolates, (188 H1N1pdm09 and 36 H3N2). The H1N1pdm09 data revealed distinct genetic variants circulating predominantly in North and South Wales, as well as identifying further variation at a city level. The H3N2 data, from season-end cases, shows some regional variation, and is currently being expanded to support forecasting efforts.

**Conclusion:** Our work provides a fully reproducible, simple to deploy, sample-to-result system that encompasses both wet- and dry-lab processes. Using our system in Wales has enabled us to detect regional variation of influenza, in real time, enhancing surveillance and creating new opportunities for in-season public health interventions.

*Keywords: Genomics; Bioinformatics; National surveillance; Real-time; Rapid*



Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11233

## MULTIPLE WAVE ALGORITHM FOR INFLUENZA EPIDEMIC TIMING AND THRESHOLDS SETTINGS

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### Introduction and objectives

Influenza seasonality is well established in temperate countries. However, in tropical countries influenza presents an endemic activity all the year round with periods of exacerbations and usually two epidemic-like waves. This makes the assessment of intensity difficult and complicates the decision about vaccination timing and formula.

The Moving Epidemic Method (MEM) is developing a new algorithm to locate the epidemic periods in data series. We present the first phase of the validation analysis with data from temperate countries.

### Methods

The multiple wave algorithm (MWA) detects epidemic waves one by one in a set of seasons treated as a single series. Starting by the highest value, the first parameter establish the minimum value for an adjacent weekly value to be part of the wave currently being detected. If the percentage is lower than the parameter, the iteration stops and continues with another epidemic wave. The second parameter determines the number of waves. If the cumulative value of the new wave represents less than a percentage of the total values, it stops. The third parameter indicates the minimum distance required between two waves to be considered independent.

Influenza series from 18 temperate countries from 1996 to 2018 were used to compare the number of waves, epidemic length, epidemic percentage, thresholds, sensitivity and specificity calculated with MEM and MWA.

### Results

Using parameters 3%, 2% and 1 week, the difference in the number of waves detected by MWA and MEM was not significant. Differences in epidemic length was 2.1% and the epidemic percentage were similar, 76% in MEM and 77% in MWA. Epidemic threshold sensitivity and specificity with MWA were similar to MEM, above 60% and 95% respectively.

### Conclusion

MWA provides the same reliable indicators in temperate countries than classic MEM. Next stage will validate this algorithm in non-temperate countries.

*Keywords: Influenza; MEM; non-temperate countries*

**Topic: Public Health: Surveillance & Forecasting**  
**Abstract No: 11241**

## FluNet and FluID - A Review

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### **Introduction and Objectives**

Real-time global data on influenza surveillance have been collected by the World Health Organization (WHO) on web-based applications such as FluNet (1997-present) and FluID (2009-present). FluNet captures virological surveillance data from countries, areas and territories. Complementary epidemiological data is captured in FLUID. This paper analyses the completeness, timeliness, and use of publicly available data in these systems.

### **Methods**

Timeliness and completeness of country reporting over time was analysed. Utilization of the data was examined with Google analytics by assessing the frequency of visits to the data repository pages as a proxy for downloads of FluNet/FLUID data and through a systematic literature search to detect publications utilizing FluNet/FLUID data.

### **Results**

The number of Member States reporting increased from 58 to 146 (FluNet) between 1997-2018, and 44 to 124 (FluID) between 2009-2018. Consistent reporting increased from 64 to 138 (FluNet), and 4 to 118 (FluID) between 2008- 2018. In 2018 56 Members states reported timely in FluNet and 41 in FluID. Yearly unique external visits to chart view page increased from 688 to 11,795.

Thirty-four publications were categorised into four themes: seasonality-18; burden of disease-3; FluNet awareness-4; modelling and advanced analytics-4; and others-5. Additionally, FluNet and FluID data are utilized at WHO bi-weekly and bi-annually to monitor and summarize seasonal activity, update vaccine composition deliberations, and support development of influenza surveillance guidance.

### **Conclusion**

FluNet and FluID are unique applications providing real-time data. They are the go-to not only for monitoring, but also to define seasonality, burden of disease and to support prediction of disease. The number of countries reporting to FluNet and FluID have doubled since inception with good completeness but enhancing the timeliness would further increase its utility.

*Keywords: FluNet, Fluid, Influenza surveillance*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11244

## GENETIC CHARACTERIZATION OF INFLUENZA A AND B VIRUSES IN RUSSIA IN 2018-2019 EPIDEMIC SEASON

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### Introduction

Sustainable and well-performing influenza surveillance system is critically important for monitoring influenza activity, selection of vaccine strains and mitigating the impact of influenza on public health. Increase in timely sequencing and sharing influenza genetic sequence data positively affects GISRS ability to forecast the spread of certain genetic groups of influenza viruses and better understand virus evolution.

### Methods

Isolated influenza viruses and clinical specimens from patients with PCR-confirmed influenza collected in 34 regions of the Russian Federation were used in this study. Next-generation sequencing (NGS) on Illumina MiSeq was performed.

### Results

We performed whole-genome sequencing of 205 influenza A viruses (A(H1N1pdm09) – 107 viruses and A(H3N2) – 98 viruses) and 6 influenza B viruses (Victoria lineage (del2) – 2, Yamagata lineage – 4).

All A(H1N1)pdm09 viruses belonged to genetic group 6B.1 (A/Michigan/45/2015-like viruses).

All A(H3N2) viruses belong to subclade 3C.2a1b. Viruses of subclade 3C.2a1b from Russia can be divided into three genetic subgroups. Subgroup 1 is defined by amino acid substitution T135N in 130-loop (antigenic site A). Subgroups 2 and 3 probably originated from one cluster of viruses with substitutions in antigenic sites E and A. Subgroup 2 is defined by amino acid substitutions T131K in HA1 and V184I in HA2. Viruses of this subgroup have truncated NS1. Subgroup 3 is defined by amino acid substitutions T128A and T135K in antigenic site A resulting in the loss of two potential N-glycosylation sites (in positions 126 and 133).

All analyzed influenza B(Yam) viruses belonged to clade 3, influenza B(Vic) viruses possessed deletion of two ( $\Delta$ 162-163) aa residues in HA molecule.

### Conclusion

Full-genome analysis of influenza A and B viruses revealed a number of amino acid substitutions in surface and internal genes. Further study is required to evaluate evolutionary significance of these substitutions.

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*Keywords: influenza virus; next-generation sequencing; MiSeq; surveillance*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11259

## POPULATION HETEROGENEITY AND INFLUENZA DYNAMICS IN ST. PETERSBURG, RUSSIA: AN AGENT-BASED MODELING APPROACH

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### Introduction and objectives

Mathematical modeling is one of the useful tools in public health that helps to explain the retrospective disease dynamics and to plan control measures for the future outbreaks. One of the important questions that could be addressed using models is how changes in contact patterns caused by day-to-day activities (public transport usage, school attendance, working schedules) in different age groups may result in variation of epidemic dynamics in different urban settings throughout different epidemic seasons. In the presented work the author investigates the matter by applying an agent-based model of influenza dynamics to synthetic population of Saint Petersburg, Russia.

### Methods

The applied approach combines three interlinked components: (1) a synthetic population of Saint Petersburg, Russia; (2) an agent-based microsimulation model of influenza dynamics implemented via open-source framework FRED; (3) the micro and macro data connected with influenza dynamics (namely, weekly incidence of acute respiratory infections) provided by Russian Influenza Research Institute. For comparison purposes, an age-structured compartmental influenza model calibrated to the same data is also employed.

### Results

The simulation results demonstrate the ability of an agent-based model to reproduce the overall flu incidence dynamics in Saint Petersburg accounting for population heterogeneity. The obtained results show the benefits of using spatially explicit models to reproduce disease dynamics in St. Petersburg, compared to age-structured compartmental models, and how uncertainty in input data affects the quality of model fitting to disease incidence.

### Conclusion

In this work, it is demonstrated that agent-based approach combined with synthetic populations makes it possible to fully consider the influence of spatial heterogeneity of the population and variations in contact patterns, caused by day-to-day activities of individuals, on influenza dynamics in urban settings, using St. Petersburg as a case study. Also the research shows the limitations of agent-based modeling approach connected with its demand in detailed data. In case of influenza modeling with scarce input data, an age-structured compartmental model proves itself more useful for planning targeted influenza control measures, because the level of uncertainty of an output generated by an agent-based model becomes too high which results in low plausibility of the influenza dynamics assessment.

*Keywords: influenza; agent-based model; contact structure*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11274

## HOSPITAL-BASED SURVEILLANCE OF INFLUENZA IN SWITZERLAND – A PILOT STUDY

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### Introduction

Until 2018, the national reporting system for influenza in Switzerland consisted of two parts: 1) voluntary reports of influenza-like illness (ILI) by selected primary care clinicians. 2) weekly reports of laboratory-confirmed cases. No national surveillance system existed for hospitals. With support from the Federal Office of Public Health (FOPH), we developed a pilot study for hospital-based influenza cases in Switzerland.

### Methods

Three university hospitals and three cantonal hospitals participated. Data collection followed WHO recommendations using a standardised questionnaire that included demographic data, information on the influenza episode and optional information about the patient's health. Data were directly entered into a secure web-based REDCap database at the participating sites. Data quality checks and descriptive analyses were done weekly, and results were reported back to the sites.

### Results

From 01.11.2018 to 26.02.2019, 1260 cases of influenza were announced. Three hospitals declared 68.8% of cases (site 1 - 35.5%; site 2 - 16.1; and site 3 - 17.2%). The Influenza epidemic started during the week 2018-47 in Western Switzerland, and three to four weeks later in other sites. Most patients were elderly (66.7% over age 65). The majority of cases (98.1%) was due to influenza A; influenza B was reported in 36 infants. Most cases were diagnosed in medicine (51.6%) and geriatrics (11.5%). The proportion of nosocomial cases was 30% during the beginning of the season, and decreased to 20% in recent weeks, with substantial variation between sites.

### Conclusions

Our pilot system allowed us to get a better understanding of the morbidity and spread of severe influenza cases in Switzerland. Simplification of the questionnaire, direct import of existing data, automated analysis, and additional tools for epidemic management will help to reduce the workload and ensure that all data are entered in time. Inclusion of other hospitals is needed.

*Keywords: Influenza, surveillance, Hospital*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11281

## Impact of school-based vaccination on primary school absenteeism in Hong Kong

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### Introduction

Annual influenza vaccination has been recommended for all school-aged children to reduce the burden of influenza in community. The potential impact of large-scale school-based vaccination programme on student absenteeism over an influenza seasonal epidemic remains poorly defined.

### Methods

As an initiative to increase vaccine uptake and coverage, subsidized school-based influenza vaccination programme was first launched by local government in the 2018-19 season. Riding on a smart-card based electronic school absenteeism surveillance system we developed since 2014, we examined student absenteeism data from local primary schools for the current academic years (2018-19) over the 2019 winter epidemic season, and stratified to analyze the impact of school-based influenza vaccination on student absenteeism.

### Results

A total of 47 local primary schools were under the regular surveillance in our school absenteeism surveillance system, covering a total of 30110 enrolled students. Among which 29 schools (20218 students) were having and 18 schools (9892 students) were not having a school-based vaccination programme in the 2018-19 season. The weekly aggregated daily student absent rates from September to November 2018 were ranged from 1.31% to 2.16%, with no significant difference between the two groups. Over the 2019 winter epidemic period, the weekly aggregated student absent rate were about 1% lower among these schools having vaccination programme (1.94% - 3.59%) comparing with those not having a school-base vaccination programme (1.87% - 4.50%), and attaining statistical significance. This figure would translate to hundreds of student absence on a daily basis if extrapolated to the 180,000 total primary school student population in Hong Kong.

### Conclusions

School-based influenza vaccination programme is effective in reducing student absenteeism rate among primary school students in Hong Kong. Other key indicators such as outbreak incidence should be also taken into consideration to evaluate the overall impact of such programme.

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11314

## CDC INFLUENZA NEXT-GENERATION SEQUENCING SURVEILLANCE SYSTEM AND ANALYTICS

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### Introduction

The influenza surveillance program at CDC determines and reports disease prevalence, severity levels, and molecular characteristics of circulating viruses based on a complex network of domestic and international data inputs and specimens. An interoperable analytics system was developed and mirrored within cloud computing infrastructure to support a distributed next-generation sequencing surveillance approach that is enhanced by collaboration between the CDC Influenza Division, the Association of Public Health Laboratories, and three state public health laboratories that act as National Influenza Reference Centers (Wisconsin State Laboratory of Hygiene, New York State Department of Health-Wadsworth Center, and California Department of Public Health).

### Methods

The CDC Influenza Division transitioned in 2014 to using next-generation sequencing (NGS) technologies for molecular surveillance and prioritizes use of original clinical specimens for genetic characterization. Following optimizations to the targeted sequencing library generation protocols and distributed implementation of the pipeline at central reference centers, workflow quality control tracking and automated data analysis components were custom-integrated with an NGS laboratory information management system and visualization suite within cloud infrastructure.

### Results

Reference center laboratories use the cloud-based system to produce high quality genetic data in parallel with CDC. Full genome sequences are assembled and analyzed automatically upon the completion of sequencing runs, and through integrated curation workflows data are often available within weeks of original specimen collection. The cloud-based mirror of these components became fully operational in 2016, and in concert with the CDC pipeline, the overall system operates year-round with near 24/7 availability. In total more than 30,000 full genomes have been submitted to public databases since 2014.

### Conclusions

This collaborative surveillance system and infrastructure framework has ensured sustainability of virologic surveillance by increasing national surveillance surge capacity, reducing turn-around times for the availability of genetic data, and has improved readiness for pandemic preparedness.

**Keywords:** Next-generation sequencing; full genome surveillance; informatics; cloud computing

Topic: Public Health: Surveillance & Forecasting

Abstract No: 11318

## CIRCULATION OF INFLUENZA B LINEAGES IN ARGENTINA, 1990-2019.

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**Background:** The trivalent vaccine (TV) was the only vaccine used to mitigate seasonal influenza in Argentina until 2018, in 2019 is available the quadrivalent vaccine (QV) in the health private system. Both lineages B/Victoria (B/Vic) and B/Yamagata (B/Yam) coexist, evolve separately, and alternate in prevalence in a so far unpredictable pattern. This implies that in case of a lineage-mismatch between the vaccine and the circulating strain or a cocirculation of both lineages the TV protection will be limited. This study shows the pattern of the influenza B viruses circulation in Argentina during the period 1990-2019.

**Materials and methods:** Influenza A or B positive clinical samples are routinely submitted to the National Influenza Centre through the National Influenza and Respiratory Viruses Laboratory Network for further characterization. Until 2012 the lineage determination was carried out by haemagglutination inhibition test using the WHO influenza reagent kit. From the season 2012, all clinical influenza B samples were tested for lineages differentiation by duplex RT-PCR real time technique.

**Results:** Circulation of Influenza B viruses has been detected in most seasons in Argentina, during the studied period: between 1991 and 2001 only B/Yam was detected and matched correctly with the vaccine; in 2002 B/Vic was re-introduced in Argentina and coexists with B/Yam until 2010, when only B/Vic was detected; in 2011 influenza B activity was low; in 2012, both lineages began to circulate in unpredictable proportions year after year; in 2015 both lineages circulated in similar proportions; in 2016 B/Vic circulated mostly and in 2017/2018 B/Yam was the predominant.

**Conclusions:** Surveillance of influenza viruses is essential for updating vaccines. Since the reintroduction of B/Vic, alternation or co-circulation of both lineages, makes the choice difficult of a unique strain as component B in VT. The use of the QV may ensure a better improved vaccine efficacy.

*Keywords: Influenza B, Surveillance, Argentina*



Topic: Public Health: Surveillance & Forecasting

Abstract No: 11319

## TEMPORAL CIRCULATION AND GENETIC CHARACTERIZATION OF INFLUENZA A VIRUSES IN ARGENTINA 2017-2019

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**Introduction and Objectives:** Human influenza is usually transmitted in the winter months in the southern hemisphere temperate regions, but the exact timing and duration of the influenza season vary by year. Influenza season can begin as early as May but usually peaks around August. This report describes the temporal distribution of influenza A (FLUA) viruses circulating in Argentina and analyzes their genetic characteristics in comparison with the vaccine formula during the period 2017-2019.

**Methods:** FLUA temporal distribution was analyzed based on the information available in the National Database managed by the Ministry of Health. FLUAH1 and H3 subtyping by rtRT-PCR was performed following CDC protocols. A total of 165,522 respiratory samples coming from pediatric and adult inpatient and outpatient were collected for respiratory virus study (77,048 in 2017; 87,437 in 2018 and 4037 until EW10 2019). A set of 198 viruses (155 H3 and 43 H1) were selected for HA1 sequencing and phylogenetic analyses.

**Results:** In 2017, influenza positivity was 7.7% and only FLUAH3 was detected, with a peak in EW 24-25 (June). In 2018, influenza positivity was 6%, mostly FLU A H1 and only a few FLU A H3 viruses with a peak in August (EW35-36). In 2019, until EW10, only sporadic FLUA H3 were detected. A complete match between FLU A circulating and the vaccine strains was observed except for FLUA H3 at the beginning of the 2018 and 2019 seasons, when clades 3C.2a1 and 3C.3a circulated, respectively.

**Conclusions:** In 2018, influenza peaked 12 weeks later in comparison with previous seasons. A mismatch between FLUA H3 circulating and vaccine strains was observed at the beginning of two consecutive seasons, 2018 and 2019. Monitoring the temporality and genetic characteristics of local influenza viruses is important to alert health authorities and establish efficient control measures.

*Keywords: Influenza A. Genetic characterization. Argentina*

Topic: Public Health: Surveillance & Forecasting

Abstract No: 11332

## WHOLE GENOME INFLUENZA SURVEILLANCE IN THE US ENHANCED WITH NATIONAL INFLUENZA REFERENCE CENTERS IN COOPERATION WITH THE CDC

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### Introduction

To inform influenza virologic surveillance in the United States (US), specimens flow through a multi-tiered system from testing at clinical and public health laboratories (PHLs), to National Influenza Reference Centers (NIRCs) and the Centers for Disease Control and Prevention (CDC). The three US NIRCs were established in 2009 as a surveillance support network to the CDC, receiving influenza-positive specimens from all states. In recent years, surveillance testing at the NIRCs has increasingly focused on genomic sequencing using next generation sequencing.

### Methods

US PHLs submit representative influenza positive specimens to assigned NIRCs, where virus is cultured and codon complete genomic sequencing performed via NGS on original specimens. Sequences are analyzed and curated through an integrated genome assembly pipeline within a cloud infrastructure and transmitted through secure channels to the CDC for further analysis.

### Results

Implementation of the NGS pipeline was initiated in the NIRCs during the 2014-15 influenza season and completed at all sites by January 2017. Since then, full genome data from 8,600 influenza samples has been generated from the NIRCs, transmitted to CDC, analyzed and contributed to public databases. Average turn-around time from receipt at a NIRC to sequence transmission to CDC is 13 days. Numerous viral changes have been detected, including influenza A/H3 3C.2a and 3C.3a clade emergence, HA gene deletions in B/Victoria viruses, reassortants, neuraminidase substitutions that impacted fitness and drug resistance, and RNA polymerase analysis for assessment of new drug targets.

### Conclusions

The use of genomics facilitates the global analysis of influenza virus changes in a single, high-resolution data set, enabling timely intervention strategies and improved data availability for vaccine strain selection. Advances in methods and platforms have enabled its application for systematic influenza surveillance and implementation at coordinated reference centers in the US, resulting in the rapid detection of evolutionary trends in circulating viruses.

*Keywords: Surveillance, consortium, next generation sequencing,*

Topic: Public Health: Surveillance & Forecasting

Abstract No: 11336

## HOSPITALIZATIONS DUE TO INFLUENZA IN MOSCOW DURING 2012-2019.

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### Background

Most of ARVI hospitalizations are related to influenza. Based on the Global Influenza Hospital Surveillance Network (GIHSN) influenza hospitalizations and influenza seasonal activity has been studied in Moscow, Russia.

### Methods

Patients with ARVI were admitted to Clinical Hospital #1 for Infectious Diseases in Moscow during seven influenza seasons 2012/13 to 2018/19. Hospitalized patients were swabbed and interviewed according to GIHSN protocol. Samples were tested by RT-PCR to detect influenza A(H3N2), A(H1N1)pdm09 and B viruses.

### Results

Totally 10176 hospitalized patients were tested, including 3102 pregnant. Influenza was confirmed in 3707 (36%). The highest level (46-51%) of positive cases was observed among pregnant, elderly and school children. A(H1N1)pdm09 was mostly detected among these compromised groups. For whole period the dominant virus was A(H3N2) – 1486 (40%). A(H1N1)pdm09 and influenza B were approximately in equal proportion – 1147 (30%) and 1121 (30%). Subtyping of influenza B revealed that most of them belonged to B/Victoria-lineage.

The proportion with confirmed influenza was higher in pregnant compared to non-pregnant women. They had influenza in all trimesters of pregnancy independently of circulating influenza virus.

Complications like pneumonia and bronchitis were often associated with A(H1N1)pdm09. Most common comorbidities with confirmed influenza were COPD, asthma and renal impairment. There were low number of vaccinated patients 2,2-6,3%.

### Conclusions

ARVI hospitalizations were often caused by influenza and accounted for from 24% to 46% depending on the season.

Influenza was revealed mostly among elderly, schoolchildren and pregnant women.

Pregnant women are at extremely high risk to be exposed to influenza infection.

Vaccination level was very low among hospitalized patients.

Influenza vaccination and early antiviral therapy are the main preventive measures against influenza hospitalizations.

**Acknowledgment.** This work is supported by GIHSN (FISABIO-Public Health, Valencia, Spain) and Fondation de France (Paris, France). A special thanks to Dr.Joan Puig-Barbera and all Spain team.

*Keywords: hospitalization, pregnancy, surveillance*

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11347

## NOTIFICATION OF SEROLOGICALLY DIAGNOSED INFLUENZA CASES ENHANCES THE MONITORING OF THE AGE-RELATED INCIDENCE AND IMPACT OF INFLUENZA

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### Introduction and objectives

Surveillance of laboratory-confirmed influenza virus infections is fundamental to monitoring of activity, measuring impact and assessing vaccine effectiveness. Most cases are identified by virus detection, but this misses cases where there are low level of virus in the upper respiratory tract, or where the patients presents after the phase of viral replication. This is particularly important for patients presenting with complications of influenza, e.g. bacterial pneumonia in the elderly. Serological diagnosis can assist in identifying these cases but as acute and convalescent serum samples are uncommon so that diagnosis by rising titres is unusual, and most seropositive samples are single high titres. There has been considerable concern about the reliability of this for surveillance purposes.

### Methods

PathWest Laboratory Medicine performs influenza testing by PCR and serology (CFT ) for hospital and community samples within Western Australia. We reviewed 12 years of data on cases notified by our laboratory.

### Results

A total of 29,495 PCR positive cases and 6,280 serologically diagnosed cases were included, with nearly all of the latter being single high titre results. The percentage of case notified based on serology varied from under 1% in children under 5y.o. to 24% in those aged 65-79y.o. The seasonal patterns for influenza diagnosis were almost identical for cases diagnosed by PCR and those diagnosed by a single high titre (Figure). These results were the same for influenza A and B, and for samples collected across the dominant winter season compared to this collected out of season.

### Conclusions

Influenza cases diagnosed by a single high titre are an indicator of influenza activity within the community. Their seasonal distribution was similar to PCR-diagnosed cases, and did reflect disease incidence rather than background antibody levels. Serological diagnoses particularly increased the recognition of influenza infections in patients aged 65 years or older.

*Keywords: Influenza; serology; surveillance*

Topic: Public Health: Surveillance & Forecasting

Abstract No: 11356

## Using the Moving Epidemic Methods(MEM) to determine the epidemic threshold for Influenza, Zambia,2013/2014 to 2017/2018

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**Introduction:** Zambia has a stable Influenza surveillance system with sentinel sites located in two highly populated Provinces. The type of surveillance implemented is both influenza like illness (ILI) and severe acute respiratory illness surveillance (SARI). Since 2011, the Influenza surveillance system has been actively reporting results the World Health Organisation through flunet and Fluid platforms. Among the objectives of the national surveillance system are to monitor the proportions of confirmed cases of influenza among SARI in-patients and/or among ILI out-patients and to monitor strains of influenza and other viral respiratory viruses circulating in Zambia and thus contribute to influenza global information.

**Aim:** Our aim was to use Moving Epidemic Methods (MEM) to determine Influenza thresholds for use in the influenza surveillance system in Zambia.

**Methods:** Historical ILI/SARI data from 2013-2018 was run separately .Epidemic threshold for 2017/2018 season was calculated using the proportional rates from 2013-2018 seasons.

**Results:** Pre-epidemic ILI threshold per 10,000 populations was 7.8, while the post-epidemic threshold was 12.2. Using MEM, we observed an epidemic of 14 weeks' duration. The sensitivity of the MEM epidemic threshold in Zambia was 74% and the warning signal specificity was 89%. The Pre-epidemic SARI threshold per 10,000 populations was 1.9, while the post-epidemic threshold was 3.9. Using MEM, we observed an epidemic of 20 weeks' duration. The sensitivity of the MEM epidemic threshold in Zambia was 91% and the warning signal specificity was 82%.

**Conclusions.** The study demonstrates that MEM can be used to determine influenza epidemic thresholds in Zambia. The results can be used to determine influenza rates in a season .The data obtained can also be applied to measure influenza severity and transmissibility in communities. Our findings also demonstrate that MEM can be used to compare current influenza seasons with previous seasons and allow for intervention measures where necessary.

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11706

## Improved accuracy of antigenic characterization of recent influenza A/H3N2 isolates by modified focus reduction assay

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### Introduction and objectives

*In vitro* virus microneutralizing (MN) tests have been conducted as an alternative to hemagglutination-inhibiting (HI) assays for antigenic analysis of recent A/H3N2 viruses in response to extremely low hemagglutination activity by HA, which precludes analysis by HI assays. Recently, we found that the receptor binding activity of neuraminidase (NA) rendered by an amino acid substitution at position 151 (D151X) seemed to affect MN titers and hinder precise evaluation of HA antigenicity by MN tests. In this study, we carried out MN/focus reduction assays (MN/FRA) in the presence and absence of oseltamivir to verify whether the receptor binding activity of NA from influenza A/H3N2 virus affected MN titers, and to assess the usefulness of oseltamivir additive in MN/FRA for improved antigenic evaluation of A/H3N2 HA.

### Methods

Recent A/H3N2 isolates and ferret antisera raised against the isolates chosen from different genetic subclades were used to conduct MN/FRA in the presence and absence of oseltamivir.

### Results

MN titers to viruses possessing D151X in NA were much lower than those to viruses with no substitution at the same position when we tested the assay without oseltamivir, as is standard. However, when oseltamivir was added to the medium, MN titers to viruses possessing D151X were typically restored and comparable to those to viruses without substitution. These results suggested that a virus could infect cells via receptor binding by NA, which was not inhibited by antiserum, resulting in low MN titers in MN/FRA; this problem could be overcome by the addition of oseltamivir.

### Conclusion

Our data suggest oseltamivir may be a useful additive to prevent the unanticipated effects of receptor binding by NA in MN/FRA. The modified MN/FRA method presented here can improve the reliability of antigenic evaluation of A/H3N2 HA by MN/FRA.

*Keywords:* A/H3N2; antigenic characterization, microneutralizing/focus reduction assay

Topic: Public Health: Surveillance & Forecasting  
Abstract No: 11742

## Genetic and antigenic analyses of influenza B viruses isolated in Japan during the 2017–2018 and 2018–2019 influenza seasons

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### Introduction and Objectives

Influenza B viruses are responsible for severe morbidity and mortality worldwide during seasonal influenza epidemics. Here, we genetically and antigenically analyzed influenza B viruses isolated in Japan during the 2017–2018 and 2018–2019 influenza seasons.

### Methods

A total of 68 influenza B viruses [61 B/Yamagata/16/88-like (B/Yamagata)-lineage and 7 B/Victoria/2/87-like (B/Victoria)-lineage] were recovered from respiratory specimens collected from patients in Japan during these two seasons. Influenza B virus isolates were antigenically and genetically characterized by using hemagglutination inhibition assays and phylogenetic analysis, respectively.

### Results and Conclusions

All 61 B/Yamagata-lineage isolates were genetically closely related to B/Phuket/3073/2013, the vaccine strain for these two seasons. Eleven B/Yamagata-lineage isolates tested were antigenically similar to B/Phuket/3073/2013. Seven B/Victoria-lineage isolates were genetically closely related to B/Texas/02/2013, the vaccine strain for the 2017–2018 season; however, they were antigenically distinct from B/Texas/02/2013. Of these 7 isolates, 4 possessed a two-amino-acid deletion at positions 162 and 163 in hemagglutinin (HA) and the other 3 had a three-amino-acid deletion at positions 162–164 in HA. Importantly, the variants with the three-amino-acid deletion appeared to be antigenically different from the B/Colorado/06/2017 virus with the two-amino-acid deletion, the vaccine strain for the 2018–2019 season. One B/Yamagata-lineage isolate carrying a G407S mutation in its neuraminidase (NA), which confers reduced susceptibility to NA inhibitors, was detected. These results highlight the need for continued monitoring for the prevalence of the antigenic variant with the three-amino-acid deletion and the variant with reduced NA inhibitor susceptibility.

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10132

## Vaccine effectiveness of 2017/18 trivalent seasonal influenza vaccine in preventing laboratory-confirmed influenza in outpatient settings: A test-negative case-control study in Beijing, China

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### Introduction and Objectives:

The objective of this study was to estimate influenza vaccine effectiveness (VE) for the 2017/18 epidemic of co-circulating influenza A(H3N2) and A(H1N1)pdm09, and a lineage mismatched influenza B(Yamagata) viruses in Beijing, the capital of China.

### Methods:

A test-negative case-control design was used to estimate VE against medically-attended laboratory-confirmed influenza in outpatient settings. Cases were influenza-like illness (ILI) patients who tested positive for influenza, and controls were influenza negative patients. ILI patients were identified through influenza virological surveillance, from November 1, 2017 to April 30, 2018.

### Results:

A total of 10627 ILI patients were enrolled and swabbed. Among them, 1235 tested positive for influenza A(H1N1)pdm09, 625 for A(H3N2), 1318 for B(Yamagata), and 48 for B(Victoria). Adjusted VE against all influenza was low at 36% (95% confidence interval (CI): 28% to 51%), with 63% (95%CI: 41% to 77%) for influenza A(H1N1)pdm09, 10% (95%CI: -44% to 44%) for influenza A(H3N2), and 25% (95%CI: -3% to 46%) for B(Yamagata). Overall VE was 34% (95%CI: 4% to 55%), 0% (95%CI: -48% to 32%), and 38% (95%CI: 14% to 56%) for 2017/18 vaccination only, 2016/17 vaccination only and vaccination in both seasons, respectively.

### Conclusions:

Our study suggested a moderate VE against influenza A(H1N1)pdm09, but low VE against influenza A(H3N2) and B(Yamagata) in Beijing, 2017/18 season. There was no evidence that repeated vaccination had a negative impact on VE for this season.

*Keywords: Influenza, Vaccine effectiveness, Influenza-like illness, China.*



Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10136

## Influenza vaccine effectiveness in preventing influenza illness among children during school-based outbreaks in the 2016–2017 season in Beijing, China

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**Introduction and Objectives:** Since 2007, trivalent inactivated influenza vaccine (TIV) has been provided free-of-charge to elementary and high school students in Beijing. However, school-based studies on influenza vaccine effectiveness (VE) were limited. In this report, we estimated influenza vaccine effectiveness against laboratory-confirmed influenza illness among school children in Beijing, China during the 2016-2017 influenza season.

**Methods:** In this study, the VE of 2016-2017 TIV in preventing laboratory-confirmed influenza among school-age children was assessed through a matched case-control study. Participants were enrolled from thirty-seven eligible school influenza outbreaks in Beijing, China from 1 November 2016 to 30 April 2017. Four asymptomatic controls were chosen randomly per case matching on class and week. Conditional logistic regression was performed on matched case-control sets to estimate VE. The effect of prior vaccination on current VE was further examined.

**Results:** The average coverage rate of 2016-2017 TIV among students across the 37 schools was 30.6%. The adjusted VE of 2016-2017 TIV against laboratory-confirmed influenza was 69% (95% confidence interval (CI): 51% to 81%), with 60% (95% CI: -15% to 86%) for influenza A(H1N1)pdm09 and 73% (95% CI: 52% to 84%) for influenza A(H3N2). The overall VE for receipt of 2015-2016 vaccination only, 2016-2017 vaccination only, and vaccinations in both seasons was 46% (95% CI: -5% to 72%), 77% (95% CI: 58% to 87%), and 57% (95% CI: 17% to 78%), respectively.

**Conclusion:** Our study during school outbreaks found that VE of 2016-2017 TIV was highly effective against both influenza A(H3N2) and A(H1N1)pdm09 for school children in Beijing, China during the 2016-2017 influenza season.

*Keywords: Influenza; Vaccine effectiveness; School; Outbreak; China*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10241

## PROGRESS ON THE DEVELOPMENT AND THE PROMOTION OF THE LAIV FOR INFLUENZA PROPHYLAXIS IN LMIC

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### Introduction and objectives

In Russia LAIV has a long history of development, stage-wise improvement, licensing and use in public health. The concept of replicating the vaccine virus in the nasal cavity and thus generating a specific immune response at the site of infection, appears to be the most appropriate model of immunization. Limited downstream processing and significantly higher yield in eggs make LAIV especially attractive for LMIC with large population.

WHO recognized this advantage LAIV in the event of a pandemic included LAIV in GAP for influenza vaccines. In 2009 WHO signed an agreement with IEM, the sole developer for reassortants strains for Russian LAIV.

### Results

During the period 2009-2018, IEM developed and transferred to WHO 11 seed LAIVs for seasonal vaccine and 7 pandemic seed-LAIV for further distribution to manufactories in India and China.

All the above mentioned pandemic LAIV candidates have been included in the Phase I clinical trials. Vaccines demonstrated a good safety profile and immunogenicity. Data generated from clinical trials suggested that the methods used to routinely measure LAIV immunogenicity should be revised to include additional immunological methods.

SII licensed LAIV "Nasovac" in 2014 and is prequalified by WHO. Today SII is conducting research on the improvement of MDCK-derived liquid, quadrivalent LAIV.

In 2011 the BCHT (China) signed an agreement with WHO and since then has built a manufacturing plant and adapted the LAIV production technology. BCHT is currently finished Phase III clinical trials among 9000 people different age. In 2019 BCHT intends to finalize registration of LAIV and begin its use for influenza prevention.

### Conclusion

Licensing of the Russian LAIV to WHO and the subsequent transfer of the technology to LMIG manufactures has proven to be highly successful and effective in providing access to vaccine production capabilities, under the supervision and guidance of WHO.

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10260

## Effectiveness of maternal influenza vaccination to protect their infants against influenza: prospective cohort study in Japan

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**Introduction and Objectives:** Infants aged less than 6 months are too young to receive influenza vaccination, although they are one of the high risk population for severe influenza-related complication. The aim of this study was to examine the effectiveness of maternal influenza vaccination in preventing infants' influenza by means of a prospective cohort study.

**Methods:** Study subjects were 3,441 infants born at the participating hospitals before the 2013/14 influenza season. At the time of recruitment, their mothers completed the questionnaire about influenza vaccination status for the 2013/14 season. The follow-up survey conducted after the end of the 2013/14 season to collect information regarding influenza diagnosis and hospitalization among infants. The primary exposure was maternal influenza vaccination which divided into prenatal vaccination and postpartum vaccination in the detailed analysis. Logistic regression model was employed to calculate odds ratios (OR) and 95% confidence intervals (CI) of the maternal vaccination for infants' influenza with adjustment for potential confounders.

**Results:** During the 2013/14 influenza season, 71 infants (2%) were diagnosed with influenza and 13 infants (0.4%) were hospitalized with influenza. Maternal influenza vaccination (especially prenatal vaccination) decreased the occurrence of infants' influenza (OR of prenatal vaccination=0.39, 95% CI=0.19-0.84; OR of postpartum vaccination=0.47, 95%CI=0.17-1.28). On the other hands, a diagnosis of maternal influenza infection elevated the OR for infants' influenza (OR=36.0, 95%CI=21.1-61.4). Maternal influenza vaccination was also associated with decreasing influenza-related hospitalization of infants, although the vaccine effectiveness could not reach statistical significance due to the limited number of infants hospitalized due to influenza (OR of maternal vaccination=0.27, 95%CI=0.06-1.24).

**Conclusion:** Maternal influenza vaccination including both prenatal and postpartum vaccination had beneficial effects in protecting infants from influenza, although maternal influenza infection was a strong risk factor for infants' influenza.

**Keywords:** Influenza; infants; maternal vaccination; prospective cohort study; vaccine effectiveness.

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10261

## Safety of influenza vaccination in pregnant women in terms of adverse birth outcomes: prospective cohort study in Japan

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**Introduction and objectives:** To promote influenza vaccination for pregnant women, information about vaccine safety is needed. The aim of this study was to examine the safety of influenza vaccination in pregnant women in terms of adverse birth outcomes.

**Methods:** This prospective cohort study included 10,631 pregnant women who were attending maternity hospitals and clinics in Osaka Prefecture before the beginning of the 2013/14 influenza season. The primary exposure was influenza vaccination during pregnancy. All subjects were followed until the end of their pregnancy. The study outcome evaluated was adverse birth outcomes, including abortion, still birth, preterm birth, low birthweight and malformation. We compared the proportion of adverse birth outcomes between vaccinated and unvaccinated pregnant women, and logistic regression model was employed to calculate odds ratios (OR) and 95% confidence intervals (CI) of the vaccination during pregnancy on the risk of adverse birth outcomes with adjustment for potential confounders.

**Results:** A total of 875 adverse birth outcomes (14%) were reported among 6,387 unvaccinated pregnant women, whereas 477 adverse birth outcomes (11%) were reported among 4,244 vaccinated pregnant women. After the adjustment for potential confounders, vaccination during pregnancy had a significantly decreasing OR for the risk of adverse birth outcomes (OR=0.82, 95%CI=0.71-0.94). Vaccination during the first trimester had no association with adverse birth outcomes (OR=1.04, 95%CI=0.80-1.35), whereas vaccination during the second or third trimester had significantly decreasing ORs for adverse birth outcomes (OR of second trimester=0.79, 95%CI=0.62-0.99; OR of third trimester=0.69, 95%CI=0.56-0.86).

**Conclusion:** Influenza vaccination during pregnancy did not increase the risk of adverse birth outcomes, regardless of the trimester of pregnancy when the vaccination was given, compared to unvaccinated pregnant women.

**Keywords:** Birth outcome; influenza; maternal vaccination; prospective cohort study; vaccine safety.

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10312

## INFLUENCE OF STUDY CHARACTERISTICS ON SEASONAL INFLUENZA VACCINE EFFECTIVENESS ESTIMATES: A SYSTEMATIC REVIEW AND META-ANALYSIS OF TEST-NEGATIVE DESIGN STUDIES

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**Introduction/Objectives:** In test-negative design (TND) study, patients presenting with influenza-like illness and testing positive for influenza virus are defined as cases and those testing negative are defined as comparators. Our objective was to summarize seasonal influenza vaccine effectiveness (SIVE) from TND studies according to source of seasonal influenza vaccination (SIV) information, study continent, and confounder adjustments.

**Methods:** We systematically searched appropriate bibliographic databases and websites from January 2011–July 2018 for full-text articles from TND studies of SIVE against laboratory-confirmed (PCR/culture) influenza in primary care settings during the 2010/11–2017/18 influenza seasons. Two reviewers independently screened retrieved citations against the eligibility criteria using a two-stage sifting approach (by titles/abstracts and full-text articles) and extracted data from included studies. Disagreements were resolved by consensus or by a third reviewer. We included only final SIVE estimates. Pooled SIVE was calculated using inverse variance, random-effects model for all–Influenza, H1N1, H3N2, influenza–A, and influenza–B.

**Results:** Seventy articles met our eligibility criteria. Except for H3N2, compared with self-reported and mixed methods (medical record or self-reported), pooled SIVE was lower for SIV confirmation from medical records: 48% (CI: 31–61%;  $I^2=84.5\%$ ) and 47 (CI: 38–55%;  $I^2=70.9\%$ ), respectively, versus 41% (CI: 34–47%;  $I^2=75.1\%$ ) for all–Influenza, 62% (CI: 46–73%;  $I^2=55\%$ ) and 56 (CI: 49–61%;  $I^2=31.3\%$ ), respectively, versus 47% (CI: 38–54%;  $I^2=55.6\%$ ) for H1N1, 41% (CI: 13–60%;  $I^2=86.6\%$ ; for medical records) versus 40 (CI: 32–48%;  $I^2=19.1\%$ ) for influenza–A, and 48% (CI: 36–59%;  $I^2=28.2\%$ ) and 50% (CI: 43–56%;  $I^2=23.2\%$ ), respectively, versus 41% (CI: 30–51%;  $I^2=72.3\%$ ) for influenza–B. Pooled SIVE across influenza types and subtypes was highest in Oceania, followed by North–America, Europe, and Asia. Pooled SIVE varied between studies that included age and those that included age and medical conditions among adjusted variables.

**Conclusion:** The available evidence suggests source of SIV information and study continent influence SIVE estimates from TND studies.

*Keywords: Test-negative design; Seasonal influenza vaccine; Effectiveness; Systematic review; Meta-analysis*

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10335

## INFLUENZA VACCINE EFFECTIVENESS AND FACTORS ASSOCIATED WITH OUTCOMES IN HOSPITALIZED ADULT PATIENTS WITH CHRONIC LUNG DISEASE

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**Objective:** Among adult patients with chronic lung disease (asthma, COPD=CLD) who were hospitalized with an acute respiratory illness (ARI), we determined the vaccine effectiveness (VE), identified the clinical characteristics associated with influenza infection, and with admission to the intensive care unit (ICU).

**Methods:** Data were from the test-negative, case-control HAIVEN study Pittsburgh site for seasons 2015-2018. Eligibility included age  $\geq 18$  years and hospital admission for ARI of  $\leq 10$  days, with new or worsening cough and with ICD-10-confirmed CLD. Influenza infection was verified using real-time polymerase chain reaction tests from nasal/pharyngeal or nasopharyngeal swabs. Influenza VE was determined using multivariable logistic regression models with  $VE = 100 \times (1 - \text{Odds Ratio})$ . Patient characteristics were associated with influenza infection and ICU admission using chi square tests, t-tests, signed rank tests and multivariable logistic regression.

**Results:** 1,367 patients with CLD were included. 61% were vaccinated, 275 (20%) patients were influenza positive. Influenza cases vs. controls more often were  $\geq 65$  years (51.2% vs 42.8%,  $p=0.028$ ), had fewer high-risk conditions (6.3 vs 7.2;  $p<.001$ ) and fewer prior ED or hospital admissions (50.6% vs 65.6%;  $p<.001$ ). In adjusted models, VE was not significant for any age group [ $\geq 65$  years VE=8% (-38%-39%); 50-64 years VE=19% (-34%-51%); 18-49 years VE=35% (-27%-66%)] or season [2015-2016 VE=57% (-5%-82%); 2016-2017 VE=11% (-40%-44%); 2017-2018 VE=19% (-25%-47%)]. Risk factors for influenza were being unvaccinated,  $\geq 65$  years, not frail, without CHF, other blood disorders or other lung conditions. Risk factors for ICU admission in influenza cases included CHF (OR, 2.78; 95% CI, 1.14-6.81), CVD (OR, 4.17; 95% CI, 1.17-14.8) and other lung conditions (OR 3.7; 95% CI, 1.48-9.65).

**Conclusion:** Influenza vaccination for those with CLD did not reduce ARI hospitalizations. Influenza cases in this vulnerable population were more likely to be healthy, but cases with high-risk conditions were more likely to be admitted to the ICU.

*Keywords: ARI; hospitalizations; Influenza; asthma and COPD; vaccine effectiveness*

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Abstract No: 10380

## ACCEPTABILITY OF SEASONAL INFLUENZA VACCINES AMONG HEALTH CARE WORKERS IN VIETNAM IN 2017

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**Introduction:** Healthcare workers (HCWs) can contract and spread seasonal influenza to vulnerable patients, and WHO recommends HCWs receive vaccine annually. As part of an influenza vaccine project among HCWs in 4 provinces of Vietnam, we conducted a survey to identify the main reasons that HCWs accept or refuse vaccination to inform and improve future immunization activities.

**Methods:** We conducted a descriptive cross-sectional survey from May to August 2017 among HCWs at 13 selected health facilities. We employed logistic regression to determine the association between demographic and professional factors, and the decision to receive seasonal influenza vaccine. We performed post-hoc pairwise comparisons among reasons for and against vaccination using Fisher's exact test.

**Results:** A total of 1,450 HCWs participated in the survey, with a higher proportion of females than males (74% versus 26%). The age group 30-39 had the greatest proportion of participants (36%). Among those surveyed, 700 (48%) were vaccinated for influenza during the first half of 2017. HCWs under 30 and 30-39 years were less likely to get vaccinated than HCWs  $\geq 50$  years old (OR=0.52; 95%CI 0.36-0.75 and OR=0.58; 95%CI 0.41-0.83 respectively). Nurses were more likely to be vaccinated than physicians (OR=1.53; 95%CI 1-2.35). The most common reason for accepting vaccination was fear of getting influenza (66%); the most common reason for refusing was concern about vaccine side effects (23%). Nurses cited side effects as the most common reason (28%) for refusing, while physicians most frequently invoked lack of time to get vaccination (25%).

**Conclusion:** Our data suggest that younger HCWs, especially physicians should be targeted for interventions to increase acceptance of vaccine. Such interventions could highlight benefits of vaccination and communicate risks effectively as well as improved vaccine access.

**Keywords:** Influenza vaccination, health care workers

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10393

## 2017-18 clade-specific vaccine effectiveness against A(H3N2) viruses in the United States, US Flu VE Network

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**Background:** 2017-18 was a severe US influenza season with circulation of several HA genetic clades of A(H3N2) viruses including the predominant clade 2a2. Clade-specific effectiveness of influenza vaccines containing A/Hong Kong/4801/2014 (genetic group 3C.2a)-like viruses provides information about vaccine-induced protection against circulating influenza A(H3N2) viruses.

**Methods:** In the US Influenza VE Network, patients ≥6 months of age seeking care for acute respiratory illness within 7 days of illness onset were enrolled at ambulatory clinics in five study sites. Influenza vaccination status was obtained from electronic records or self-report. Respiratory specimens were tested for influenza by RT-PCR; influenza positive specimens were sequenced using whole genome sequencing. Influenza A(H3N2) and clade-specific VE was estimated using a test-negative design as  $100\% \times (1 - OR)$ , where OR is the odds ratio from adjusted logistic regression models.

**Results:** A total of 1761 influenza A(H3N2)-positive and 5386 influenza-negative patients were enrolled in the US Flu VE Network from November 2017—February, 2018. Of these, 45% of A(H3N2)-positive patients and 53% of influenza-negative patients were vaccinated; VE against any A(H3N2) virus was 19% (95% confidence interval [CI]: 5, 30). HA clade was determined for 948 A(H3N2) cases: 816 (86%) 2a2, 40 (4%) 2a1 and 87 (9%) 3a. By HA clade, VE was 19% (CI: 4, 31) against clade 2a2, 7% (CI: -80, 52) against 2a1 and 39% (CI: 3, 62) against 3a.

**Conclusion:** Although differences were not statistically significant, higher VE point estimates against A(H3N2) clade 3a viruses compared to the predominant 2a2 viruses were unexpected and merit further study. Clade-specific VE estimates from more studies are needed.

*Keywords: vaccine effectiveness, genetic group, clade*



Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10406

## REAL WORLD OUTCOMES OF ADJUVANTED TRIVALENT INFLUENZA VACCINE COMPARED TO FLUZONE-HD AND EGG-BASED QUADRIVALENT AND TRIVALENT VACCINES AMONG THE US ELDERLY DURING 2016-2018 FLU SEASONS USING CLAIMS DATA

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### Objective

There is limited real-world data describing subject characteristics and vaccine effectiveness among elderly receiving influenza vaccine in matched and mis-matched seasons. Adjuvant trivalent vaccine (aTIV) was introduced in the US in 2015 and has proven to increase and broaden immune response in older populations. This study aimed to describe baseline characteristics among subjects 65+ years vaccinated with aTIV, Fluzone High-Dose (TIV-HD), egg-based quadrivalent vaccine (QIVe), or trivalent vaccines (TIVe) and rates of influenza-related hospitalizations/ER visits and office visits for the 2016/17 and 2017/18 flu seasons.

### Methods

A retrospective cohort analysis was conducted using professional fee claims, prescription claims and hospital charge data in the US. Baseline characteristics included age, gender, payer type, region, Charlson Comorbidity Index (CCI), location of vaccine receipt, comorbidities, indicators of frail health status, and pre-index hospitalization rates and costs. Differences in baseline characteristics were evaluated using standardized mean difference. The number of events and rates of influenza-related hospitalizations/ER visits and office visits were calculated per 1,000 vaccinated subject-seasons.

### Results

During the 2016/17 and 2017/18 flu seasons, we studied 58,402 and 234,313 recipients of aTIV; 1,256,490 and 1,269,855 of TIV-HD; 210,602 and 212,287 of QIVe; and 281,096 and 106,491 of TIVe, respectively. In both seasons, a greater proportion of aTIV recipients were vaccinated at pharmacies compared to other cohorts; aTIV recipients were also healthier (lower CCI) and more often Medicare-insured compared to QIVe or TIVe cohorts. Unadjusted outcomes showed that aTIV had fewer influenza-related hospitalizations/ER visits and office visits compared to other vaccines in both seasons. For season 2017/18, univariate odds ratios (95% confidence intervals) for influenza-related hospitalizations/ER visits were: 0.92 (0.87-0.98), 0.89 (0.82-0.96) and 0.84 (0.77-0.93) for aTIV vs. TIV-HD, QIVe and TIVe, respectively.

### Conclusions

Unadjusted analysis shows that aTIV reduced influenza-related hospitalizations/ER visits and office visits compared to other standard egg-based vaccines and TIV-HD.

*Keywords: Adjuvanted Influenza Vaccine, Fluzone High-Dose, Real World Evidence, Vaccine Effectiveness, Elderly*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10407

## HOSPITALIZATION ENCOUNTERS FOLLOWING VACCINATION WITH ADJUVANTED TRIVALENT INFLUENZA VACCINE COMPARED TO FLUZONE-HD AND EGG-BASED QUADRIVALENT AND TRIVALENT VACCINES AMONG THE US ELDERLY USING CLAIMS DATA

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### Objective

Older adults experience a higher rate of suffering cardio-respiratory events during influenza seasons due to immunosenescence and lower vaccine effectiveness. Adjuvant trivalent influenza vaccine (aTIV) was recently introduced in the US and has proven to reduce the risk of hospitalization in older adults against trivalent egg-based vaccines (TIVe). However, little is known about its effectiveness compared to other current alternatives. This real-world study describes the rates of all-cause hospitalizations and serious cardio-respiratory hospitalization encounters among subjects 65+ years old vaccinated with aTIV, Fluzone High-Dose (TIV-HD), egg-based quadrivalent vaccine (QIVe) or TIVe for the 2016/17 and 2017/18 flu seasons.

### Methods

A retrospective cohort analysis was conducted using professional fee claims, prescription claims and hospital charge data in the US. Serious cardio-respiratory hospitalization encounters were defined as the first hospitalization or ER visit with a discharge diagnosis code for serious cardio-respiratory events of interest (pneumonia, asthma/COPD/bronchial events, myocardial infarction [MI], congestive heart failure [CHF], etc.). Rates for all-cause hospitalizations and serious cardio-respiratory hospitalization encounters were calculated per 1,000 vaccinated subject-seasons.

### Results

We studied 58,402 and 234,313 recipients of aTIV; 1,256,490 and 1,269,855 of TIV-HD; 210,602 and 212,287 of QIVe; and 281,096 and 106,491 of TIVe during the 2016/17 and 2017/2018 flu seasons, respectively. In unadjusted analyses, aTIV was associated with lower rates of all-cause hospitalizations and serious cardio-respiratory hospital encounters compared to the other vaccine cohorts. For 2016/17 and 2017/18 flu seasons, univariate odds ratios (95% confidence intervals) for all-cause hospitalizations were 0.90 (0.87-0.92) and 0.96 (0.95-0.98) for aTIV vs. TIV-HD, 0.92 (0.89-0.94) and 0.97 (0.96-0.99) for aTIV vs. QIVe, and 0.84 (0.82-0.86) and 0.91 (0.89-0.93) for aTIV vs. TIVe, respectively.

### Conclusions

For the 2016/17 and 2017/18 seasons, aTIV was more effective in preventing all-cause hospitalizations and cardio-respiratory hospital encounters among US elderly in unadjusted analyses.

*Keywords: Adjuvanted Influenza Vaccine, Cardio-Respiratory Hospitalizations, Vaccine Effectiveness, Real World Evidence, Elderly*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10414

## The Israeli winter of 2018-19: Unique influenza viruses

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**Introduction and Objectives** Influenza infections, lead to an acute respiratory disease, causing substantial morbidity and mortality. While vaccination is considered the most effective mean to prevent influenza morbidity, influenza vaccine effectiveness varies yearly, depending on the circulating influenza strains.

**Methods, Results** Seasonal surveillances allow the identification of the circulating influenza virus strains in Israel. In winter of 2018-19, the dominate influenza strain was influenza A/H3N2 virus, detected in 76% of influenza-positive samples obtained in outpatient sentinel clinics and in 63% of hospitalized influenza-positive patients in Sheba medical center; some of the positive cases were vaccinated.

Interestingly, while the dominant strain circulating this season in most parts of the northern hemisphere was influenza A/H1N1pdm09, in Israel it was detected only in 22% of the outpatient influenza-positive cases and in 30% of the hospitalized influenza-positive patients. Influenza B morbidity was negligible in Israel, as in the rest of the northern hemisphere.

Molecular analysis revealed substantial differences between the circulating influenza A/H3N2 strain and the vaccine strain, with approximately 18 amino acid mutations; a total of 10 amino acid mutations occurred in antigenic sites. Phylogenetic analysis indicated that the circulating strain belongs to clade 3C.3a, while the vaccine strain belongs to a different clade-3C.2a1. Unlike influenza A/H3N2, molecular analysis of influenza A/H1N1pdm09 revealed fewer mutations in the circulating strains compared with the vaccine strain. However, phylogenetic analysis revealed that the circulating strain belong to a new sub-clade, different from the vaccine strain clade-6B.1.

**Conclusion** Additional molecular analysis of the Israeli influenza strains and the influenza strains worldwide are necessary to further understand the unique widespread and most importantly, the significance of the influenza A/H3N2 mutations in Israel. The ongoing genetic changes that occur among influenza viruses, in general, and influenza A/H3N2 in particular highlight the potential advantage of a universal influenza vaccine.

*Keywords: Influenza, Unique, vaccine*

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10419

## IMMUNOGENICITY OF CANDIDATE INFLUENZA A(H3N2) VACCINE VIRUS STRAINS IN JAPAN

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**Background:** For the 2017/18 season, A/Saitama/103/2014(CEXP002) (Saitama) was antigenically more similar to prior circulating strains than A/Hong Kong/4801/2014(X-263) (Hong Kong) in the ferret model and was selected as the H3N2 vaccine virus strain in Japan. However, the Saitama strain yielded poor growth during production, and the Japanese government switched to the Hong Kong strain. This change raised public concern of poor effectiveness of the 2017-18 influenza vaccine.

**Methods:** We compared the immunogenicity, a surrogate of the effectiveness, of one dose of monovalent influenza A(H3N2) vaccine containing either the Saitama or the Hong Kong strain in a randomized controlled trial in 100 healthy adults aged 20-64 years. Virus neutralization assay was performed on sera collected on days 0 (pre-vaccination) and 21 (post-vaccination). Geometric mean titer (GMT), mean fold rise (MFR), seroconversion proportion (SCP) and seroprotection proportion (SPP) were calculated for the vaccine strains and a representative circulating A(H3N2) virus strain (A/Osaka/188/2017).

**Results:** For the Hong Kong strain, post-vaccination GMT was significantly higher for Hong Kong vaccine recipients (1:546 vs 1:260,  $p < 0.01$ ), but MFR (5.5 vs. 4.5,  $p = 0.34$ ), SCP (50% vs. 40%,  $p = 0.31$ ), and SPP (96% vs. 92%,  $P = 0.68$ ) were similar for both vaccine groups ( $n = 50$  per group). For the Saitama strain, post-vaccination GMT (1:116 vs 1:61,  $p = 0.01$ ) and SPP (86% vs. 68%,  $p = 0.03$ ) were significantly higher in Hong Kong vaccine recipients, but MFR (5.3 vs. 4.2,  $p = 0.60$ ) and SCP (50% vs. 46%,  $p = 0.69$ ) were similar for both vaccine groups. Against A/Osaka/188/2017, post-vaccination GMT (1:17 vs 1:9,  $p = 0.20$ ) and MFR (2.7 vs. 1.3,  $p = 0.16$ ) were similar in both vaccine groups, but SCP (32% vs. 4%,  $p < 0.01$ ) and SPP (28% vs. 6%,  $p < 0.01$ ) were significantly higher for Hong Kong vaccine recipients.

**Conclusion:** The switch from the Saitama to the Hong Kong strain resulted in improved or equivalent H3N2 antigen immunogenicity by multiple parameters.

*Keywords: Influenza A(H3N2) vaccine antigenicity immunogenicity*

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10465

## Influenza vaccine effectiveness against hospitalizations in South America during 2013–2017: Are two doses essential for young children? Do older adults receive protection from current and prior vaccination?

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**Introduction and Objectives:** In 2013, the Pan American Health Organization established a multi-site, multi-country network to evaluate influenza vaccine effectiveness (VE). We pooled data from five consecutive seasons in five countries to conduct an analysis of southern hemisphere VE against hospitalization in young children and older adults.

**Methods:** We used a test-negative case-control design to estimate adjusted VE against laboratory-confirmed influenza in hospitalized young children (aged 6–24 months) and older adults (aged ≥60 years) in Argentina, Brazil, Chile, Colombia, and Paraguay. Hospitalized persons with severe acute respiratory infections (SARI) at 48 sentinel hospitals (March 2013–December 2017) were tested for influenza virus infection by rRT-PCR. VE was estimated for young children and older adults using logistic random effects models accounting for cluster (country), adjusting for sex, age (months for children and age categories for adults), year, preexisting conditions, and month of illness onset.

**Results:** We included 7,259 SARI cases (2,389 children and 4,870 adults) in the VE analyses. Among young children, VE against any influenza virus was 32% (95%CI: 15%, 45%) for children who received two doses and 11% (95%CI: -32%, 40%) for those who received one dose in a given season. By type, only VE against B viruses among those who received two doses was significant (52%; 95%CI: 19%, 72%). Among older adults, overall VE against any influenza virus was 43% (95%CI: 29%, 54%); 44% (95%CI: 29%, 55%) for those consecutively vaccinated in two seasons, 39% (95%CI: 21%, 53%) for those vaccinated in the current season only, and 23% (95%CI: 4%, 39%) for those vaccinated in the prior season only.

**Conclusion:** Our results suggest that over the five-year study period, influenza vaccination programs in five South American countries potentially prevented one-third of hospitalizations in young children receiving the recommended two doses and older adults vaccinated in a given season.

**Keywords:** Influenza vaccine effectiveness; children; adults; southern hemisphere; Latin America

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10470

## INFLUENZA VACCINE EFFECTIVENESS IN THE INPATIENT SETTING; EVALUATION OF POTENTIAL BIAS IN THE TEST NEGATIVE DESIGN BY USE OF ALTERNATE CONTROL GROUPS

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### Introduction and Objectives

The test negative design (TND) is used to estimate influenza vaccine effectiveness (VE) and is well validated in outpatient but not inpatient settings, where specific biases may differ. For example, the high prevalence of chronic pulmonary disease among enrollees of inpatient studies may lead to a non-representative control group. TND estimates are biased if influenza vaccine administration is associated with incidence of non-influenza viruses. We evaluated potential biases correlated with inpatient control group selection and effects of influenza vaccination on the incidence of other respiratory viruses.

### Methods

Patients with acute respiratory infection were enrolled from two hospitals during the 2014-15 and 2015-16 influenza seasons and tested for a variety of respiratory viruses. VE against influenza was estimated using three control groups: influenza negative (those who test negative for influenza), other respiratory virus positive (those who test negative for influenza and positive for a different respiratory virus), and pan-negative individuals (those who test negative for all respiratory viruses tested). VE at preventing other common respiratory viruses was also estimated.

### Results

In 2014-15, VE was 41.1% (95% CI: 1.7%, 64.7%) using the influenza negative control group, 24.5% (95% CI: -42.6%, 60.1%) using the other-virus positive group, and 45.8% (95% CI: 5.7%, 68.9%) using the pan-negative group. In 2015-16, VE was 68.7% (95% CI: 44.6%, 82.5%) using the influenza negative control group, 63.1% (95% CI: 25.0%, 82.2%) using the other-virus positive group, and 71.1% (46.2%, 84.8%) using the pan-negative group. Influenza vaccination effectiveness against respiratory syncytial virus, rhinovirus, or all pooled respiratory viruses did not vary significantly from the null value in either season.

### Conclusions

We did not find evidence of substantial bias related to control group selection or vaccine effects on the incidence of non-influenza viruses, supporting the use of the TND in inpatient studies.

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10512

## AN FDA-APPROVED ANTI-PARASITIC AS AN ADJUVANT OF SEASONAL INFLUENZA VACCINE

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### Introduction

Vaccine has been the main strategy for the prevention and control of influenza, but its overall effectiveness is only about 50%. We have previously shown that a topical toll-like receptor 7 agonist, imiquimod, can accelerate and broaden immune response after intradermal influenza vaccine. In this study, we sought to assess the efficacy of an approved anti-parasitic drug (AP) as an influenza vaccine adjuvant.

### Methods

BALB/c mice were immunized with a commercially available trivalent influenza vaccine with or without AP, followed by influenza virus challenge 7 days after vaccination (Figure 1A). Survival and body weight were monitored. Histopathological changes and viral titer were assessed on the mice lungs. Antibody titers were determined using hemagglutination inhibition assay (HAI), microneutralization assay (MN) and enzyme-linked immunosorbent assay (ELISA). Cytokine responses were assessed by RT-qPCR, ELISA and enzyme-linked immunospot (ELISpot).

### Results

Upon lethal virus challenge, survival rate of mice was significantly higher in the AP-adjuvanted influenza vaccine (AP+V) group than that in the non-adjuvanted vaccine (NAV) group (Figure 1B). After virus challenge, the mice body weight loss was significantly reduced in the AP+V group comparing to NAV group (Figure 1C). When compared with NAV group, the AP+V group has less severe lung damage (Figure 1D), although there was no significant difference in the lung viral load (Figure 1E). HAI, MN, total IgG, and IgM titers were higher in the AP+V group than those of NAV group post infection. IgG1 to IgG2a ratio was also significantly higher in the AP+V group (Figure 1F and 1G). The number of antigen-specific IFN- $\gamma$  secreting splenic cells was higher in the AP+V group than the NAV group 7 days after vaccination (Figure 1H).

### Conclusion

AP improved the immunogenicity and effectiveness of influenza vaccine.

*Keywords: Influenza vaccine; adjuvant; lung damage; antibody*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10520

## ASSESSING PARENTAL ATTITUDES TOWARD INFLUENZA VACCINATION

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**Introduction and Objectives:** Seasonal influenza imposes a significant burden on the public, both clinical and economic. Despite the availability of an annual vaccine to prevent influenza infection and reduce disease severity, influenza vaccination rates remain suboptimal. A growing body of research suggests personal experience, perceived effectiveness, and concerns regarding vaccine safety and side effects are the most influential factors in predicting a parent's decision to vaccinate their child and themselves. In order to assess parental attitudes and beliefs regarding the influenza vaccine, a 50-question, mixed-methods survey was developed and optimized for use with Qualtrics.

**Methods:** The Health Belief Model informed survey design and data analysis. Questions were classified into five core concepts: knowledge, barriers, benefits, experience, and severity. Survey participants were solicited from a population of parents whose children had previously participated in a school-based influenza surveillance study (n = 244, 73% response rate). Associations of children's vaccination the prior season with categorical questions were tested using chi-square tests and with numerical or ordered questions were tested using Mann-Whitney tests. P-values were corrected using the Bonferroni method.

**Results:** Concerns about negative side effects, doubting effectiveness, inconvenience, and believing the vaccine is unnecessary for health were barriers negatively associated with parents' decision to vaccinate their children during the 2017-18 flu season (p<0.001). Knowledge that the vaccine is effective in lowering risk, duration, and severity of influenza, self-vaccination as an adult, and recognizing the importance of vaccination as a means to prevent influenza transmission in high-risk populations were positively associated with parents' decision to vaccinate (p<0.001).

**Conclusion:** Understanding the barriers and motivators behind parents' decision to vaccinate provides valuable insight that has the potential to shape vaccine messaging, recommendations, and policy. The motivation to vaccinate in order to prevent influenza transmission in high-risk populations is a novel finding that warrants further investigation.

*Keywords: influenza; vaccine; KABs; attitudes; parents*



Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10542

## VACCINE EFFECTIVENESS IN PARTIALLY AND FULLY VACCINATED CHILDREN IN HONG KONG

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**INTRODUCTION:** To provide adequate protection against influenza, two-doses of influenza vaccination are recommended for children  $\leq 8$  years old who have not previously been vaccinated. We aim to assess the vaccine effectiveness (VE) of partial and full vaccination among young children in Hong Kong.

**METHODS:** Using the test-negative design (TND) study we enrolled children 6 months to 8 years of age admitted to four hospitals and recorded laboratory testing results for influenza and vaccination history. Fully vaccinated children included those vaccinated with two doses, or one dose if they had received vaccination in previous years, with the most recent dose within 6 months and  $\geq 14$  days before admission. Partially vaccinated children included those who should have received two doses but had only received one dose within 6 months and  $\geq 14$  days before admission. We estimated VE overall and by age and influenza type/subtype using conditional logistic regression models matching on epidemiological week and adjusting for age and age squared.

**RESULTS:** Of 23,187 children admitted during the influenza seasons 2011/12 to 2018/19, 3,852 (16.6%) tested positive for influenza A or B while 19,335 (83.4%) tested negative. Of the influenza-negative controls, 1,924 (10.0%) were fully vaccinated, while 373 (1.9%) were partially vaccinated. Overall VE estimates among fully and partially vaccinated children were 73% (95% CI: 69%, 77%) and 31% (8%, 48%) respectively. Overall VE for partial vaccination in children 6 months-2 years, 3-5 years and 6-8 years of age were 18% (-20%, 43%), 47% (13%, 67%) and 36% (-72%, 76%), respectively. Partial vaccination was only significantly protective against influenza A(H1N1) in children 3-5 years of age (56%, 95% CI: 8%, 79%).

**CONCLUSION:** Partial vaccination was significantly less effective than full vaccination in children  $\leq 8$  years. Our study supports the current recommendation of two-doses vaccine for young children.

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10584

## THE VICTORIA CLADE 1A VACCINE STRAIN PROVIDES PROTECTION IN SUBSEQUENT INFLUENZA SEASONS DESPITE LINEAGE MISMATCH

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### Introduction and objectives

In the 2010/11 and 2017/18 influenza seasons, B-Yamagata comprised 80% and 97%, respectively, of all influenza B infections in Denmark. Only the trivalent influenza vaccine containing the influenza B virus of the Victoria lineage and genetic clade 1A was administered, and this vaccine strain was unchanged from the previous season. We explored the duration of protection within and from one season to the next of the Victoria clade 1A vaccine strain against influenza B Yamagata.

### Methods

Individuals aged  $\geq 65$  years in season 2010/11 and 2017/18 were included. The Danish vaccination register holds real-time influenza vaccine records at individual level. Laboratory confirmed influenza B virus infections were diagnosed using RT-PCR. Influenza B virus lineage was determined by real-time duplex RT-PCR differentiating between the Yamagata and Victoria lineage. Influenza vaccine effectiveness (IVE) was estimated using test-negative case-control design (VE=1-OR). Within the 2017/18 season, IVE as a function of time from vaccination was estimated using a quadratic spline model.

### Results

Similar IVE against influenza B was observed among individuals vaccinated in current only or current and previous season compared to those not vaccinated in any of the two seasons 38% (95%CI:29;45) and 32% (95%CI:27;37%), respectively in 2017/18 and 56% (95%CI:5;80) and 54% (95%CI:32;69), respectively in 2010/11. In contrast, no IVE was observed if vaccinated in current and previous seasons compared with those vaccinated in previous season only, -2% (95%CI:-23;16) in 2017/18 and 6% (95%CI: -88;54) in 2010/11. The estimated IVE against influenza B showed no signs of waning immunity during the 2017/18 season.

### Conclusions

Unchanged Victoria clade 1A vaccine strain provided protection against B-Yamagata. The lack of waning immunity towards influenza B support our findings that no additional effect against influenza B was observed by subsequent vaccination with Victoria clade 1A in two seasons.

*Keywords: influenza vaccine effectiveness; influenza B; duration of protection; lineage mismatch*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10654

## REDUCED VACCINE EFFECTIVENESS RESULTING FROM CANDIDATE INFLUENZA VIRUS VARIATION DURING EGG-BASED MANUFACTURE: LITERATURE REVIEW AND EXPERT SURVEY

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### INTRODUCTION AND OBJECTIVE:

Influenza is associated with significant morbidity and mortality despite influenza vaccination programmes. Reduced vaccine effectiveness from candidate virus variation due to manufacturing in eggs is the subject of debate, with direct evidence slow to emerge. The objective was to review evidence and expert opinion supporting a mechanistic basis for reduced vaccine effectiveness due to the egg-based manufacturing.

### METHODS:

Ten European influenza specialists (three virologists, three public health and four primary care physicians) were recruited to the expert panel.

The objective was deconstructed into the following component principles: 1) presence of antigenic drift (virus variation), 2) stages in egg-based influenza vaccine manufacture and candidate virus vaccine selection, 3) egg-adaptation changes in influenza vaccine manufacturing process and 4) stage(s) in the manufacturing process most likely to impact influenza vaccine effectiveness. Each was examined, in series, using a two-stage online questionnaire. The first stage involved generating a list of supporting references for each component principle from a literature search and the expert panel independently. In the second stage, a summary of each reference was circulated amongst the experts who rated their agreement (5-point Likert scale) that each reference supported the component principle. Finally, the panel were asked if they agreed the evidence reviewed, as a whole, supported a mechanistic basis for reduced vaccine effectiveness due to egg-based manufacturing.

### RESULTS:

Component principles 1-4 were rated as being supported by strong, or very strong evidence in: 95/123 (77%), 22/27 (82%), 19/21 (90%) and 7/10(70%) of all references identified for each principle respectively. 10/10 (100%) panellists agreed with the mechanistic basis of reduced vaccine effectiveness.

### CONCLUSIONS:

On reviewing the evidence, supporting the component principles, experts were in unanimous agreement that there is a mechanistic basis for reduced vaccine effectiveness resulting from candidate influenza virus variation due to egg-based manufacturing, particularly in A/H3N2.

*Keywords: influenza, egg-adaptation, vaccine, manufacturing, effectiveness*

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10674

## INFLUENZA ILLNESS AND HOSPITALIZATIONS AVERTED BY INFLUENZA VACCINATION IN CHILDREN AGED 6 MONTHS-59 MONTHS IN SUZHOU, CHINA, 2011-2016

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**Introduction and Objectives:** Estimates of vaccine-averted influenza-associated illnesses are unknown in China.

**Method:** We estimated averted influenza-associated hospitalizations, influenza-associated medically-attended influenza-like illnesses (MA ILI) and all influenza-associated ILI from October 2011–September 2016 in children aged 6–59 months in Suzhou. We estimated influenza-associated hospitalizations and MA illness using surveillance data from Suzhou University Affiliated Children's Hospital (SCH), which detected laboratory-confirmed influenza among children 6–59 months. Non-medically attended illness was determined through 7 health utilization surveys conducted in the SCH catchment area in 2011–2014. We obtained influenza vaccine coverage and population estimates in Suzhou from Expanded Program on Immunization data and vaccine effectiveness estimates for children 6–59 months from field and published studies. We calculated the number of averted cases as the difference between the expected burden if there were no vaccine and the observed burden with vaccination.

**Result:** In ~250,000 children, influenza vaccination prevented 403 (95% confidence interval (CI): 175-607) influenza-associated hospitalizations over five years (4% of expected hospitalizations) and 7,267 (95%CI: 3,268-10,317) ILI cases (6% of expected), of which 6,045 (95%CI: 2,732-8,578) were medically-attended (6% of expected). Most cases were prevented in 2011–2012 when vaccination coverage was highest (19.6%): 303 (95% CI:138-402) cases of influenza-associated hospitalizations (9% of expected) and 6,060 (95% CI: 2,777-8,026) ILI cases (10% of expected), of which 5,043 (95%CI: 2,323-6,669) were medically-attended (10% of expected). The fewest cases were prevented in 2015–2016 when vaccination coverage was lowest (5.6%): 10 (95% CI:0-32) influenza-associated hospitalizations (0.6% of expected) and 124 (95% CI: 0-442) ILI cases (0.7% of expected), of which 103 (95% CI:0-365) were medically-attended (0.7% of expected).

**Conclusion:** As vaccine coverage decreased due to changes in vaccine deployment, fewer influenza-associated hospitalization and ILI cases were averted. Increasing influenza vaccine coverage in children 6–59 months should be prioritized to reduce influenza-associated illness.

*Keywords: Influenza, vaccination, Averted, Effectiveness*

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 10676

## EFFICACY AND EFFECTIVENESS OF HIGH-DOSE INFLUENZA VACCINE FOR OLDER ADULTS BY CIRCULATING STRAIN AND ANTIGENIC MATCH: A SYSTEMATIC REVIEW AND META-ANALYSIS

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**Introduction and Objectives:** Influenza vaccine efficacy/effectiveness can vary from season to season due in part to the dominant circulating strains and antigenic matching. This study reviews the relative vaccine efficacy/effectiveness (rVE) of high-dose inactivated trivalent influenza vaccine (HD-IIV3) compared to standard-dose influenza vaccines (SD-IIV3) in adults  $\geq 65$  years against influenza-associated outcomes across all influenza seasons, during seasons where A/H3N2 or A/H1N1 strains predominantly circulated, and where there was an antigenic match or mismatch of the vaccine and circulating strains.

**Methods:** A systematic review was conducted for studies assessing the rVE of HD-IIV3 against probable/laboratory-confirmed influenza-like illness (ILI), hospital admissions, and death in adults  $\geq 65$  years. Results from individual seasons were extracted from the identified studies, and surveillance data from each season were used to determine the dominant circulating strains and antigenic match. Results were then stratified based on clinical outcomes and seasonal characteristics and meta-analyzed to estimate pooled rVEs of HD-IIV3.

**Results:** 11 studies were meta-analyzed after screening 1,018 studies, providing data on 9 consecutive influenza seasons and over 12 million individuals receiving HD-IIV3. Across all influenza seasons, HD-IIV3 demonstrated improved protection against ILI compared to SD-IIV3 (rVE=15.9%, 95% CI: 4.1-26.3%). HD-IIV3 was also more effective at preventing hospital admissions from all-causes (rVE=8.4%, 95% CI: 5.7-11.0%), as well as influenza (rVE=16.1%, 95% CI: 7.4-24.1%), pneumonia (rVE=27.3%, 95% CI: 15.3-37.6%), pneumonia/influenza (rVE=13.4%, 95% CI: 7.3-19.2%) and cardiorespiratory events (rVE=17.9%, 95% CI: 15.0-20.8%). Some numerical differences were observed in the pooled rVE of outcomes in matched and mismatched seasons and in seasons where A/H3N2 or A/H1N1 strains were predominantly circulating (table 1).

**Conclusions:** Evidence over 9 influenza seasons suggest that HD-IIV3 is consistently more effective than SD-IIV3 at reducing the clinical outcomes associated with influenza infection irrespective of circulating strain and antigenic match.

*This study was funded by Sanofi Pasteur*

*Keywords: influenza vaccine; high dose; vaccine effectiveness; vaccine efficacy; elderly*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10746

## Impact of repeated influenza vaccination on the vaccine effectiveness among the elderly in the country with high annual vaccine uptake rates during A/H3N2 epidemic

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**Introduction.** Annually, about 80% of the Korean elderly aged  $\geq 65$  years receive influenza vaccination. However during A/H3N2 epidemics, repeated annual vaccination has been suggested as an important factor of poor influenza vaccine effectiveness (VE) with conflicting results. The impact might be variable according to the age, vaccine-matching degree and annual vaccine uptake rates.

**Methods.** During poorly vaccine-matched A/H3N2-dominant influenza season from October 2014 to May 2015, we comparatively evaluated the VE (repeated vs. current season only) against laboratory-confirmed influenza, pneumonia and hospitalization in the elderly aged  $\geq 65$  years with influenza-like illness (ILI). Using a hospital-based influenza morbidity and mortality (HIMM) registry, demographic and clinical data were collected prospectively. Vaccination status of prior and current seasons was verified using immunization registry data of Korean Centers for Disease Control and Prevention.

**Results.** During study periods, 1039 ILI cases were analyzed, including 535 influenza A and 118 influenza B cases. Among enrolled cases, 65.1% (676 cases) received influenza vaccination in two consecutive seasons, and 35.4% (365 cases) got vaccinated in three consecutive seasons. The adjusted VE was indistinguishable between repeated vaccination versus vaccination in current season only: influenza A, -25% (-91 to 17%) vs. -18% (-99 to 30%); influenza B, 8% (-75 to 52%) vs. 22% (-80 to 66%); pneumonia, 10% (-48 to 46%) vs. 10% (-68 to 53%); and hospitalization, 0% (-51 to 33%) vs. 14% (-51 to 33%).

**Conclusion.** During influenza A/H3N2 epidemic, poor influenza vaccine effectiveness might be more pronounced among the elderly in the country with high annual vaccine uptake rate. Contrary to the young ages, the negative interference from prior seasonal vaccination might not be distinct in the elderly who had exposed to diverse viruses.

**Keywords:** Influenza vaccine; vaccine effectiveness; repeated vaccination; elderly people; H3N2

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 10786

## AVIAN INFLUENZA A(H7N9) IMMUNIZATION PROVIDES BROAD CROSS-PROTECTION AGAINST HETEROLOGOUS VIRUS CHALLENGE IN FERRETS

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### Introduction

Low pathogenicity avian influenza (LPAI) A(H7N9) viruses caused annual epidemic waves of human infections in China since emerging in 2013. The fifth wave marked an increase of human infections with antigenically distinct viruses, a wider geographical distribution, and emergence of a highly pathogenic avian (HPAI) A(H7N9) virus.

### Methods

Three antigenically distinct candidate vaccine viruses (CVVs) were developed as reverse genetic reassortants with PR8 internal gene segments and HA and NA gene segments targeting viruses from the 1<sup>st</sup> and 5<sup>th</sup> waves, including both LPAI and HPAI viruses. Ferrets were immunized with a live, intranasal inoculation of each CVV and challenged with both homologous and heterologous wild-type viruses. Nasal washes were collected for up to 9 days post-challenge (dpc) and tissues were collected at three dpc to determine infectivity.

### Results

Ferrets immunized with LPAI-like CVVs seroconverted by 14 days post-vaccination (dpv) and, prior to challenge at 28 dpv, had HI titers ranging from 80-640. Lower pre-challenge antibody titers (40-80, on average) were detected in ferrets immunized with the HPAI-like CVV. Challenge of mock-immunized ferrets with LPAI and HPAI viruses resulted in significantly higher nasal wash titers (up to 4-fold higher log<sub>10</sub> vRNA copies/mL) compared to immunized ferrets, with infections persisting until at least 9 dpc and severe morbidity and mortality in HPAI virus infected animals. In contrast, all immunized ferrets challenged with LPAI or HPAI viruses showed little morbidity and 100% survival irrespective of homologous or heterologous vaccination.

### Conclusion

While vaccination provided good protection against homologous virus challenge, heterologous challenge illustrated that broad cross-protection against antigenically divergent A(H7N9) viruses could be achieved. Despite lower antibody titers in the HPAI-like vaccinated group, these ferrets were protected from heterologous and lethal virus challenge. Collectively, these findings highlight the breadth of coverage possible with currently available A(H7N9) CVVs against diverse A(H7N9) viruses.

*Keywords: H7N9, candidate vaccine virus,*

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## Parallel assessment of enhanced seasonal influenza vaccines in mice finds an Ad-vantage for FluAd for antibody and cellular responses

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**Introduction:** The vaccination strategy with standard inactivated influenza vaccine (IIV) may provide sub-optimal protection in locations with prolonged influenza virus exposure or in immune-compromised subjects. Enhanced influenza vaccines including the MF59-adjuvanted vaccine (FluAd), Recombinant HA-protein vaccine (FluBlok) and High-Dose vaccine (FluZone), may increase the durability and quality of vaccine mediated protection.

**Objectives:** To investigate the protective potential and mechanism of action of enhanced vaccines in parallel a mouse vaccination and challenge model was used in order to assess vaccine superiority.

**Methods:** BALB/c mice were vaccinated with either one of the three enhanced IIV or with the standard IIV, and subsequently challenged with influenza virus for homologous (H1N1) or heterologous (H3N2-1968) protection. Mice were monitored for survival, weight loss, and immune responses, from local and peripheral sites assessed at acute and memory timepoints.

**Results:** The MF59-adjuvanted vaccine induced a higher and earlier induction of a Germinal Centre reaction and B memory activation versus other vaccine groups. As a result, mice that received the MF59-adjuvanted vaccine displayed an earlier immune response during lethal influenza challenge with higher magnitude and breadth for cellular and antibody responses. This was especially evident for high avidity anti-HA, anti-HA-Stem, and anti-NP antibodies, in conjunction with increased protection against the heterologous H3N2-1968 virus. In addition, High-Dose IIV reduced lung inflammation and also showed early recruitment of IFN $\gamma$ <sup>+</sup> CD4<sup>+</sup> T cells. Whilst the Recombinant HA-protein vaccine did not show immune superiority over the standard vaccine in our mouse model.

**Conclusion:** The MF59-Adjuvanted vaccine induces a higher increase in the quantity, breadth and quality of antibody production via an enhanced and earlier germinal-centre reaction compared to the standard IIV vaccine. This early and broadly reactive antibody response may be advantageous in the case of a vaccine mismatch, leading to prioritization of FluAd in locations with frequent influenza epidemics.

*Keywords: vaccines; adjuvant; antibodies; avidity; memory*



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## INFLUENZA VACCINE EFFECTIVENESS AGAINST A(H3N2) HOSPITALIZATION IN THE 2016-2017 AND 2017-2018 SEASONS: RESULTS FROM THE U.S. HAIVEN STUDY

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**Introduction.** The Hospitalized Adult Influenza Vaccine Effectiveness Network (HAIVEN) study conducts active surveillance in four sites to evaluate VE in prevention of influenza-associated hospitalization annually in the United States. The 2016-2017 and 2017-2018 influenza seasons were notable for the widespread circulation of and high numbers of hospitalizations for influenza A(H3N2).

**Methods.** We conducted a multi-center, test-negative evaluation of VE against influenza A(H3N2) hospitalization. Adults with acute respiratory illness meeting a standard case definition were enrolled from ten hospitals at four study sites. Nasal-throat swabs were tested by RT-PCR for influenza. Vaccination, including product, medical history, demographics and clinical characteristics were obtained from surveys and electronic medical records. Vaccine effectiveness estimates were calculated from GEE models adjusted for multiple enrollments, hospital, age, calendar time, self-reported health, previous hospitalization, and day of illness.

**Results.** A total of 2,065 participants from 2016-2017 and 3,524 participants from 2017-2018 were included. In 2017-2018, overall influenza prevalence was higher (25.8% versus 17.8%) and vaccination was less frequent (66.7% versus 72.4%) compared to the 2016-2017 season. Adjusted VE against A(H3N2) was 18.0% (95% C.I. -9.2, 38.3; 309 total infections) in 2016-2017 and 23.8% (95% C.I. 5.3, 38.7; 530 total infections) in 2017-2018. In 2017-2018, 67 (2%) of participants received a cell-based vaccine; the VE point estimate against A(H3N2) for this vaccine was higher than for egg-based vaccines (43.0% (95% C.I. -36.1, 76.2) versus 24.6% (95% C.I. 4.7, 40.4), respectively), but was not statistically significant.

**Conclusions.** Recent VE for H3N2 has been lower than previously reported (seasons 2010-2013: 42-54%). Similar VE for the A/Hong Kong/4801/2014 vaccine virus in both A(H3N2) seasons emphasizes concerns for continued changes in H3N2 antigenic epitopes, including changes that may impact glycosylation and ultimately reduce VE. Although not significantly different, the estimate for the cell-based vaccine was higher than for the egg-based vaccine.

**Keywords:** vaccine effectiveness, influenza A(H3N2), hospitalization

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## The effect of influenza vaccination history on changes in hemagglutination inhibition titers following receipt of the 2015/16 influenza vaccine in older adults in Hong Kong

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**Introduction and Objectives:** Immune responses to influenza vaccination can be weaker in older adults than in other age groups. Based on the antigenic distance hypothesis, we hypothesized that antibody responses would be particularly weak among repeat vaccinees when the current and prior season vaccine components are the same.

**Methods:** An observational study was conducted among 827 older adults aged  $\geq 75$  years in Hong Kong. Sera were collected immediately before and one month after receipt of the 2015/16 quadrivalent inactivated influenza vaccine. We measured antibody titers with the haemagglutination inhibition assay, and compared mean fold rise in titers from pre- to post-vaccination, and the proportion with post-vaccination titer  $\geq 40$ .

**Results:** Participants who reported receipt of vaccination during either of the previous two years had lower mean fold rise against all strains compared to those who were not. Mean fold rises for A(H3N2) and B/Yamagata were particularly weak following repeated vaccination with the same vaccine strain, but we did not generally find significant differences in the proportion of participants with post-vaccination titers  $\geq 40$ .

**Conclusion:** Overall, we found that reduced antibody responses in repeat vaccinees were particularly reduced among older adults with prior vaccination against the same vaccine strains, consistent with the antigenic distance hypothesis.

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## SEASONAL INFLUENZA VACCINE EFFECTIVENESS IN PATIENTS WITH UNDERLYING MEDICAL CONDITIONS AND AGED 65+ BETWEEN 2015-2018 IN LITHUANIA

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**Introduction and Objectives:** Due to lack of knowledge about seasonal influenza vaccine effectiveness (SIVE) against laboratory-confirmed influenza in patients with underlying conditions and aged 65+, a study to measure SIVE in hospitalised persons due to severe acute respiratory infection (SARI) in Lithuania during the 2015-2018 was conducted. The co-circulation of other respiratory viruses was also described.

**Methods:** A test-negative case-control study was performed, with cases positive, and controls negative for laboratory-confirmed influenza. Crude SIVE and its 95% confidence intervals (95%CI) were calculated as (1-odds ratio)\*100%. The analysis was not adjusted for sociodemographic and clinical characteristics, as they did not change SIVE estimate by ≥10%. Influenza and other respiratory pathogens were detected with multiplex RT-PCR.

**Results:** Overall 586 participants were recruited, 55(9.4%) of whom were vaccinated against influenza ≥2 weeks before the onset of SARI symptoms. Influenza was confirmed in 293(50%) subjects. Influenza A(H1N1)pdm09 was predominant in 2015-2016(69.4%), influenza A(H3N2) dominated in 2016-2017(93.2%), while influenza B/Yamagata was detected most often in 2017-2018 (78.8%).

SIVE in 2015-2016 influenza season was 57%(95%CI:-41%,87%), and 70%(95%CI :-43%,94%) against any influenza and influenza A(H1N1)pdm09 respectively.

SIVE in 2016-2017 influenza season was 42%(95%CI: -37%,76%) and 34%(95%CI:-58%,72%) against any influenza and influenza A(H3N2) respectively.

SIVE in 2017-2018 influenza season was 62%(95%CI:-27%,88%) and 56%(95%CI:-43%,87%) against any influenza and influenza B/Yamagata respectively.

All in all, respiratory pathogens found were similar in all the seasons: RSV 23(3.9%), rhinovirus 12(2.1%), metapneumovirus 13(2.2%), coronavirus 9(1.5%), adenovirus 9(1.5%), parainfluenza 2(0.3%).

**Conclusions:** Half of the hospitalized SARI cases were confirmed with influenza, which shows high influenza disease burden in this population. Although the results should be interpreted with caution due to broad confidence intervals, the point estimates suggest moderate SIVE in 2015-2016 and 2017-2018, and low SIVE in 2016-2017.

Co-circulation of the other viruses tested was low and did not differ through the seasons.

**Keywords:** Influenza; Vaccine, Effectiveness, Lithuania

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## EVALUATING THE IMPACT OF STATIN USE ON INFLUENZA INFECTION AND INFLUENZA VACCINE EFFECTIVENESS IN OLDER ADULTS

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**Introduction and Objectives:** Statins have immunomodulatory properties that might mitigate the effects of influenza infection but could also reduce influenza vaccine effectiveness (VE). The objectives of this study were to: (1) determine the association between statin use and laboratory-confirmed influenza; and (2) evaluate whether statins are an effect modifier of VE in older adults.

**Methods:** We studied adults aged >65 years living in Ontario, Canada during the 2010-11 to 2015-16 influenza seasons. We linked laboratory and health administrative databases, including data on physician- and pharmacist-administered influenza vaccines and statin prescriptions. Statin users were defined as those with continuous exposure between October 1<sup>st</sup> of the enrolment season up to the specimen collection date, and we excluded those with periodic statin use. We used the test-negative design and logistic regression to estimate odds ratios (OR) for influenza associated with statin use separately among vaccinated and unvaccinated individuals, and VE in statin users and non-users.

**Results:** Among 58,304 older adults tested for influenza, 22,820 were continuous statin users, 27,853 were non-users, and 7,631 were excluded because of periodic statin use. Statin users were older, had more comorbidities (particularly cardiovascular conditions, chronic kidney disease, and diabetes), and used more healthcare services compared to non-users. Both the proportions vaccinated and influenza-positive were higher among statin users than non-users (vaccinated: 60% vs. 51%; influenza-positive: 21% vs. 19%). Statin use was associated with increased odds of influenza infection among both vaccinated (OR=1.28; 95%CI, 1.20-1.37) and unvaccinated (OR=1.19; 95%CI, 1.12-1.28) individuals. We observed no difference in VE between statin users (VE=19%; 95%CI, 13%-24%) and non-users (VE=24%; 95%CI, 19%-29%) (test for interaction p-value=0.21).

**Conclusion:** Statin use was associated with increased odds of influenza infection and was not a significant effect modifier of influenza VE.

**Keywords:** influenza; statins; vaccine effectiveness

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## Psychological determinants of seasonal influenza vaccination uptake among healthcare workers

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**Introduction and Objectives:** Although annual seasonal influenza vaccination is recommended for healthcare workers (HCWs), vaccination uptake remained suboptimal in this priority group. This study aimed to examine the psychosocial determinants of influenza vaccination among HCWs in Hong Kong using a longitudinal study design based on behavioral change theories.

**Methods:** Participants were invited to complete a survey before the influenza vaccination program to measure their baseline perceptions and vaccination intention, and a follow-up survey after the vaccination program to measure the actual vaccination uptake. Structural equation modelling was used to examine factors associated with HCWs' decision on influenza vaccination.

**Results:** Of the 845 participants who completed the follow-up survey, 43% showed intention to vaccinate and 31% reported actual receipt of vaccination. Attitude towards influenza vaccination ( $b=0.69$ ), perceived susceptibility to influenza virus infection ( $b=0.34$ ), anticipated regret for not vaccinating ( $b=0.31$ ), and cues to action ( $b=0.29$ ) were strongly associated with vaccination intention which directly predicted vaccination uptake ( $b=0.82$ ). Norms were observed to have indirect association to vaccination intention through the mediation of attitude towards influenza vaccination ( $\beta=0.63$ ). Self-efficacy was associated with the actual receipt of influenza vaccination ( $\beta=0.13$ ) but not vaccination intention. The model explained 85% of the variance in HCWs' intention to receive influenza vaccination during the next season and 70% of the variance in the actual receipt of influenza vaccination.

**Conclusion:** Attitude towards influenza vaccination was the strongest predictor of HCWs' intention and actual receipt of influenza vaccination. Public health interventions targeting norms may be an important strategy to shape HCW's attitudes towards influenza vaccination and their subsequent decision-making for influenza vaccination.

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## Best use of a limited vaccine supply in a pandemic: what does "best" actually mean?

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### Introduction

Public health decision-makers face many uncertainties in pandemic response, including identification of risk groups, and prediction of impact. Pandemic plans need to be flexible to accommodate these uncertainties, while providing pragmatic advice on strategies to achieve 'best' outcomes, particularly when interventions (like vaccines) are in short initial supply. This study aimed to identify (a) overarching objectives of the public health response; and (b) given a limited vaccine supply, how decisions can be made on which groups should receive priority vaccination, for different impact scenarios.

### Methods

We used an existing model of influenza in the Australian population to simulate pandemic scenarios of varying transmissibility and severity, and evaluated the relative merits of two vaccination strategies:

1. Direct protection of groups anticipated to experience severe disease; and
2. Indirect protection via a transmission-reduction strategy focused on primary-school children.

Intervention impact was measured as reductions in both overall harms (hospitalisations, ICU admissions, and deaths) and inequity of health outcomes by risk group. The influence of vaccine factors, including timeliness of availability and schedule requirement (one versus two dose), on achievable outcomes was also evaluated.

### Results

In low transmission scenarios, indirect protection was most effective at reducing all harms, while both vaccine strategies reduced health inequities. In all other scenarios the two strategies had similar effects, and could not reduce inequities. Vaccine timeliness was critical for intervention impact. Administering only one dose of a two-dose vaccine schedule increased population coverage, but diluted benefit, preventing less harms overall.

### Conclusions

Defining the objectives of pandemic response is critical when preparing for uncertain events. Our approach has defined strategies most likely to achieve desired outcomes, for different population, pandemic, and vaccine scenarios. The next phase of this work will engage Citizen Juries to consider the relative merits and potential problems of a number of vaccination strategies.

*Keywords: pandemic; pandemic response; vaccine; impact; targeted interventions*

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## INFLUENZA VACCINE EFFECTIVENESS: ADDRESSING MISCLASSIFICATION BIAS IN THE TEST-NEGATIVE STUDY DESIGN

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**INTRODUCTION:** Case misclassification associated with delay in seeking care could bias vaccine effectiveness (VE) estimates in test-negative design (TND) studies. Influenza viral shedding in infected persons usually lasts 4-5 days. However, patients may stop shedding virus by the time they seek care, resulting in false negative test results. Controlling delay since illness onset is often attempted by restriction or statistical adjustment. We examined the validity of correcting for case misclassification using several different analytical approaches.

**METHODS:** We simulated a cohort with 4 age groups 0-5, 6-17, 18-65 and 65+ at risk of influenza and non-influenza infections, then randomly drew cases and controls among those who seek care. Simulation parameters were based on the Hong Kong population profile and from the literature. We assumed 35% of cases who sought care delayed presentation. The proportion of cases delayed, "true" VE for each age group (50%, 43%, 35% and 24%), and test sensitivity were varied. We conducted 100 simulations and compared mean VE using: logistic regression, both unadjusted and adjusted for delay since onset, restriction (excluding delayed cases), and using Bayesian models with flat and Cauchy priors on the odds of exposure among cases versus controls.

**RESULTS:** Misclassification bias by delay since onset was greatest in both unadjusted and adjusted logistic regression models and in the Bayesian model with a flat prior. However, restriction and the Bayesian model with a Cauchy prior minimised the degree of bias. When there was a high proportion of cases delaying seeking care, adjusting for delay greatly underestimated VE, whereas restriction may over- or underestimate VE.

**CONCLUSION:** Case misclassification bias VE. Caution should be taken in analyses to produce reliable VE estimates. While Bayesian approaches were able to reproduce true VE, those reluctant to use strong priors could consider using restriction instead.

*Keywords: vaccine effectiveness; test-negative design; misclassification*

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## STRONG INCREASE IN INFLUENZA VACCINE COVERAGE IN NORWAY, 2014-2018

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### Introduction and objectives

In Norway, seasonal influenza vaccination is recommended to target groups, including children and adults with underlying chronic diseases, all persons above 64 years of age, health care personnel, household contacts of immunosuppressed patients and pig farmers/others who have regular contact with live pigs. Estimations show that the target population for vaccination in Norway count 1.9 million. Vaccination coverage decreased in the years following the 2009 pandemic and increased coverage has been a priority for the Norwegian health authorities. Several measures have been implemented over the last years in order to increase the vaccination coverage, including an improved and available knowledge base on influenza vaccination, text messages to the elderly and local campaigns in hospitals to increase awareness. We aimed to measure the impact of these measures on vaccine sales.

### Methods

For the period 2014-2018, we retrieved national data from the Norwegian Institute of Public Health (NIPH) on influenza vaccine distribution. NIPH are responsible for providing vaccines to the National Influenza Vaccination Programme. The NIPH and other wholesalers also supply vaccines for vaccination outside the programme. Indication for vaccination is not reported to the NIPH.

### Results

The number of vaccines distributed has increased annually since 2014, from 479 437 doses in the 2014/15 influenza season to 885 144 doses in the 2018/19-season, which is the highest number ever recorded in Norway. Most of the increase in vaccine distribution occurred over the last three seasons, with an increase of about 60 % in total number of doses distributed.

### Conclusion

Seasonal influenza vaccination coverage is increasing in Norway. Continued work is needed in order to reach the target of at least 75% coverage in the target groups.



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## Assessment of H7N9 vaccine immunity against highly pathogenic A(H7N9) infection in ferret model

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### Introduction

The novel A(H7N9) influenza viruses that have been circulating in China since 2013 remain a threat to global public health. The large number of human infections, geographical expansion and emerging highly pathogenic avian influenza (HPAI) A(H7N9) strains in humans have highlighted the fifth epidemic wave and urged continuous investigation on effective vaccines. In this study, we generated low-pathogenic candidate vaccine viruses (CVVs) from the HPAI, and assessed the protection of the immunity elicited by the CVVs in ferret model.

### Methods

The low pathogenic reassortant CVVs were generated by reverse genetics and propagated in eggs. Ferrets were immunized (i.n.) with one dose of CVV, and then challenged by HPAI A/Guangdong/17SF003/2016 wt ( $5 \times 10^7$  PFU) at day 14 post immunization, followed by close observation of body weight, temperature, and illness signs for two weeks. Post immunization and post challenge serum specific antibody titers were determined by hemagglutinin inhibition (HI) and microneutralization (MN) assays.

### Results

Intranasal inoculation with live H7N9 CVVs in ferrets induced specific antibody response against the parental HPAI in two weeks, while no obvious illness signs were observed. Compared with the naïve control ferrets on that the HPAI caused severe illness signs including neural disorders with two thirds of mortality, none of the immunized ferrets showed obvious illness signs after challenge.

### Conclusion

The ferret study demonstrated that the low pathogenic H7N9 reassortants induced specific immune response that effectively protected the immunized ferrets from lethal HPAI infection.

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## VACCINE EFFECTIVENESS AGAINST THE 2018-19 BIMODAL EPIDEMIC OF INFLUENZA A(H1N1)PDM09 AND A(H3N2) IN CANADA

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### INTRODUCTION/OBJECTIVES

The 2018-19 influenza season in Canada has been characterised by two successive waves of influenza A: an early primary epidemic due to A(H1N1)pdm09 followed by a secondary late-season wave of A(H3N2), with a paucity of influenza B. Vaccine effectiveness (VE) estimates for the 2018-19 northern hemisphere influenza vaccine are reported from Canada's community-based Sentinel Practitioner Surveillance Network, together with the clade distribution of contributing viruses.

### METHODS

VE was derived using a test-negative design comparing the adjusted odds ratio for influenza test-positivity among vaccinated and unvaccinated participants. Nasal/nasopharyngeal specimens collected between November 2018 and March 2019 from patients presenting within 7 days of influenza-like illness onset were tested for influenza by RT-PCR. Viruses were characterised and clade distribution assessed by Sanger sequencing of the haemagglutinin gene and phylogenetic analysis.

### RESULTS

Among 2,863 eligible specimens, 1,283(45%) tested influenza-positive (98% influenza A); 80% of subtyped influenza A viruses were A(H1N1)pdm09. Sequence information was available for 369/946(39%) A(H1N1)pdm09 and 118/201(59%) A(H3N2) viruses. All sequenced A(H1N1)pdm09 viruses belonged to clade 6B.1A; however, genetic heterogeneity among circulating viruses was observed. The majority (65%) of A(H3N2) viruses belonged to subclade 3C.2a1b, with a minority (16%) belonging to clade 3C.3a.

VE against any influenza, foremost driven by A(H1N1)pdm09 viruses, was 61% (95%CI:53-69%), and for A(H1N1)pdm09 alone was 69% (95%CI:60-76%). This substantial protection against A(H1N1)pdm09 was observed in all age groups. Conversely, little or no vaccine protection against medically-attended outpatient A(H3N2) illness has been observed (23%; 95%CI:-9-46), particularly among working-age adults (-16%; 95%CI:-76-23%).

### CONCLUSIONS

Late-season estimates from Canada for the 2018-19 influenza season indicate substantial VE of ~70% against predominant A(H1N1)pdm09 viruses but low to nil VE against late-season A(H3N2) viruses. Ongoing monitoring is warranted for further evolution in circulating variants and potential impact on vaccine protection. Findings will be updated, and clade-specific analyses explored, in end-of-season analysis.

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## IDENTIFYING AND ESTABLISHING IMMUNE CORRELATES OF PROTECTION FOR NEXT GENERATION INFLUENZA VACCINES – A REVIEW OF EPIDEMIOLOGICAL METHODS

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**Background:** Immune correlates of protection (CoPs) are important in the development and evaluation of next generation influenza vaccines. However, there are few established CoPs for current influenza vaccines. In this study, we aim to review the common analytical frameworks to establish CoPs when evaluating an immune marker as a potential CoP for next generation influenza vaccines.

**Methods:** We reviewed the literature on analytical frameworks to establish immune CoP for vaccines and documented the common methods used to identify and establish CoP for influenza vaccines. We recorded immune CoPs that have been identified and established for influenza vaccines, as well as CoPs under investigation for next generation and universal influenza vaccines.

**Results:** Statistical methods that assess the association between an immune marker and influenza vaccination, or an immune marker and clinical endpoints (e.g. laboratory-confirmed influenza virus infection) are most commonly used to identify and establish an immune marker's potential as a CoP. However, due to increasing awareness of the need to distinguish between statistical associations and causal relationships, causal inference approaches have recently been used to assess the causal contribution of existing CoPs for influenza vaccines towards vaccine-induced protection against specific clinical endpoints. Systems biology and machine learning methods have also been applied to identify novel CoPs for influenza vaccines.

**Conclusions:** Statistical methods to assess the association between individual immune markers and clinical endpoints are still the most common approach for the identification and establishment of CoPs. Less consideration is given to whether the CoPs are measuring part of the immune system which provides protection, which has been referred to by some authors as "mechanistic CoPs". The standardization of terminology used to describe CoPs with and without causal contributions to vaccine-induced protection could be a first step to identify basic characteristics of appropriate CoPs for next-generation influenza vaccines.

*Keywords: Influenza; vaccines; correlates of protection*

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## A RETROSPECTIVE TEST-NEGATIVE DESIGN CASE CONTROL STUDY OF SEVERE INFLUENZA AMONG VACCINE RECIPIENTS IN SINGAPORE

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### Introduction and Objectives

Several studies have reported waning vaccine protection against influenza during winter season in temperate countries. We investigated influenza vaccine effectiveness in Singapore, a tropical city-state where influenza often circulates year-round with >1 seasonal epidemic occurring each year.

### Method

A retrospective case-positive, control test-negative design was adopted for this study. Vaccine recipients at Tan Tock Seng Hospital and government primary care clinic under the National Healthcare Group were identified and matched with their electronic medical records. All cases and controls had received standard dose influenza vaccine 14-365 days prior to the influenza test.

Cases and controls were matched on date of influenza test (+/-7days) in a 1:4 ratio with replacement. Odds ratios (OR) and 95% confidence interval (CI) were estimated using conditional logistic regression where the dependent variable was a dichotomous indicator of whether the first influenza PCR test was positive.

### Result

We identified 5,279 patients (median age 75years, interquartile range 65-83) who were vaccinated against influenza from January 1, 2013 to October 31, 2017 and subsequently tested for influenza. The 462 patients who tested positive were matched with 1,370 unique patients who tested negative.

No significant difference in age, gender, ethnicity or Charlson score between cases and controls was found on univariate analysis. There was also no significant difference in quarter/year of vaccination and number of vaccinations received in the preceding 2 years. Cases had significantly fewer hospital admission in the year before vaccination ( $p=0.002$ ).

A multivariable model of time since vaccination identified a significant increase in the odds of influenza more than two months after vaccination; days 61-107: OR 1.68 (1.19-2.36), days 108-154: OR 1.48 (1.02-2.13), days 155+: OR 1.48 (1.11-1.97).

### Conclusion

Duration of seasonal influenza vaccine effectiveness warrants further investigations, as it has implication on prevention and control strategies, including the appropriate time for vaccination.

*Keywords: Test-negative design; vaccine effectiveness; duration; protection; tropics*

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## RELATIVE VACCINE EFFICACY: TRANSLATION INTO ABSOLUTE VACCINE EFFICACY TO INFORM INCREMENTAL POPULATION BENEFIT AND PUBLIC HEALTH RECOMMENDATIONS

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### INTRODUCTION/OBJECTIVES

Clinical studies to demonstrate enhanced protection provided by a new over a standard vaccine often lack a placebo group and can only provide relative vaccine efficacy (VE) estimates. However, an understanding of the absolute improvement in VE conferred by the new over the standard product is required in order to quantify and understand its actual incremental population benefit. We describe the relationship between absolute VE and relative VE (rVE) and the impact on population benefit.

### METHODS

Varying  $VE_{New}$  and  $VE_{Standard}$ , we calculated the rVE and absolute difference in VE ( $\Delta VE$ ) between the two vaccines. We also calculated the impact of rVE in the population varying attack rates (AR) from 1% to 10%.

### RESULTS

Unless the standard vaccine provides no protection, the rVE will always exceed the  $\Delta VE$  as a measure of a new vaccine's added benefit. Furthermore, for a given rVE, the corresponding  $\Delta VE$  becomes smaller as the absolute VE becomes greater. For example, with  $VE_{Standard}$  of 10%, 50% and 80%, a given rVE of 20% would translate into a  $\Delta VE$  of 18%, 10% and 4% respectively. Varying the AR has no effect on the relationship between rVE,  $\Delta VE$  or absolute VE but directly affects the population impact of the new vaccine, notably the number needed to vaccinate with the new versus standard vaccine in order to prevent one additional case.

### CONCLUSIONS:

Because rVE estimates always exceed  $\Delta VE$ , they may amplify the impression of a new product's advantage, particularly where the comparator product already provides substantial protection. rVE estimates from different settings or seasons cannot be compared or extrapolated because they depend upon the specific study conditions, notably the starting VE of the standard product. The  $\Delta VE$  should also be taken into account before making public health (or preferential use) recommendations on the basis of rVE findings.

*Keywords: relative vaccine efficacy, vaccine effectiveness, vaccine efficacy*

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## NEURAMINIDASE ANTIBODY RESPONSES AFTER PANDEMIC AND SEASONAL VACCINATION: A FIVE-YEAR STUDY

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Seasonal influenza epidemics and occasional pandemics are significant global health threats and vaccination is the main method of prophylaxis. Neuraminidase (NA) antibodies with the ability to inhibit NA enzyme activity have been correlated with reduction of symptoms and viral shedding. There is limited knowledge on vaccine-induced NA antibody responses and the impact of repeated vaccination is unclear. Here we show the NA antibody response after single and multiple influenza vaccinations over a 5-year period.

Health care workers (HCWs) were immunised with AS03-adjuvanted pandemic H1N1pdm09 vaccine in 2009 and subsequently vaccinated with non-adjuvanted seasonal vaccines containing H1N1pdm09 for 3 seasons (seasonal group) or received no further vaccinations (single group). Blood samples were collected on day 21 and 3, 6, and 12 months after each vaccination, or annually after the 2009 season for the single group. ELISA was used to quantify the binding antibody titre to NA Cal/09. ELLA is a fetuin-based microplate assay that quantifies the NA enzyme activity of influenza virus in the presence of antibodies and was used to determine the NA inhibition (NI) titres. Plaque reduction assay was used to assess viral infectivity in the presence of antibodies *in vitro*.

NI titres persisted above the pre-pandemic vaccination level for both the single and seasonal group after 60 months. NI titres for the single group were maintained at a stable level during the 5-year study, indicating that long-lasting NA-specific antibody responses were generated, whereas the seasonal group had a gradual increase with each seasonal vaccination. There was a positive correlation between the number of vaccinations and NI and binding antibody titres measured 12 months after vaccination.

The monovalent AS03-adjuvanted H1N1pdm09 vaccine generated long-lasting NA specific antibody responses that were further boosted by non-adjuvanted seasonal vaccines.

*Keywords: Neuraminidase; H1N1pdm09*

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## INFLUENZA VACCINE EFFECTIVENESS IN ≥60 YEARS OLD HOSPITALIZED PATIENTS FROM THE VALENCIA REGION OF SPAIN IN THE 2018/2019 SEASON. PRELIMINARY RESULTS.

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### Introduction

Influenza vaccines are reformulated every season resulting in a variable influenza vaccine effectiveness (IVE). We estimated the IVE in ≥60 years old hospitalized patients using preliminary data from the 2018/2019 season in Valencia.

### Methods

The Valencia Hospital Network for the Study of Influenza (VAHNSI) conducts a prospective, active-surveillance hospital-based study. This analysis was restricted to ≥60 years old admitted patients from 2019-01 to 2019-11 weeks.

Non-institutionalized resident patients, hospitalized within 7 days of the ECDC ILI-case symptoms onset were swabbed and tested by real-time reverse transcription polymerase chain reaction (RT-PCR). Patient information was collected by interview and clinical records review. Vaccination status was ascertained from a population-based registry. Patients with unknown status or vaccinated less than 15 days prior to their symptoms' onset were excluded. IVE was estimated overall, by strain and prior vaccination following a test-negative design.

### Results

We included 837 patients; 165 (20%) influenza cases: 54 A(H1N1)pdm09, 99 A(H3N2) and 12 not subtyped. Preliminary data indicated that 14 A(H1N1)pdm09 viruses sequenced fell into A/Michigan/45/2015 (vaccine virus) clade 6B.1 within the A/Paris/1447/2017 subgroup, while the 5 influenza A(H3N2) sequenced viruses belonged to the A/Singapore-16-0019/2016 (vaccine virus) subclade 3C.2a1, but more closely related to the A/Alsace/1746/2018 subgroup 3C.2a1b.

The IVE was 36% (95% confidence interval, CI: 7, 57), 49% (7, 72) and 24% (-24, 53) against overall influenza, A(H1N1)pdm09 and A(H3N2). IVE was 73% (15, 92) for individuals vaccinated in the current but not in the prior 2 seasons, however, it was lower and not significant in revaccinated patients.

### Conclusions

The vaccine prevented 36% of the cases in hospitalized elderly patients. The IVE was moderate against A(H1N1)pdm09 and lower and not significant against A(H3N2). The IVE was higher in patients not vaccinated in prior seasons.

### Funding statement

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*Keywords: influenza; vaccine; effectiveness; hospitalizations; epidemiology*

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## THE CONSEQUENCES OF EGG ADAPTATION IN THE H3N2 COMPONENT TO THE IMMUNOGENICITY OF LIVE ATTENUATED INFLUENZA VACCINE

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### Introduction and objectives

Adaptations in egg-passaged vaccine strains leading to the loss of a putative glycosylation site (T160) in haemagglutinin (HA) of H3N2 are suggested to be a key reason for the recent loss of vaccine efficacy against H3N2. The impact of egg adaptations in inactivated vaccines for H3N2 immunogenicity have been well described. We explored the consequence of these adaptations in live attenuated influenza vaccine (LAIV) on serum and mucosal antibody responses and whether there is an associated fitness cost to replication in the human nasopharynx, resulting in reversion.

### Methods

244 Gambian children aged 24-59 months were immunised with intranasal Russian-backbone LAIV. Seroconversion and mucosal HA1-specific IgA response to egg- and wild-type H3N2 A/Hong Kong/2014/4801 (HK14) strains were assessed by haemagglutination inhibition and IgA protein microarray assays. Next generation sequencing with Primer ID was performed on the vaccine and 20 samples from children shedding H3N2 at day 2 and/or 7 post-LAIV.

### Results

25.8% of children seroconverted to egg-adapted and 15.2% to wild-type HK14. In children seronegative at baseline, these proportions were 56.0% and 28.6% respectively. A 2-fold HA1-specific IgA response was seen in 16.4% to egg-adapted and 20.6% to wild-type HK14. The egg adaptations T160K, L194P and T203I were identified in LAIV. No reversion of these adaptations and no fixed mutations were observed at either day 2 or 7 post-LAIV, including in children who showed wild-type HK14-specific immune responses.

### Conclusion

The presence of H3N2 A/Hong Kong/2014/4801 egg-adaptations in LAIV reduced seroconversion, but not mucosal IgA responses, to wild-type HK14. The mechanisms of seroconversion to wild-type HK14 despite immunisation with egg-adapted HK14, especially in children seronegative at baseline, warrants further investigation. Our data show this is not due to reversion during nasopharyngeal replication, which in turn suggests no significant fitness cost of egg adaptations to replication in the human mucosa.

*Keywords: Vaccine, Next generation sequencing, LAIV, serology, egg adaptations, H3N2*



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## Evaluation of the use of administrative data in test-negative vaccine effectiveness studies

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**BACKGROUND:** Test-negative design (TND) vaccine effectiveness (VE) studies typically use surveillance data, which allows the sample to be limited to subjects with clinically confirmed Acute Respiratory Infection (ARI). If VE is estimated using administrative data, such as hospital records or insurance records, it is not always possible to distinguish between subjects with and without confirmed ARI, which can lead to selection bias. Moreover, the probability of misclassifying vaccination or influenza status may be higher in administrative data, further biasing VE estimates.

**METHODS:** A simulation study was performed to estimate the bias in VE estimates obtained from administrative versus surveillance data. Thirteen parameters likely to differ among data sets were compared, including: the measurement of vaccination and influenza; the probabilities of vaccination, infection, clinical presentation and testing; and true VE. Simulation parameters were varied within plausible ranges, both overall and for 3 population age groups.

**RESULTS:** Under the range of parameter estimates tested administrative estimates were generally more biased than surveillance estimates, and for both data sources bias was generally towards the null. Among the most prominent influential parameters were the proportion vaccinated, true VE, and specificities of vaccination and influenza measurement. Age-unadjusted estimates tended to lie within the range of their corresponding age-specific estimates, except under certain conditions where they had the potential to be greatly biased in either direction.

**CONCLUSION:** Estimates of VE with administrative data can be expected to be more biased towards the null. This bias will be small as long as administrative sample is close to the corresponding surveillance sample in composition (i.e. if administrative data does not contain a large amount of non-ARI subjects). Age-unadjusted estimates are unreliable for both kinds of data. When measurement errors vary within levels of a confounder (here, age), the bias is not guaranteed to be towards the null.

*Keywords: vaccine effectiveness; test negative; simulation*

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## KNOWLEDGE, ATTITUDES, AND PRACTICES TOWARD INFLUENZA VACCINATION AMONG GUARDIANS OF YOUNG THAI CHILDREN

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**Background:** Thailand offers a free influenza vaccination through national campaigns to children aged 6 months-2 years. We implemented a KAP survey to understand determinants of influenza vaccination in children.

**Methods:** Within a network of seven government hospitals, Children's guardians at outpatient departments were interviewed using a structured questionnaire. Data on demographics, guardian's awareness, knowledge of influenza illness, national vaccine recommendations, perceptions toward influenza vaccine, trusted information sources were collected. Children's influenza vaccination was verified using vaccine books. Significant independent determinants of children's influenza vaccination were assessed using logistic regression.

**Results:** During August 2018-January 2019, 65 (9%) children of 745 guardian-child pairs were vaccinated for influenza. Sixty-one (94%) guardians of vaccinated children and 495 (73%) guardians of unvaccinated children had heard of influenza (p-value<0.01). Guardians of vaccinated children (59/61, 97%) were also more likely than guardians of unvaccinated children (423/495, 85%) to be aware of influenza vaccine availability (p-value=0.05); to know of national recommendations (55/59, 93% vs. 334/423, 79%; p-value<0.05). The child's doctor was the most common source of information about influenza (238/556, 43%) and influenza vaccine (264/482, 55%); a doctor was also the most trusted source (388/482, 81%). A higher proportion of guardians of vaccinated children perceived vaccine to be completely safe (26/59, 44% vs. 113/423, 27%, p-value=0.02); and agreed with the national recommendations (46/59, 78% vs. 189/423, 54%, p-value<0.01). Factors independently associated with children having received influenza vaccinations were guardians' agreement with national recommendations (adjusted Odds ratio (aOR), 95% CI= 2.4, 1.1-5.5), free vaccine provided (aOR, 95% CI= 0.4, 0.1-0.9), and history of previous influenza vaccination (aOR, 95% CI= 2.6, 1.2-6.0).

**Conclusion:** Our findings provide insights into the decision making of Thai guardians related to influenza vaccine. For this reasons, Thai Public Health is better to consider to provide a free influenza vaccine through national campaigns for all children.

*Keywords: Children, Influenza vaccine, Knowledge, Attitude, Practice*

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## INFLUENCE OF REPEATED VACCINATION ON INFLUENZA VACCINE EFFECTIVENESS ESTIMATION

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**INTRODUCTION:** Annual influenza vaccination is recommended to prevent infection. However, some studies using the test-negative design (TND) have reported reduced vaccine effectiveness (VE) for people vaccinated in consecutive seasons compared with people vaccinated in the current season only. We used simulations to examine the role of various potential biases on valid estimation of VE when participants are repeatedly vaccinated.

**METHODS:** We simulated a population at risk of influenza and non-influenza respiratory virus infections, where a proportion were enrolled in a TND after two influenza seasons. We simulated various TNDs based on real scenarios seen in recent years, considering: 1) matching of vaccine strain and circulating strain in both seasons; 2) partial protection from prior vaccination (V1) against current circulating strains (C2) if V1=C2; and 3) matching of V1/prior circulating strain (C1) and current vaccine strain (V2). We assumed: 1) prior vaccination or infection predicts current vaccination; 2) blunting of immune response if V1=V2 or C1=V2; 3) true VE=60% if vaccine strain matches circulating strain, else 10%; and 4) prior infection provide equal protection as an effective current vaccine (V2=C2). We calculated mean VE and relative percentage bias (significant if >10%) from 100 simulations.

**RESULTS:** When current VE=60%, vaccine strains and circulating strains match one another (V1=C2; V1=V2; C1=V2), unadjusted logistic regression underestimated VE significantly (mean VE=48.7%, bias=18.8%). VE was over- or underestimated when there was mismatch between current vaccine and circulating strains. When current VE=10%, bias was greatest when there was 1) blunting of immune response by both previous vaccination and infection (mean VE=0.9%, bias=90.8%), and 2) partial protection from previous vaccination (mean VE=21.2%, bias=112.5%). Adjusting for prior vaccination alone did not correct for biases significantly.

**CONCLUSION:** Prior vaccination and infection significantly bias current VE estimate. Careful interpretation is required when interpreting the effects of repeated vaccination.

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 Abstract No: 11176

**Estimates of seasonal influenza vaccine effectiveness in preventing laboratory-confirmed influenza in the Hospital-based Influenza Morbidity and Mortality surveillance, South Korea: 2018-2019 season**

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**Introduction:** During the early phase of the 2018-2019 influenza season, A(H1N1) influenza viruses dominantly circulated in South Korea. The effectiveness of seasonal influenza vaccine in preventing laboratory-confirmed influenza was evaluated through the Hospital-based Influenza Morbidity and Mortality (HIMM) surveillance system.

**Methods:** Among patients who visited emergency room or outpatient clinic with influenza-like illness or who were hospitalized with influenza, vaccine effectiveness (VE) was estimated by case (PCR-positive)-control (PCR-negative) design. VE was defined as  $[100 \times (1 - \text{odds ratio for influenza in vaccinated versus non-vaccinated persons})]$ . Samples and data collected from week 42, 2018 to week 5, 2019 in adult ( $\geq 19$  years old) patients and from week 52, 2018 to week 4, 2019 in pediatric patients were analyzed.

**Results:** During the early period of the 2018-2019 season, total 49 pediatric patients and 148 adult patients were enrolled. Among 148 respiratory specimens from adult patients, 96 were influenza A. Among 96 influenza A samples, 78 (81.2%) were A(H1N1)pdm09. Influenza vaccination rate was 46.6% in adults. Adjusted VE was 66.9% (95% CI, 3.9 to 88.6) in preventing influenza A in adult patients. Among patients aged 19-64 years, adjusted VE for prevention of influenza A was 66.6% (95% CI, 0.7 to 88.7). For prevention of A(H1N1) influenza, adjusted VE was 61.9% (95% CI, -10.9 to 86.9) in adults.

**Conclusion:** Seasonal influenza vaccine was found to be effective in preventing laboratory-confirmed influenza A in adults during the early period of the 2018-2019 influenza season in South Korea.

**Keywords:** influenza; influenza vaccine; vaccine effectiveness

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 11196

## ESTIMATED HEALTH AND ECONOMIC IMPACT OF USING HIGH DOSE INFLUENZA VACCINE IN OLDER AUSTRALIANS ON RESPIRATORY HOSPITALIZATIONS

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### Introduction and Objectives

Older adults (≥65 years old) are at increased risk of influenza-associated hospitalization and death. Since 2018 two influenza vaccines have been registered in Australia exclusively for this population: high dose trivalent unadjuvanted vaccine (HD-TIV; Fluzone<sup>®</sup> High-Dose) and standard dose trivalent MF59-adjuvanted vaccine (aTIV; Fludax<sup>®</sup>). In 2019 only aTIV is publicly funded for older Australians.

A recent retrospective cohort study directly comparing HD-TIV with aTIV in the USA estimates that HD-TIV was 12% (95% confidence interval 3.3–20%) more effective in preventing respiratory hospitalizations in older adults. Here we applied this finding to estimate incremental benefits of HD-TIV over aTIV in Australia.

### Methods

A decision tree model was developed for the analyses. The model accounted for the incidence rate of respiratory hospitalization for older-Australians. We estimated this over a 7-month period encompassing the peak of influenza circulation from International-Classification-of-Diseases (ICD-10<sup>th</sup> Revision)-coded admissions (J00-99 as the principal diagnosis) over 12 seasons in Australia. Key model inputs also included the national influenza vaccine coverage, and the unit cost of hospitalization from Australian Refined Diagnosis-Related Groups. In the absence of direct efficacy data for aTIV, a range of potential values were explored (30-50%) against the outcome of respiratory admissions.

### Results

The average respiratory hospitalization rate for older Australians when influenza circulates was 3,566/100,000 population. The average cost of such a hospitalization was AU\$7,171. A publicly funded program in Australia using HD-TIV may avert an additional 9,444–10,570 respiratory admissions amongst older adults each year. Accordingly the incremental cost avoidance per year may be AU\$67.7–75.8 million.

### Conclusion

Using HD-TIV instead of aTIV for older Australians could lead to substantial incremental health and economic gains due to reduction in respiratory hospitalizations. Respiratory admissions complement other important influenza-related complications that drive the value of HD-TIV.

### Funded by Sanofi Pasteur

*Keywords: influenza, influenza vaccine, older adults, public immunization program, Australia*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 11210

## Comparison of results obtained with HAI, protein micro array, and ADCC from influenza vaccine studies.

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**Introduction:** Induction of hemagglutination inhibition (HAI) and virus neutralizing antibodies by vaccination are regarded the major correlates of protection from re-infection with matching influenza strains. However cross-reactive and/or non-neutralizing antibodies also contribute to protective immunity. The role of these cross-reactive antibodies is poorly investigated, but might play an important role in vaccine effectiveness.

**Methods:** Paired serum samples were obtained pre- and post- vaccination during clinical trials evaluating trivalent or quadrivalent influenza. First, antibody titers were measured using the conventional HAI assay. In addition we tested paired serum samples using the multiplex protein-microarray, which allows in-depth characterization of antibody responses to a broader range HA1 antigens of historic and recent influenza virus strains. Third, we tested a selection of serum samples for antibody-dependent cellular cytotoxicity (ADCC) activity against the homologous vaccine HA.

**Results:** Serum titers measured using the protein-microarray showed a median titer increase against all vaccine strains. The induction of titer increase to other strains was most prominent for the most closely related viral antigens. Interestingly a clear median titer increase was shown against both the Victoria and Yamagata like influenza B strains upon trivalent vaccination. ADCC activity, detectable prior to vaccination, increased significantly after vaccination against the vaccine strains. These Abs were mainly directed to the HA-stem whereas HA1 Abs were induced at low levels. Similar to the PA, we showed ADCC activity to both homologous and heterologous influenza B strains by trivalent vaccination. The correlation between ADCC and HI and PA were both moderate to good.

**Conclusion:** The different serological approaches used in this study provide the opportunity to look into the induction of cross-reactive Abs induced by vaccination possibly related to historical infections, which might play an important role in vaccine effectiveness. The combination of these datasets will likely contribute to define possible new correlates of protection.

Topic: Public Health: Vaccine Effectiveness/Impact

Abstract No: 11218

## Model-based comparison of annual and biannual childhood vaccination strategies

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**Introduction:** Currently, some public health organisations recommend annual influenza vaccination for children. However, vaccination in the previous season may affect an individual's susceptibility in the current season in different ways. Past vaccination may still afford some protection in the current season if the circulating strain has not drifted far from the prior vaccine strain. On the other hand, those who were not vaccinated in the previous seasons have had more chances to become infected and this past infection may increase a person's immune response in the current season. Here, we aim to determine how different vaccination strategies in children impact annual attack rates. Specifically, we compare annual vaccination to biannual vaccination.

**Methods:** We have developed a multi-annual, individual-based, stochastic model of infection and vaccination. Within our model we account for each individual's infection and vaccination history as well as disease dynamics influencing susceptibility, namely antigenic drift. Our model allows a) immunity from exposure (by either natural infection or vaccination) to wane as the exposing virus drifts over time, and b) the waning of the protective effects of natural infection and vaccination to be different. We simulate infection and vaccination in birth cohorts over 17 years under two vaccination strategies beginning in year 3: annual vaccination and biannual vaccination. We compare annual attack rates from the different vaccination strategies under different assumptions of immunity waning, vaccine efficacy, and vaccine coverage.

**Results:** Overall, annual attack rates for biannual vaccination were similar to attack rates for annual vaccination, regardless of vaccine coverage. We compared the number of lifetime infections since vaccination and found that the average number of lifetime infections from ages 3 to 17 between the two vaccination strategies were almost the same.

**Conclusion:** Biannual vaccination may achieve similar annual attack rates to annual vaccination.

*Keywords: Influenza; modelling; vaccination; children; annual; biannual; birth cohort; drift*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 11221

## COMPARISON OF SIDE EFFECT PROFILES OF INACTIVATED AND LIVE ATTENUATED SEASONAL INFLUENZA VACCINES IN PRIMARY SCHOOL CHILDREN OF HONG KONG

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### Introduction

Although the effectiveness of inactivated influenza vaccines (IIV) and live attenuated influenza vaccine (LAIV) is generally regarded to be comparable, a comprehensive head-to-head comparison of their side effects (SE) profiles is lacking.

### Methods

We conducted a school-based influenza vaccination programme, covering 9410 children in 33 primary schools, with 57.6% and 42.4% received IIV and LAIV respectively, in the 2018/19 season in Hong Kong. SE profile was examined for 4 days (Day 0-3), with a severity scale of 0-3 (0=no symptom, 1=mild symptom, 2=moderate symptom causing disturbance to daily activities, 3= severe symptom causing significant disturbance to daily activities).

### Results

3138 children returned the post-vaccination questionnaire (33.3%). 53.5% of children receiving IIV and 52.7% of children receiving LAIV reported  $\geq 1$  symptom. IIV group reported more systemic symptoms including fatigue (24.9%) and myalgia (32.7%), LAIV group reported more respiratory symptoms including rhinorrhea (41.0%) and cough (19.0%) ( $p < 0.01$ ). Mean symptom score was lower for LAIV on D0 (0.7 vs 1.1) and higher on Day2 (0.8 vs 0.4) & 3 (0.8 vs 0.3) comparing with IIV ( $p < 0.01$ ). Median duration of SE was 1.0 day for both groups. There was no overall difference between the two groups on number of vaccinee reporting SE  $\geq$  grade 2. 59.5% of IIV vaccinee reported local reactions at the injection site, while 6% of LAIV vaccinee experienced epistaxis. Total symptom severity estimated by median AUC was comparable between the two groups ( $p = 0.26$ ). SE profile was similar for first and second dose for both vaccines.

### Conclusions

SE profile was comparable between IIV and LAIV among primary school children. SE were predominately systemic and more severe on D0 for IIV, and mainly respiratory and delayed on D2-3 for LAIV. Duration and odds of moderate to severe SE with disturbance to daily activity was comparable in the two groups.



Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 11226

## INFLUENZA VACCINE EFFECTIVENESS AGAINST HOSPITALISATION DUE TO INFLUENZA IN CHILDREN IN ENGLAND: A COMPARISON OF METHODS

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**Introduction:** England has started a paediatric influenza vaccine programme using live attenuated influenza vaccine (LAIV). Assessing vaccine effectiveness (VE) is an important component of assessing the impact of vaccination programmes. Two observational methods to assess VE are the case test-negative design (TND) and screening method. This study set out to compare VE against hospitalisation in children using these methods.

**Methods:** The TND and screening method were used to estimate VE in children in preventing laboratory-confirmed influenza hospitalisation in the 2015/16 season.

Using the TND, cases and controls were identified from Datamart, a national sentinel laboratory surveillance system. Logistic regression was used to estimate the odds ratio for influenza vaccination in cases compared to controls adjusting for potential confounding variables.

Using the screening method cases were identified from USSS, a national surveillance system which collects data on individuals hospitalised due to influenza from a sentinel network of hospitals in England. Vaccination coverage in these hospitalised children was compared with vaccination coverage in children in the general population.

**Results:** In the TND study, the overall adjusted VE against laboratory confirmed influenza hospitalisation in 2-6 year olds was 30.0% (95% CI -10.7, 55.7) for all vaccine types. Clinical risk group was the only confounder to reduce the VE estimate more than 5%. The overall adjusted VE in the screening study in 2-6 year olds was 54.5% (95% CI 31.5, 68.4) for LAIV.

**Conclusion:** Both studies provide evidence of the effectiveness of influenza vaccination in preventing hospitalisation in children in 2015-16, continuing to support the rollout of the childhood programme. The non-significant estimate found using the TND is likely to be explained by inclusion of participants who received inactivated influenza vaccine; the VE for which is likely lower than for LAIV. The availability of possible confounding variables is a strong advantage for the TND.

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 11249

## ACCEPTABILITY AND DETERMINANT OF INFLUENZA VACCINATION AMONG HEALTH CARE WORKERS

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**Introduction:** Influenza virus is one of the most contagious respiratory pathogens. Influenza vaccination is recommended by WHO for Health Care Workers (HCWs) who are at increased risk of its exposure, with potential threat for their health and their patients'. The study aimed to know the vaccination acceptability among nurses in Dr.Hasan Sadikin General Hospital Bandung and the determinant factors.

**Method:** After the approval by Universitas Padjadjaran Ethical Committee, this cross-sectional study was conducted from October 2017 to January 2018 by inviting all nurses to enroll. Only willing nurses included as subjects after signing the informed consent.

**Result:** Only 920 nurses (73.1%) from total 1.258 gave response and 787 of 920 (85.5%) enrolled and completed questionnaires. Of 920 nurses, 233 of them (25.3%) refused vaccination after further discussion. Reasons for vaccination rejection were: "no time for vaccination"(19.3%), "doesn't like needles" (12.9%), pregnant, delivery, and breastfeeding (14.6%), "not in a good health condition" (11.2%), "not a high risk group for severe influenza" (11.2%), "no reason" (9%), "fear of illness after vaccination" (7.3%), "belief that vaccine is ineffective" (6%), and other reasons (8.6%). Factors associated with increased likelihood of acceptance were: higher education ( $p=0.00$ ), receipt of advice for vaccination ( $p=0.00$ ), vaccination offered in workplace ( $p=0.00$ ), higher numbers of respiratory infection in the last year ( $p=0.00$ ), absence from work due to respiratory infection ( $p=0.02$ ) and presence of children <5 years of age at home ( $p=0.00$ ).

**Conclusion :** Almost quarter of nurses (21.3%) refused vaccination, posing a threat to their health and increasing the likelihood of influenza acquisition and secondary spread in the workplace. Education to address misperceptions regarding vaccine efficacy, provider's advice regarding vaccination importance, and interventions to make vaccine accessible in the workplace, are important to improve the rates of vaccination among HCWs.

*Keywords: acceptability;determinant factors;HCW;influenza vaccination*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 11258

## IMPROVED SHARING OF INFLUENZA-POSITIVE SAMPLES IN THE WHO EUROPEAN REGION TO BETTER INFORM VACCINE STRAIN SELECTION

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### **Introduction**

The production of seasonal influenza vaccines requires continuous global monitoring of influenza viruses to detect emergence and circulation of new antigenic drift variants. Sharing of viruses by National Influenza Centres (NICs) within the Global Influenza Surveillance and Response System (GISRS) is the cornerstone of this process.

### **Objectives**

Analysis of timeliness and geographic representativeness of influenza viruses shared by NICs in the WHO European Region over seven seasons (2010-2017).

### **Methods**

Data from NICs on influenza-positive specimens shared with the WHO Collaborating Centre (CC) in London were analysed for geographic representativeness and timeliness of sharing with respect to a 31 January deadline for inclusion in February WHO consultations on the composition of influenza virus vaccines for the Northern Hemisphere.

### **Results**

Over seven influenza seasons, NICs representing 48 countries shared 13,704 influenza-positive samples (viruses or clinical specimens). Since 2010-2011, the number of countries sharing samples with GISRS increased from 38 to 48. During 2010-2011, 606 (33%) of 1,837 samples were received by the deadline, compared to 1,231 (71%) of 1,726 samples during 2016-2017 (Chi-squared test,  $p < 0.0001$ ). The geographic representation of countries sharing by the deadline increased from 20 to 43. Average times between specimen collection date and shipment decreased from 90 to 43 days between 2010-2011 and 2016-2017, but varied by subregion. Most viruses were shared from eastern, northern, southern, and western Europe, while Western Asia and, in particular central Asia, submitted fewer samples. During the 2016-2017 season, central Asia and western Asia each contributed <10% of all samples for the season; however, they sent 100% and 75%, respectively, of their samples by the deadline.

### **Conclusions**

Our data demonstrate significant improvements in terms of timeliness and representativeness of viruses shared with GISRS over the last seven seasons, reflecting the commitment of personnel involved.

*Keywords: Influenza vaccine virus selection; WHO recommendations; GISRS; surveillance; virus sharing*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 11311

## DEFINING A MECHANISM FOR H1N1 LIVE ATTENUATED INFLUENZA VACCINE EFFECTIVENESS

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### Introduction

In the 2013-14 and 2015-16 influenza seasons, the 2009 pandemic H1N1 (A/H1N1pdm09) component of the quadrivalent live attenuated influenza vaccine (QLAIV) gave low vaccine effectiveness (VE). Sustained multi-cycle replication in human respiratory epithelial cells was identified as a key determinant of H1N1 VE.

### Methods

Mechanisms behind reduced replicative fitness were investigated by comparison of A/H1N1pdm09 A/Bolivia/559/2013 (2015-16, low VE), with clinically effective, pre-2009 H1N1 A/New Caledonia/20/1999 (A/NC99). Non-infectious and defective interfering (DI) viruses were quantified by single/multi-cycle infectivity assays and digital PCR. HA/NA reassortant viruses assessed contributions of HA and NA proteins to fitness. Site directed mutagenesis and *in silico* modelling were applied to optimising HA protein properties. Assessment of *in vivo* efficacy utilised a ferret challenge model.

### Results

A/BOL13 generated more non-infectious virus than A/NC99, offering a potential mechanism for reduced fitness. DI viruses were found not to be responsible, suggesting a non-DI defect in viral assembly. HA/NA reassortant viruses identified the HA rather NA protein as the driver of fitness. An A/SLOV15 virus with an optimised HA protein was then developed. With increased human cell receptor binding and a more flexible HA head structure, A/SLOV15 possessed increased replicative fitness and a reduced non-infectious virus population. In QLAIV formulation, A/BOL13 suffered from inter-strain competition, leading to a loss of efficacy in ferrets. Optimised A/SLOV15, conversely, was effective in QLAIV formulation.

### Conclusion

There are multiple potential mechanisms driving A/H1N1pdm09 LAIV fitness and VE. A/BOL13 is suggested to have suffered from a non-DI deficiency in virus assembly. The HA protein was found to be key to fitness, with enhanced human receptor binding and HA structural flexibility of A/SLOV15 resulting in improved fitness, non-infectious virus content and efficacy *in vivo*. Comprehensive understanding of the factors influencing fitness and VE will be essential to the reliable selection of effective H1N1 vaccine strains.

*Keywords: live attenuated influenza vaccine, LAIV, mechanism of action, vaccine effectiveness, efficacy*

Topic: Public Health: Vaccine Effectiveness/Impact  
 Abstract No: 11325

**INFLUENZA BURDEN OF DISEASE AND 2017/18 END-OF-SEASON  
 INFLUENZA VACCINE EFFECTIVENESS ESTIMATES FOR PREVENTING  
 INFLUENZA-ASSOCIATED HOSPITALIZATION AMONG CANADIAN  
 ADULTS: AN UPDATE FROM THE CIRN SERIOUS OUTCOMES  
 SURVEILLANCE (SOS) NETWORK**

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**Introduction:** Influenza surveillance is important in order to understand patterns of disease burden and vaccine effectiveness (VE). In Canada, the Serious Outcomes Surveillance (SOS) Network conducts active surveillance for influenza hospitalizations at 13 adult and community hospitals in four provinces (Ontario, Quebec, New Brunswick and Nova Scotia). We contribute these Canadian data to the Global Influenza Hospital Surveillance Network (GIHSN).

**Methods:** Active surveillance for influenza infection in adults ( $\geq 16$  years of age) was conducted January 1st to April 30th, 2018. For laboratory confirmation, all patients with acute respiratory illness or unexplained sepsis had nasopharyngeal swab PCR testing for influenza A & B. Clinical and demographic data included age, vaccination status and frailty. Comparing influenza cases with test-negative controls, VE was calculated as  $VE = 1 - OR \times 100\%$ .

**Results:** 2134 lab-confirmed influenza cases were enrolled during the 2017-2018 influenza season. Influenza A was the predominant strain (55.2%), though there was substantial circulation of influenza B throughout the season. Most patients were older adults, with 54.9% being aged 75+. Half of the patients were at least mildly frail. Overall, 10.8% of patients with laboratory-confirmed influenza were admitted to ICU and 6.7% died. ICU admission generally decreased with increasing frailty, while mortality increased. The overall preliminary VE estimate was 40.1% (22.8,53.6). Notably, VE was higher in older adults (65+) at 38.8% (17.3,54.7) vs. 34.1% (-21.9,64.4) for adults under age 65.

**Conclusions:** The SOS Network contributes to influenza surveillance in Canada and internationally through the GIHSN. Focus on outcomes and health measures relevant to older adults (particularly frailty and function) continues to contribute to our understanding of influenza in this important and vulnerable population.

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 11725

## Passage adaptation correlates with the reduced efficacy of the influenza vaccine

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### Background

As a dominant seasonal influenza virus, H3N2 virus rapidly evolves in human and is a constant threat to public health. Despite sustained research efforts, the efficacy of H3N2 vaccine has decreased rapidly. Even though antigenic drift and passage adaptation (substitutions accumulated during vaccine production in embryonated eggs) have been implicated in reduced vaccine efficacy, their respective contributions to the phenomenon remain controversial.

### Methods

We utilized mutational mapping, a powerful probabilistic method of studying sequence evolution, to analyze patterns of substitutions in different passage conditions for an unprecedented amount of H3N2 hemagglutinin sequences (n=32278).

### Results

We found that passage adaptation in embryonated eggs is driven by repeated convergent evolution over 12 codons. Based on substitution patterns at these sites, we developed a metric, Adaptive Distance (AD), to quantify the strength of passage adaptation and subsequently identified a strong negative correlation between AD and vaccine efficacy.

### Conclusions

The high correlation between AD and vaccine efficacy implies that passage adaptation in embryonated eggs may be a strong contributor to the recent reduction in H3N2 vaccine efficacy. We developed a computational package called MADE to measure the strength of passage adaptation and predict the efficacy of a candidate vaccine strain. Our findings hence shed light on strategies that reducing Darwinian evolution within the passaging medium can potentially restore an effective vaccine program in the coming future.

*Keywords: H3N2 influenza virus; vaccine efficacy; passage adaptation; mutational mapping*

Topic: Public Health: Vaccine Effectiveness/Impact  
Abstract No: 11767

## **rVP3-ADJUVANTED VIRUS-LIKE PARTICLES VACCINE EFFICACY AGAINST TAIWAN HPAI H5N2 IN CHICKEN**

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Development of new and effective vaccines to protect humans and animals from influenza virus infection and to control the spread of influenza viruses is an important quest worldwide. Both antigen preparation and adjuvant are important components of vaccine. Virus-like particle (VLP) which represents the structure and antigen compositions of whole virus is a potent vaccine antigen and lacks the risks of growing virus. We found the combination of VLP and rVP3 and alum as vaccines against bird flu yielded the best protection. Chicken immunized with H5N2-VLP adjuvanted with rVP3 and alum protected 100% of chicken from H5N2 virus challenge resulting in less virus shedding than chicken immunized with inactivated virus adjuvanted with rVP3 and alum. Our data suggest that VLP adjuvanted with VP3 and alum as novel vaccines against bird flu.

*Keywords: virus-like particles; adjuvant; protective efficacy; avian influenza H5N2*

Topic: Public Health: Vaccine Effectiveness/Impact  
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## AS03 and MF59 adjuvants in pandemic influenza A(H1N1)pdm09 vaccines: a systematic review and indirect comparison meta-analysis

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### Introduction and objectives

Pandemic influenza vaccines can have improved immunogenicity by adding oil-in-water adjuvants, allowing haemagglutinin antigen sparing for mass production early in a pandemic. However, there is limited evidence to justify choice of adjuvant in A(H1N1)pdm09 pandemic vaccines. We conducted a systematic review and meta-analysis to determine which adjuvant allowed the most antigen sparing.

### Methods

A systematic review and indirect-comparison meta-analysis of randomised controlled trials (01/01/2009–09/09/2018) was conducted, extracting immunogenicity and safety data of AS03- or MF59-adjuvanted vaccines. Pooled comparisons of log-transformed haemagglutination inhibition geometric mean titre ratio (GMTR), risk ratio of seroconversion rate and adverse events (pain, fatigue and fever), for each adjuvant versus its unadjuvanted counterparts was estimated. Then, we indirectly compared different AS03 versus MF59 regimens via a test of subgroup differences. Separate analyses were conducted for articles using 7.5 and 15µg haemagglutinin antigen in the unadjuvanted vaccine comparator group (hence two results per comparison below).

### Results

In adults, AS03-adjuvanted vaccines (containing 3.75µg haemagglutinin antigen) achieved superior GMTR compared with unadjuvanted vaccines; mean difference (MD)=0.94 (95%CI 0.36 to 1.52, p=0.001), 0.56 (95%CI 0.33 to 0.80, p<0.001). There was evidence of MF59 (full dose)-adjuvanted vaccine (containing 7.5µg haemagglutinin) superiority versus unadjuvanted vaccine in studies using 7.5µg haemagglutinin as an unadjuvanted comparison (MD=0.73, 95%CI 0.34 to 1.11, p<0.001), but not those using 15µg haemagglutinin as an unadjuvanted comparison (MD=0.24, 95%CI -0.06 to 0.53, p=0.123). Indirect comparison of GMTR weakly trended towards AS03-adjuvanted over MF59 (full dose)-adjuvanted vaccines in studies using 15µg haemagglutinin as an unadjuvanted control (p=0.088), but not in those using 7.5µg haemagglutinin as an unadjuvanted control (p=0.545).

All adjuvanted vaccines caused increased adverse events.

### Conclusion

Compared with unadjuvanted and MF59-adjuvanted vaccines, AS03-adjuvanted vaccines achieved similar immunogenicity in adults with less antigen, at the cost of higher rates of pain and fatigue (only compared to unadjuvanted vaccines).

*Keywords: Influenza A; H1N1 subtype; Adjuvants; Influenza vaccines; Pandemics*



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## The causal interpretation of “overall” vaccine effectiveness in test-negative studies

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### Introduction and Objectives

The test-negative studies usually reported an overall vaccine effectiveness (VE) by considering all individuals with an equal weight. However, younger population might more likely to be included while the estimated VE were also higher for these population, which challenges the causal interpretation of “overall” VE. The objective of this study is to examine whether there would be variation in overall VE estimates after considering the underlying population structure with/without vaccination coverage.

### Methods

We first used simulations to examine how “overall” VE estimates would be biased when a certain age group was over-represented. Then we abstracted published VE estimates by the U.S. Flu VE Network for 2015/16, 2016/17 and 2017/18 seasons. For both methods, we re-estimated overall VE by weighting age-specific VE estimates using population structure and population who were vaccinated in each age group.

### Results

From the simulation study, we found VE estimates tended to be higher than the weighted estimates when children were over-represented while lower than the weighted estimates when elderly were over-represented. Compared with underlying population, higher proportion of children were included for overall VE estimation by the U.S. Flu VE Network. After accounting for the population structure with/without vaccination coverage, some discrepancy in overall VE estimates were observed in 2015/16 season but not others. In 2015/16, we estimated an overall VE of 31% (95% Confidence intervals (CI): 22%, 39%) against influenza A or B, which did not cover the point estimate of unweighted overall VE (40%, 95% CI: 32%, 46%).

### Conclusion

To increase study power, some surveillance networks used standardized protocols and pooled overall VE estimate across different study regions. The “true” weight of each participant needs to be considered to estimate a valid “overall” VE.

*Keywords: influenza; vaccine effectiveness; pooling; causal inference; weighting*