ABSTRACTS
Orals & Posters

Options for the Control of Influenza VI

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Canada

Options for the Control of Influenza VI

Since its inception in 1985, the *Options for the Control of Influenza* conference has grown in scope and size to become the international meeting we have today: *Options VI* will be the largest of the series to date in both participation and presentation of scientific information. The reemergence and expansive geographic spread of avian H5N1 viruses since late 2003 has had devastating consequences for agriculture and trade and posed a clear and present threat to public health. The circulation of H5N1 and other avian subtypes have spurred enhanced surveillance efforts, research for improved measures for influenza detection, control, and prevention, and extensive pandemic preparedness strategies. The accumulation of new information on seasonal and pandemic influenza is reflected in the *Options VI* Scientific Program. The fourteen workshops encompass every aspect of surveillance, diagnosis, policy-making, prevention, and control efforts, as well as basic research on the virus and/or the host response. New workshops have been added to *Options VI* that reflect the ongoing outbreak and pre-pandemic responses to avian influenza worldwide, and growing interest in the use of mathematical modeling to predict virus evolution and spread or to guide control efforts.

On behalf of the Scientific Committee, I would like to thank all authors of abstracts for *Options for the Control of Influenza VI*. Your contributions, over 600 in total, have made this event the most comprehensive *Options* meeting to date and will ensure its success. The abstracts have been published in this volume according to the topics in which they were presented.

Finally, my sincere thanks go to the members of the Scientific Committee for their tireless efforts in organizing the *Options VI* scientific program and to the workshop chairs for their review of a very large number of abstracts. Together, their efforts have created an outstanding program that provides us with state-of-the-art knowledge on influenza and a forum for the exchange of ideas and generation of new strategies for research.

I wish all participants an interesting, productive, and enjoyable meeting.

Sincerely,

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Workshop Abstracts:

ORAL PRESENTATIONS

Workshop Topic #1

Disease Surveillance and Impact

Oral Abstracts

Abstract O1

Epidemiology of Influenza in India

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Background: Influenza, one of the most frequent causes of acute respiratory disease, has a major impact on public health. Prevention and control of disease is important in the high-risk pediatric and geriatric age groups and those with underlying pathologies. Continued surveillance is necessary to track minor and major antigenic shifts in the circulating strains for forthcoming vaccine strains. Objective: Epidemiological and virological surveillance of influenza in different geographical areas of India, to contribute information to the global influenza surveillance network. Methodology: Indian Council of Medical Research has established an influenza surveillance network in India, in collaboration with the CDC. Five regional laboratories located in Pune, Delhi, Chennai, Dibrugarh and Kolkata; identified patients with upper/ lower respiratory tract infections, using a uniform case definition. A questionnaire with demographic characteristics, symptoms, clinical examination etc was recorded. A throat/nasal swab or naso-pharyngeal aspirate was obtained from these ambulatory/ hospitalized patients. Madin-Darby Canine Kidney cell line was used for virus isolation. Further, the isolate was antigenically characterized by hemagglutination inhibition test, using WHO reference sera. Results: From 15th 2004 to 14th September 2006, 4112 patients were examined. Presenting symptoms were fever (94%), cough (96%), nasal discharge (34.4%), sore throat and headache in 15% patients respectively. Tachypnoe was found in 30% of patients below 5 years. Male to female ratio was 1.2:1. 56% of the patients were children below 5 Years of age. A marked seasonality was observed in different areas of the country; acute respiratory disease and influenza activity coincided with the rainy season or the winter months. 202 isolates (isolation rate-4.9%) of Influenza virus were obtained from the 5 regions. In 2004-05, A(H3N2) was isolated in Pune, Delhi and Chennai, A(H1N1) and type B were isolated in Pune Delhi and Dibrugarh. In 2005-06, A(H1N1) predominated in Chennai and Dibrugarh, A(H3N2) in New Delhi,type B in Pune and Kolkata had both A(H3N2) and type B 131. Conclusions: Extensive influenza activity continues in India, with influenza being the causative agent in at least 5% acute respiratory infections. Influenza A(H1N1), A(H3N2) and type B co-circulated in the community with characteristic, marked seasonality in different geographical areas.

Abstract 02

SCHOLARSHIP Promising Junior Investigator Scholarship

Burden and Seasonality of Influenza-like Illness in a Cohort of Nicaraguan Children

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Background: Many studies have been performed to assess the burden of influenza-like illness in temperate regions, however little is known about the burden and epidemiology in the tropics. Methods: To study the burden and epidemiology of influenza-like illness in Nicaragua, a Central American country, we performed a prospective community-based cohort study examining the incidence of influenza-like illness (ILI), febrile acute respiratory illness (fARI), and acute respiratory illness (ARI). Results: A total of 3,999 children between the ages of 2 and 12 years were followed for every primary medical appointment for a one-year period between January 11, 2006 and January 10, 2007. Out of a total of 3,708 person-years, 1156 episodes of ILI were recorded, yielding an incidence rate of 31 per 100 person-years. A marked biphasic pattern was seen in influenza-like illness as well as fARI and ARI episodes, with one peak in July and another peak in November. Restricting the analysis to children 5 years and over and to high probability ILI (fever of ≥38.5°C with cough) resulted in the same biphasic pattern. Conclusions: Our results suggest that the influenza burden is high in Nicaragua and that Nicaragua experiences two seasonal influenza periods during the year. These peaks correspond in timing to those observed in the Southern hemisphere in July and the Northern hemisphere in November.

Abstract O3

An Assessment of the Implementation of the First Comprehensive Influenza Surveillance Activity in Kenya

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Continuous, global influenza surveillance is critical for the detection of viral antigenic drift and shift, vaccine strain selection, and early warning of the circulation of new and dangerous influenza subtypes. No consistent influenza

surveillance has been conducted in east and central Africa despite strong evidence of frequent ILI among its population. To address this surveillance gap, the United States Army Medical Research Unit—Kenya (USAMRU-K) in collaboration with the Kenya Medical Research Institute (KEMRI), and the Centers for Disease Control (CDC), Nairobi developed an influenza surveillance network in Kenya. Jointly planned to avoid unnecessary overlap with future CDC sites, hospital study sites were selected based on representation of the major population centers, frequency of demographic movement, and reports of frequent respiratory disease. ILI cases are recruited by dedicated clinicians from the outpatient population. Nasopharyngeal specimens are transported in liquid nitrogen to the completely refurbished National Influenza Center in Nairobi. USAMRU-K and KEMRI laboratory technicians jointly process all samples. Identification is performed by virus isolation. Beginning in July 2006 and through March 2007, six of eight surveillance sites have been activated. 67 viral isolates were obtained from 839 specimens. The majority of influenzas were influenza B (Malaysia-like). All influenza infections detected by this surveillance occurred from July through October in two urban environments, Nairobi and Kisumu. Not previously maintained, a sustained ILI surveillance effort will significantly assist the Kenya MoH in monitoring and responding to potential epidemics. The first eight months have seen a rapid expansion with numerous successes despite expected difficulties with physical and scientific infrastructure. The close interagency cooperation between USAMRU-K, KEMRI, and the CDC is a significant and successful new model for conducting challenging surveillance in Africa. Most parts of the country are now represented. The cold-chain has been successfully maintained. Two influenza A (H3N2) viruses—the first ever recorded in Kenya—were unique drift variants which suggest the importance of connecting Kenya influenza surveillance to WHO global influenza surveillance. Pending milestones will be the institution of screening by real-time RT-PCR technology, improvement in site baseline clinical data to better define disease burden, expansion to Uganda and Cameroon.

Abstract O4

A Frequent West-East Spread of Influenza Activity Across Europe

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Background: The European Influenza Surveillance Scheme has collected epidemiological and virological data on influenza activity since 1996 (www.eiss.org). We performed an analysis of the EISS database to assess the validity of sentinel physician reports, the geographical spread of influenza activity across Europe and the overall length of increased clinical activity. Methods: We first assessed whether consultation rates for influenza-like illness (ILI) or acute respiratory infection (ARI) reported by sentinel physicians peaked at the same time as the virological data obtained from sentinel physicians. This analysis was performed for seven winters and 17 countries. We then assessed the seasonal spread of influenza activity by plotting the week when consultation rates peaked in each country against the approximate geographic centre (longitudes and latitudes) of the countries (26 countries). Finally, the length of increased influenza activity in Europe was calculated for each season by assessing the difference between the earliest and latest week when consultation rates peaked. Results: We found a very good match between the consultation rates and the sentinel virological reports (overall match of 80%; 1 week difference in peaks). The match was the same for countries reporting ILI and ARI. The spread analysis for the past seven seasons indicated there was a west-east spread in four winters (2001-02, 2002-03, 2003-04 and 2004-05; R^2=0.331, 0.428, 0.598 and 0.689, respectively) and a south-north spread in two winters (2001-02 and 2004-05; R^2=0.481 and 0.250). During the 2005-2006 season there was no west-east or south-north spread. The period of increased influenza activity ranged from 3-5 months. Conclusions: We found that: 1) the ILI and ARI consultation rates reported by sentinel physicians are a valid indicator of influenza activity; 2) during four out of seven seasons there was a west-east spread of influenza activity across Europe; 3) the length of increased influenza activity in Europe was surprisingly long, ranging from 3-5 months. These results have implications for pandemic planning and have led to the establishment of a more detailed mapping project which currently includes five countries and will further assess the west-east and south-north spread findings.

Abstract O5

Intrafamilial Transmission of Influenza A and Influenza B

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Background: In studies of influenza involving children and adults, a high incidence in children is well documented as is a longer viral persistence in children than in adults. However, few large-scale studies have addressed possible differences in the intrafamilial transmission of influenza A and influenza B. Methods: Influenza infection was observed from the five seasons from 2001/2002 to 2005/2006. 990 families with 3,261 family members were surveyed. Influenza was diagnosed

with a viral antigen detection kit; Capilia Flu A,B or Capilia Flu A+B. Within family pairs of patients diagnosed positive for the identical virus type and with onset within 8 days of each other were analyzed. Results: The incidence rate of at least one influenza infection during the five years was 59.7 % for children under 6 years of age and 55.1 % for children aged 7 to 12 years. 257 influenza patient pairs were identified; 161 influenza A pairs and 96 influenza B pairs. The mean time from the onset of the first patient to the onset of the second patient was 2.45 days for influenza A and 3.35 days for influenza B. The patients were grouped by age; 0–3, 4–6, 7–9, 10–12, 13–15, and over 16 years. For the first influenza patient of each pair the percentages by age were 16.4 %, 25.4%, 12.9%, 10.5%, 5.5% and 29.3 %, respectively. For the second influenza patient of each pair the percentages by age were 21.0%, 12.8%, 9.3%, 5.1%, 2.3%, and 49.4%, respectively. Discussion: The time between the onset of the first patient to the onset of the second patient was longer for influenza B than for influenza A. The fact that viral persistence is longer for influenza B than for influenza A may explain these results. That children in the 0 – 3 and 4 – 6 age groups were most frequently the first patient suggests that these children play an important role in the intrafamilial transmission of both influenza A and influenza B and may be key to controlling influenza epidemics.

Abstract O6

Socioeconomic Disparities in the Burden of Influenza Among Children in the United States

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Many studies have recognized the significant burden of influenza in pediatric populations. However, whether social factors modify this association is not well understood. In particular, the impact of socioeconomic disparities on influenza illness among children has not been quantified. We conducted a multi-scale retrospective study to measure the impact of poverty on utilization of healthcare services by children with influenza. We tested whether proportion of individuals below poverty level is associated with the geographical distribution of influenza in both a major metropolitan area and across the United States among children independent of other known or suspected risk factors. The study included 1,308,904 inpatients at 20 pediatric hospitals across the United States (2000-2005) and 298,956 patients presenting to one emergency department (ED) of a pediatric hospital in Miami, Florida (2001-2005). Hospitalizations at the national level and ED visits at the local level were aggregated by hospital city and patient ZIP code, respectively, and compared to census-based measures of socioeconomic status. Healthcare use attributed to influenza was significantly predicted by the proportion of households below the poverty, with a 1% increase in individuals below the poverty level linked to an increase in hospitalizations due influenza at the national scale of 2.0 per 1,000 visits (95% confidence interval: 0.7, 3.3; P=0.004) and emergency department visits at the local scale of 1.7 per 1,000 visits (95% confidence interval: 1.3, 2.1; p<0.0001). After controlling for factors that may be linked to disease transmission such as race and ethnicity, population density, large household size and close living quarters, we find that income is the main determinant of utilization. While barriers to low-income populations receiving influenza vaccination have been well-described, this is the first study to report a clear association of socioeconomic status with the burden of health care utilization. Although causality is not proven in a geographical association study, documenting empirical spatial patterns of influenza and their relationship to population-level demographic characteristics nonetheless represents an important step towards formulating policy to address health disparities. The recognition that socioeconomic disparities are a risk factor for influenza infection should inform resource planning and control strategies.

Abstract O7

Comparing Methods for Estimating Influenza-associated Deaths in the United States: 1976/1977 Through 2002/2003 Respiratory Seasons

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Background: Using data from the 1990/1991 through 1998/1999 respiratory seasons, we previously estimated that an average of 36,000 underlying respiratory and circulatory (R&C) deaths were associated with the circulation of influenza viruses in the United States. We provide an update of these estimates using data from 1976/1977 through 2002/2003 respiratory seasons, and compare methods for estimating these deaths. Methods: We used weekly data for the 1976/1977 through 2002/2003 respiratory seasons from the National Center for Health Statistics for underlying R&C deaths (International Classification of Diseases 9th revision (ICD-9)

codes 390-519, ICD-10 codes I00-I99, J00-J99) by age group (< 65 and ≥ 65 years). Population data were obtained from the US Census. Numbers of influenza tests and total specimen tested were obtained from weekly reports from the World Health Organization (WHO) collaborating virology laboratories. We compared estimates of influenza-associated deaths by age group for four models (Serfling Poisson regression, Serfling linear regression, peri-season risk-difference, and auto-regression-moving average (ARIMA)). Results: The Serfling Poisson regression models estimated an annual average of 26,000 (range 5,000 – 59,000) influenza-associated underlying R&C deaths from the 1976/1977 through 2002/2003 seasons. For ages < 65 and ≥ 65 years, estimates of annual influenza-associated underlying R&C deaths were 2,700 (range 600 – 5,400) and 23,000 (range 0 – 54,000), respectively. For influenza A(H3N2), B, and A(H1N1) viruses, the Poisson regression models estimated annual averages of 19,800, 4,600, and 1,300 deaths, respectively. The Serfling linear regression models estimated an annual average of 22,000 (range 0 – 43,000) deaths. The peri-season risk-difference models, using either a 10% or a 15% difference threshold, estimated an annual average of 34,900 (range 0 – 66,800) and 22,000 (range 0 – 56,000) deaths, respectively. The ARIMA model, using a threshold of either 1 or 2 standard deviations, estimated an annual average of 26,000 (range 0 – 49,000) and 24,000 (range 3,000 – 47,000) deaths, respectively. All model estimates were significantly correlated. Conclusions: The four models yielded similar estimates of influenza-associated deaths (annual averages ranging from 22,000 – 34,900 deaths from the 1976/1977 through 2002/2003 seasons). We also found that estimates of influenza-associated deaths varied by age group and virus type and subtype.

Abstract O8

Hospital Acquired Influenza in Ontario, Canada, 2005-2007

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Background: Although many patients with influenza are admitted to acute care hospitals, few influenza outbreaks in acute care hospitals have been described, and little is known about the epidemiology of hospital-acquired influenza. Objective: To describe the clinical features, treatment and outcomes of hospital acquired influenza. Methods: Population-based surveillance for laboratory confirmed influenza acquired in acute care hospitals has been on-going in Toronto/Peel region, Ontario, CA, (population 4M) since January 1, 2005. Hospital acquired influenza is that with onset >72 hours after hospital admission, cases readmitted to hospital with influenza <96 hours after discharge are also included. Laboratory confirmation is defined as having a positive direct test, PCR, or culture for influenza A or B. Consenting, eligible patients are enrolled. Results: From 1/2005 to 1/2007, 85 cases of laboratory confirmed, hospital acquired cases of influenza have been identified. Rates per hospital per influenza season range from 0 to 1.7 per 1000 admissions (median 0.2 per 1000 admissions). Most cases are sporadic, rather than outbreak associated. The median age of patients was 78 (range <1 month - 95 years); 43 were female. Seventy-two cases (85%) were due to influenza A and 13 (15%) to influenza B. Prior influenza vaccination status was known for 69 patients; 34 (49%) had been vaccinated. Seventy-one patients (84%) had an underlying illness qualifying them for influenza vaccine. The specimen (all NP swabs) yielding influenza was obtained a median of 1 day (range 0-7 days) after the onset of symptoms. Forty-nine patients (58%) were treated with antivirals (all with oseltamivir); 21 of these received their first dose of oseltamivir within 48 hours of the onset of symptoms. Twelve patients (14%) died during their hospitalization; 6 of these deaths occurred within 7 days of the onset of symptoms of influenza. Three of these deaths occurred in 33 untreated patients, and three in 49 oseltamivir treated patients. Conclusion: Hospital-acquired influenza is a relatively common disease, which is most often sporadic and is associated with a significant mortality rate. Even in a hospital setting, early diagnosis and treatment was not easily achieved.

Workshop Topic #2

Virus Structure/Function and Receptor Binding

Oral Abstracts

Abstract O9

Expression of Avian Influenza Virus Receptors and H5N1 Virus Infection in Human Respiratory Tract

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While human infections caused by avian H5N1 virus have raised concern regarding a potential pandemic, genetic studies indicate that the H5N1 viruses causing human infections are still of the avian type. Receptor specificity is known to restrict virus cross-species transmission, with SA a2,6 Gal and SA a2,3 Gal sialic acids recognized by human and avian influenza viruses, respectively. Previous studies indicated that the lower respiratory tract is more susceptible to H5N1 avian influenza virus infection. However, H5N1 virus infection of upper respiratory tract is less defined. This study examined 144 sections of tissues from different respiratory tract sites, obtained from 88 individuals, for the distribution of SA a2,6 Gal and SA a2,3 Gal along the human respiratory tract. We found SAa2,3Gal expression is more regularly observed in lower, rather than upper respiratory tract epithelial cells, being most prevalent in lung alveolus cells, with 79% of cases scoring positive. Expression of SAa2,3Gal was comparatively rare in human trachea and bronchus sections, with 20% and 42.5% of cases positive, respectively. In contrast, expression of human influenza virus receptor, SA a2,6Gal, was mainly detected in the upper respiratory tract and to a lesser degree in the alveolar epithelium. Although the majority of H5N1 human cases have been under 40 years old, we found no significant age-associated difference in the expression of SAa2,3Gal and SAa2,6Gal in the respiratory tract. Immunohistochemical examination of respiratory tract tissues obtained postmortem from a 2003 H5N1 human case in Hong Kong demonstrated virus infection in the trachea and bronchus cells with no apparent expression of the avian type receptor, SAa2,3Gal. Ex vivo infections, using both human and avian H5N1 virus isolates to infect tissues surgically removed from the respiratory tract of patients suffering non-infectious diseases, found avian H5N1 virus is able to infect human trachea and bronchus epithelial cells which do not express SA a2,3 Gal receptor. It will be important to study other factors which may contribute to the currently-observed pattern of susceptibility to H5N1 infection.

Abstract O10

SCHOLARSHIP Promising Junior Investigator Scholarship

Influenza Virus Structure by Cryo-electron Tomography

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To date, influenza virus has been intractable to 3-D structural analysis because its pleiomorphy has prevented visualization by image reconstruction of electron micrographs via methods that rely on averaging many identical particles, which have been applied successfully to many icosahedrally symmetric viruses. Therefore, we have used cryo-electron tomography (cryo-ET) - a technique capable of rendering the 3-D structures of individual macromolecular particles in their native states - to visualize influenza virions of the type A, egg-adapted, X-31 (H3N2) strain. The tomograms resolve the two viral glycoproteins, hemagglutinin (HA) and neuraminidase (NA), the matrix protein layer lining the viral envelope, and internal configurations of ribonucleoprotein (RNP) complexes. The data also reveal the stems that link the glycoprotein ectodomains to the membrane and interactions among and between the glycoproteins, the matrix, and the RNPs that presumably coordinate the assembly of nascent virions from host cells. Five classes of virions are distinguished by features of their matrix layer and RNP organization. Some virions have substantial gaps in their matrix layer (molecular "fontanels"), and others appear to lack a matrix layer entirely, suggesting the existence of an alternative budding pathway in which matrix protein is minimally involved. Also, the potential application of cryo-ET to gain structural information on virus assembly and entry will be discussed briefly.

Abstract O11

Structural Comparison of the Nucleoprotein From Influenza A Virus With Other NSV Nucleoproteins

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Structures of the nucleoprotein of three negative strand RNA virus families, borna disease virus, rhabdovirus and influenza A virus, are now available. Structural comparisons of the nucleoprotein (NP) of FLUAV with others showed that the topology of the RNA binding region from the three proteins is the same. The RNA was shown to fit into a cavity

between the two domains of the RNA binding region in the rhabdovirus nucleoprotein. Two helices connecting the two domains characterize the center of the cavity. The nucleoproteins contain at least 5 conserved helices in the N-terminal domain and 3 conserved helices in the C-terminal domain. Since all negative strand RNA viruses are required to have the ribonucleoprotein (RNP) complex as their active genomic templates, it is perceivable that the (5H+3H) structure is a common motif in the nucleoprotein of negative strand RNA viruses. The conformation of the influenza A virus nucleoprotein theoretically could change when it encapsidates the genomic RNA. The structure of FLUAV RNP may actually be more similar to that of rhabdovirus than previously thought.

Abstract O12

The Mechanisms By Which Influenza A Virus Nucleoprotein Forms Oligomers And Binds RNA

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Influenza A viruses pose a serious threat to world public health, particularly the currently circulating avian H5N1 viruses. The influenza viral nucleoprotein forms the protein scaffold of the helical genomic ribonucleoprotein complexes, and has a critical role in viral RNA replication. Here we report a 3.2 angstrom crystal structure of this nucleoprotein, the overall shape of which resembles a crescent with a head and a body domain, with a protein fold different compared with that of the rhabdovirus nucleoprotein. Oligomerization of the influenza virus nucleoprotein is mediated by a flexible tail loop that is inserted inside a neighbouring molecule. This flexibility in the tail loop enables the nucleoprotein to form loose polymers as well as rigid helices, both of which are important for nucleoprotein functions. Single residue mutations in the tail loop result in the complete loss of nucleoprotein oligomerization. An RNA-binding groove, which is found between the head and body domains at the exterior of the nucleoprotein oligomer, is lined with highly conserved basic residues widely distributed in the primary sequence. The nucleoprotein structure shows that only one of two proposed nuclear localization signals are accessible, and suggests that the body domain of nucleoprotein contains the binding site for the viral polymerase. Our results identify the tail loop binding pocket as a potential target for antiviral development.

Abstract O13

Probing of Specific Residues Near the Fusion Peptide of the Neutral pH Influenza Hemagglutinin Structure for Roles in Triggering Membrane Fusion

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Influenza virus entry occurs in endosomes, where acidification triggers irreversible conformational changes of the hemagglutinin glycoprotein (HA) that are required for membrane fusion. The acid-induced HA structural rearrangements have been well documented, and several models have been proposed to relate these to the process of membrane fusion. However, details regarding the role of specific residues in the initiation of structural rearrangements and membrane fusion are lacking. Here we report the results of studies on mutants with changes at several ionizable residues that are accessible to solvent in the HA precursor structure, but become buried or occluded by the newly-formed fusion peptide following cleavage into HA1 and HA2. The amino acids at these HA positions near the fusion peptide of the neutral pH structure are either highly conserved or fall into one of two groupings that segregate along clade-specific lines. Several of the mutant HAs examined displayed altered membrane fusion properties. Among the phenotypes obtained were examples of substitutions that lead to an increase in HA stability at reduced pH, suggesting a role for particular residues in providing the initial trigger for membrane fusion. The results are discussed in relation to new information on potential HA structural intermediates for the fusion process, and the role particular residues near the fusion peptide that vary along clade-specific lines.

Abstract O14

Hemagglutinin Glycosylation Promotes Spread of Infection of a Highly Pathogenic Avian Influenza Virus (H7N1)

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In 1999/2000 there was an outbreak of avian influenza A/H7N1 viruses among different avian species in poultry farms in Northern Italy. The viruses which were still low pathogenic

for domestic birds in the beginning of the outbreak became highly pathogenic in late 1999. In addition to a multibasic cleavage site, most of the later isolates also acquired glycans close to the receptor binding site of the HA either at position 123 or 149. In order to evaluate wether these glycosylation sites affect pathogenicity, we generated recombinant viruses with no (G0), one (G1, G2) or both of these glycans (G1+2). The G0 mutant grew to 100 times lower titers than G1+2 in cultured chicken fibroblasts as well as in the allantoic cavity of infected chicken embryos. G0 differed also from G1, G2 and G1+2 by a significantly higher mean death time of chicken embryos and by a lower elution rate from cell cultures These observations indicate that the glycans at position 149 and 123 contribute to pathogenicity by facilitating virus release from infected cells.

Abstract O15

Deletions of Neuraminidase in a Fujian-Like Influenza Virus (A/Oklahoma/323/03, H3N2)

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Influenza neuraminidase (NA) is recognized as a receptor destroying enzyme, necessary to cleave sialic acid and release newly budded virus from self-aggregation by hemagglutinin (HA). Influenza virus A/Oklahoma/323/03 (H3N2) agglutinates human erythrocytes but does not elute from them, indicating that NA does not cleave the receptor bound by the HA. To investigate if replication requires NA activity, we passaged A/Oklahoma/03 at different multiplicities, and found that NA activity was retained at limiting dilution but lost when cells were infected at higher multiplicity. Sequences of PCR products showed that the NA RNA segment was deleted to fragments of 300-800 nucleotides with retention of both the 5′ and 3′ ends. The virus grows efficiently in MDCK cells without NA activity indicating there is no dependence on receptor destroying activity, and replication of A/Oklahoma/03 in MDCK cells is resistant to oseltamivir. There was no change in HA activity or sequence. There may be positive selection for shorter NA segments, with little or no requirement for NA activity, or negative selection against NA activity but a requirement for at least the 3′ and 5′ NA RNA sequences. Receptors in humans are presumably different to those on MDCK cells, but there is potential for H3 HA to change binding specificity such that NA is not required and NA inhibitors will be ineffective.

Abstract O16

SCHOLARSHIP International Scholarship

Neuraminidases of H9N2 Influenza Viruses Isolated From Different Hosts Display Various Substrate Specificity

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Using the fluorescent neuraminidase (NA) assay developed by us earlier [Anal. Biochem., 34: 190 (2005)] we evaluated specificity of seven influenza virus neuraminidases towards seven BODIPY-labeled sialyloligosaccharides: 3`SiaLac, 3`SiaLacNAc, SiaLe^C, SiaLe^A, SiaLe^X, 6`SiaLac and 6`SiaLacNac. Substrates differed by: the type of linkage (α2-3 or α2-6) between the Neu5Ac and Gal residues, the presence of N-acetamide group at position 2 of the Glc residue, core type (1-3 or 1-4), and the presence of fucose connected to GlcNAc residue. Specificity profiles of duck, geese, turkey, chicken, swine and human viruses studied were unique and no identical profiles were found. Nevertheless, the NAs may be divided into distinct groups on the base of hydrolytic activity towards α2-3/α2-6 sialosides. First group is formed by a duck virus. High hydrolytic activity towards SiaLe^A and, on the contrary, very low towards α2-6 sialosides are the main features of this virus. Goose and turkey virus NAs fall into the second group characterized by low activity towards α2-6 sialosides and SiaLe^A. Besides, NAs of two turkey viruses distinguished the inner core of α2-3 oligosacharides. The amino acid sequences of all NAs forming this group were similar, but significantly different from other neuraminidases. Chicken, swine and human virus NAs form the third group. Low level of the α2-3/α2-6 ratio (about 10) is the main difference of the third group NAs, although, similar to the second group, hydrolysis efficacy towards SiaLe^A within this group was equal to efficacy towards the α2-6 sialosides. NA sequences inside the third group were very similar, but differed from those for the duck, turkey and goose viruses. Thus influenza viruses isolated from different hosts demonstrate different NA substrate specificity profiles. The obtained results may suggest that viral replication in different organisms require different substrate specificity of the neuraminidase.

Workshop Topic #3

Outbreak and Pre-Pandemic Response

Oral Abstracts

Abstract O17

Successful Control of Institutional Outbreaks of Influenza 2003/2004 Using Anti-virals Tamiflu in Taiwan: Can This Experience Be Applied to H5N1 Control?

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The common clinical manifestations shared by severe respiratory virus infection (SARS) and influenza constitute a big challenge for the successful control of SARS during the winter flu period 2003/2004 in Taiwan. In view of the belief that the far majority of flu-like illness (ILI) will actually represent influenza in the flu season and to avoid the confusion and panic of SARS suspect in the post-SARS period, the Center for Disease Control of Taiwan implemented a national policy of ILI control measure which includes the stockpile of antivirals-Tamiflu for the control of flu-like illness (ILI) in the 2003/2004 winter period, particularly in the institutional outbreak of ILI illness. The control policy includes the real-time reporting of ILI clustering in the institutions such as nursing home, school, military, and jail. Upon receiving the reporting, a task force team was immediately dispatched to investigate the outbreak. After sampling the throat swab specimens, anti-virals were given to the patients and close contacts. The transmission and clinical outcome were then followed up in the coming weeks. A total of 68 institutional ILI outbreaks were reported in the 2003/2004 winter period. Among which, 24 % of the cases (311 samples taken, 76 positive) were laboratory-proved to be caused by influenza by the laboratory by a combination of ELISA kit for influenza, PCR for influenza and SARS-CoV, and viral culture. None of them were caused by SARS-CoV. All the ILI outbreaks were successfully controlled by this measure and no more transmission was reported following the application of anti-virals. In parallel to this control measure, the ILI prevalence and institutional mortalities due to ILI reduced remarkably in 2003/2004 as compared to the periods before SARS. Therefore, the control policy of ILI illness by combining public health measures and the implementation of stockpile of antivirals Tamiflu proves to be effective and successful measures to control the institutional outbreaks of influenza. This strategy can be considered to be applied during the H5N1 pandemic flu.

Abstract O18

Public Health Response to an Outbreak of Highly Pathogenic Avian Influenza (H5N1) on a Poultry Farm in Suffolk, UK

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On 3rd February 2007, an outbreak of highly pathogenic avian influenza (HPAI) A/H5N1 of asian lineage was confirmed on a poultry farm in Suffolk, UK. Turkeys in one of 22 sheds on site had shown signs of sudden increasing mortality. Culling of all 160,000 turkeys at the farm commenced rapidly and ended on 5th February 2007. A local Incident Management Team (IMT) consisting of the Health Protection Agency (HPA) and its local public health partners was formed immediately to address the human health implications, supported by expert advice from the HPA Centre for Infections. Following risk assessment all people requiring antiviral prophylaxis and seasonal flu vaccination were identified. Within 12 hours, 115 people had received oseltamivir prophylaxis, dispensed at a local community hospital near the farm, as well as on the infected premises. In total, 482 people received oseltamivir, and were offered seasonal influenza vaccine with 68% uptake. 17 exposed persons had various symptoms that required further assessment. In particular 4 were tested for H5N1- all were negative. 1 person tested positive for influenza A/H3N2. The public health response to this outbreak involved the largest ever mass provision of oseltamivir prophylaxis in the UK. We will describe in further detail the practical issues of delivering the public health response to a serious H5N1 avian influenza outbreak in poultry.

Abstract O19

Rapid Assessment of an Avian Influenza Outbreak–South Sudan, 2006

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Background: The global spread of Avian Influenza (AI) H5N1 virus among poultry and humans has raised concern that the virus will cause the next pandemic. In South Sudan, poor public and animal health infrastructure make detection of AI outbreaks in birds and humans especially difficult. In Juba, South Sudan, H5N1 virus was identified in backyard chickens in September, 2006. To assess for human cases of AI and additional AI cases in poultry in Juba, we conducted a house-to-house survey. Methods: From September 20-25, 2006, 26 investigators from the state and national Ministry of Health and Ministry of Animal Resources and Fisheries, interviewed households in Juba using a questionnaire to assess whether people had sick or dead chickens in their home and whether people were sick themselves. Households were identified by cluster sampling. We defined a suspected human case of AI as anyone who had fever and cough, shortness of breath or difficulty breathing within the prior three weeks along with a history of contact with sick or dead birds. The Ministry of Health was informed if any people reported symptoms consistent with AI, and veterinary officers were informed if sick or dead poultry were found. GPS coordinates were recorded for households reporting sick humans or sick or dead chickens. Results: The survey was conducted for four days, and investigators covered an area of approximately 9 km^2. A total of 1,613 households were surveyed. 80.6% (1300/1613) of the households reported having either chickens or ducks. In all, 14% (226/1613) of households reported having chickens that died within the previous two weeks. Seventy-nine sick chickens and ducks were reported. Oneperson met the case definition for suspected AI and tested negative for AI. Conclusions: Our rapid survey did not identify any human AI cases in Juba. In countries with poor surveillance where new AI outbreaks are reported, rapid assessments through door-to-door household surveys can be effective tools in identifying suspected human cases and evaluating the extent of AI outbreaks in poultry.

Abstract O20

Risk of Occupational Transmission of Avian Influenza A (H5N1) Virus, Northern Nigeria, March 2006

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Background: The unprecedented spread of avian influenza A (H5N1) among poultry poses an increasing influenza pandemic risk. Sporadic human H5N1 cases with high mortality have occurred after contact with diseased poultry infected with H5N1 virus. H5N1 was confirmed among poultry in February 2006. We conducted the first serologic survey in Africa to assess the occupational risk of H5N1 virus infection. Methods: From March 28 through April 3, 2006, poultry workers (PWs) in Kano State, Nigeria who worked at a farm or market during a poultry outbreak that met the Ministry of Agriculture's case definition for suspected or confirmed H5N1 were invited to enroll. Participants were asked about types of poultry exposures from January through enrollment, medical history, and health care utilization. Serum collected from participants was tested for H5N1 antibodies by microneutralization assay. Results: Among 295 PWs, the median age was 28 years (range 12 - 58), 275 (93%) were male, and 141 (48%) had secondary school education. PWs reported a median of 14 days of direct physical contact with suspected or confirmed H5N1-infected poultry. Most PWs (75%) reported that they would seek care at a hospital for worsening symptoms of influenza-like illness (ILI), but 52% reported that they would not always take gravely ill family members to a hospital. All participants tested negative for H5N1 antibodies. Conclusion: Despite widespread exposure to poultry likely infected with H5N1 virus, no risk of H5N1 virus infections were was identified among survey participants. Additional sero-surveys will be useful to assess the risk of human H5N1 virus infection as the virus continues to evolve and to spread among poultry.

Abstract O21

Seroprevalence of Antibodies to Avian Influenza A (H5N1) Virus Among Residents of Villages With Confirmed Human H5N1 Cases, Thailand, 2004-05

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Background: Nearly 300 human cases of highly pathogenic avian influenza A (H5N1) virus infection resulting in severe respiratory disease with high mortality have been reported in 12 countries since 1997. Studies conducted in 1997 reported a seroprevalence of H5N1 antibodies of 2-10% among poultry workers, cullers and health care workers. However, limited data are available on the extent of mild and asymptomatic H5N1 virus infections since 1997. We conducted a cross-sectional study among village residents to 1) determine the seroprevalence of H5N1 antibodies, and to 2) describe frequencies of specific risk behaviors for H5N1 in this population. Methods: Participants were recruited from villages in four Thai provinces, each of which had a resident with confirmed H5N1 during 2004-05. Eligible participants were randomly selected or enrolled by convenience sampling. Standardized questionnaires were used to gather demographic, symptoms and risk behavior data. Sera were collected and tested for H5N1 neutralizing antibodies by microneutralization assay. Results: Of the 901 participants enrolled from four villages, the median age was 40 years, range 2-101 years, and 42.4% were male. Most (85.5%) of participants were selected by convenience sampling. Established risk factors for H5N1 reported by participants included contact with backyard poultry (68.1%), and contact with sick or dead chickens (33.3%) without use of any protective equipment. All participants tested negative for H5N1 neutralizing antibodies. Conclusions: We found no evidence of mild or asymptomatic H5N1 virus infections despite exposure to H5N1 among study participants. This suggests that H5N1 viruses circulating in Thailand during 2004-05 were transmitted inefficiently to humans. However, ongoing H5N1 surveillance and further seroprevalence studies in Thailand are needed as H5N1 viruses continue to evolve. Further efforts are needed to reduce exposures to sick and dead backyard poultry among rural villagers in Thailand.

Abstract O22

"Pandemic Quality" Surveillance: How the UK is Preparing for Daily Health Information During a Flu Pandemic

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Introduction: The Health Protection Agency (HPA) has national responsibility for monitoring influenza in England and for coordinating influenza surveillance within the United Kingdom (UK). Major steps have been taken to ensure that the UK surveillance strategy will meet the information needs of those involved in the pandemic response. The UK Government Health Departments require daily data to inform measures taken to slow the spread of the disease; inform decisions for managing the pandemic's impact; provide accurate information to the National Health Service on the current and forecasted situation and to provide accurate and regular information to the public. Methods: A UK wide pandemic surveillance strategy has been developed by the HPA, the Department of Health (DH), England and devolved administrations. Exercises have been planned to test the feasibility of daily data collection and reporting. Results: Existing UK national influenza data collection systems have been evaluated and modified to ensure harmonisation of data collection, recording and reporting and also for capturing real-time information (such as the new QFLU network). New systems are under development to capture information on treatments that may be delivered outside recognised primary care facilities together with pilot schemes to investigate collecting supplementary sentinel hospital data on pressures on hospital services. A 'one-stop integrated framework' web based portal created by the DH will be used with appropriate security, by those submitting, managing and reporting information during the pandemic. Templates for data collection have been agreed, ranging from a daily and weekly UK wide epidemiological report, reports from an enhanced dataset of the first few hundred avian or pandemic cases, a daily report of modelled estimates of cases and deaths and a weekly antibiotic susceptibility report. Daily reports will be supplemented with data on wider health service pressures and disseminated to the government office responsible for ministerial and national briefings. Once daily key messages are

in the public domain, data will be accessible through the DH website for professional users. Conclusion: Pre-planned and pre-prepared surveillance methods have been shown to be feasible within the UK for meeting information needs at local, regional and national levels during the pandemic period.

Abstract O23

Assessing the Preparedness Status for an Influenza Pandemic in the European Union

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As soon as the European Centre for Disease Control (ECDC) was established in 2005, its Preparedness and Response Unit started a process of assessing the pandemic influenza preparedness status in the European Union (EU) in collaboration with the WHO office for Europe and the European Commission (EC). An assessment tool has been developed and revised to allow for adaptation of evolving plans in the EU Member States (MS). The tool follows the structure of the components of the EC communication on pandemic influenza, the WHO global influenza preparedness plan and checklist for pandemic influenza preparedness planning and helps countries to identify gaps and plan future work. A set of 20 key indicators has been identified to objectively monitor the MS preparedness status, including protection against human seasonal influenza and transmission of highly pathogenic avian influenza. The assessment is conducted through country visits usually lasting three to four days associating the national authorities, ECDC, EC and WHO EURO. The visit results in an agreed action list for strengthening preparedness and a follow-up programme identifying additional support needed from ECDC and partner organisations. In March 2007, a report has been published describing the status of preparedness in the European Union, Norway and Iceland. The main findings are that considerable progress has been made in strengthening preparedness against pandemic influenza. All MS have developed preparedness plans and much is done to make them operational at local level. However, the main challenge lies in further engaging with non-health sector issues and across governments. As pandemic preparedness planning is a continuous process, the monitoring of progress in strengthening preparedness in MS will continue in the coming years, aiming at reaching a level of preparedness that the primary health care system will be able to timely deliver treatment, the hospital health care system to deliver acute care to influenza patients as well as continuing essential treatment for non-influenza related conditions and essential services will continue to function at local level.

Abstract O24

The Vaccine Industry's Contribution to WHO's Global Pandemic Influenza Action Plan to Increase Vaccine Supply

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As vaccines are likely to play the most important role in minimizing the public health impact of a future pandemic, influenza vaccine manufacturers represent a key partner to assist WHO achieve its global pandemic preparedness objectives. In October 2006, WHO issued a "global pandemic influenza action plan to increase vaccine supply", which describes three priority areas for action: 1) To increase seasonal influenza vaccine use; 2) To increase vaccine production capacity; 3) To further R&D of (pandemic) influenza vaccines. Influenza vaccine manufacturers have already contributed to these objectives by investing in increasing production capacity, and also in R&D of pandemic prototype vaccines. Indeed, industry has increased global production capacity for influenza vaccines from 300 million doses in 2003 to 350 million doses in 2005, and it is expected to increase to 500 million doses by 2008. In addition, industry is making considerable progress in the development of new adjuvants as well as alternative production technologies such as cell-culture. However, industry's role in increasing the use of seasonal influenza vaccines is limited to ensuring that production capacity meets market demand, which is driven by vaccination guidelines and more importantly their effective implementation. Therefore, the responsibility for this part of the WHO global action plan lies predominantly with National Health Authorities. In many countries, less than one third of the elderly and patients at risk receive an annual influenza vaccination. Therefore increasing this level of usage relies on more robust implementation of national immunization guidelines. This situation presents a concerning paradox: despite expectations of vaccine shortages in the event of a pandemic, there remains serious under-utilization of seasonal influenza vaccines. Expansion of global vaccine production to levels of "pandemic needs", as suggested in the WHO global action plan, will only be sustainable, if there is a balance between global vaccine production capacity and seasonal vaccine usage. Pandemic presents society with a global challenge, requiring global access to vaccines. Therefore, the international community, WHO, National Health Authorities and industry must come together in partnership to achieve a comprehensive level of pandemic preparedness, ensuring timely access to vaccine for those who need it.

Workshop Topic #4

Replication and Assembly

Oral
Abstracts

Abstract O25

Control of Influenza Viral Replication and Transcription

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The mechanisms regulating the synthesis of mRNA, cRNA and vRNA by the influenza A virus RNA-dependent RNA polymerase are not fully understood. Early results suggested that the RNA polymerase "switched" from a transcriptase to a replicase during the viral life cycle in response to the expression of viral proteins. However, recently we proposed an alternative model suggesting that replication of influenza virus is regulated by stabilization of the replicative intermediates. According to this model, the virion-associated polymerase is capable of synthesizing both mRNA and cRNA. We now provide evidence that virion-derived vRNPs (vvRNPs) synthesize both mRNA and cRNA, in vivo and in vitro. In vivo, we demonstrate that cRNA is bound by expressed viral polymerase and NP, whereas the in vitro assay is enabling us to explore in detail the mechanisms of primer-dependent and primer-independent transcription. For example, the de novo synthesis of cRNA was found to be more sensitive than the capped primer-dependent synthesis of mRNA to the concentration of ATP, CTP and GTP. We conclude that vvRNPs intrinsically possess both transcriptase and replicase activities and that there is no switch in the synthesis of mRNA to cRNA during the influenza virus life cycle.

Abstract O26

Intranuclear Dynamics of the Influenza A Virus RNA Polymerase as Revealed by Live Cell Imaging Studies

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Influenza A virus replicates and transcribes its genome in the nucleus of infected cells. The viral genome (vRNA) and its replicative intermediate (cRNA) both associate with nucleoprotein (NP) oligomers and the heterotrimeric viral polymerase (PA, PB1, PB2) to form viral ribonucleoproteins vRNPs and cRNPs. These viral RNPs are known to undergo a complex set of interactions with host cell nuclear components, including cellular RNA Pol II, histones and various mRNA processing and export factors. We have established a live cell imaging system using transfected cells that allows for the observation of fluorescently tagged influenza polymerase subunits on their own, in combination with other polymerase subunits or in the setting of a model c or vRNP. We have employed this system in FRAP (Fluorescence Recovery After Photobleaching) studies in which a defined area of the nucleus is selectively and irreversibly photobleached by exposure to high laser intensities. Subsequent monitoring of fluorescence recovery using low intensity laser illumination allows for analysis of the mobility and dynamic behaviour of influenza virus proteins and ribonucleoproteins in the nuclear environment. While individual polymerase subunits display very high nuclear mobility, the full polymerase complex shows a marked decrease in mobility. The fluorescently tagged polymerase in RNP settings displays an equally, if not even more, reduced mobility phenotype. Ongoing experiments are probing the involvement of cellular RNA Pol II as well as the host-range adaptation mutation PB2-E627K in the intranuclear dynamics of the viral polymerase complex.

Abstract O27

Role of a Host Factor in the Intracellular Transport and Assembly of the Influenza Virus RNA Polymerase Complex

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The influenza A virus RNA-dependent RNA polymerase is a heterotrimeric complex of PB1, PB2, and PA subunits. It performs transcription and replication of the viral RNA genome in the nucleus of infected cells. We have identified a nuclear import factor, Ran Binding Protein 5 (RanBP5), also known as karyopherin β3, importin β3, or importin 5, as an interactor of the PB1 subunit. RanBP5 interacted with either PB1 alone or with a PB1-PA dimer, but not with a PB1-PB2 dimer or the trimeric complex. The interaction between RanBP5 and PB1-PA was disrupted by RanGTP in vitro allowing PB2 to bind to the PB1-PA dimer to form a functional trimeric RNA polymerase complex. We propose a model in which RanBP5 acts as an import factor for the newly synthesized polymerase by targeting the PB1-PA dimer to the nucleus. In agreement with this model, siRNA mediated knock down of RanBP5 inhibited the nuclear accumulation of the PB1-PA dimer. Moreover, siRNA knock down of RanBP5 resulted in the delayed accumulation of viral RNAs in infected cells confirming that RanBP5 plays a biological role during the influenza virus life cycle.

Abstract O28

Mutations at Alternative Splice Sites of M1 mRNA Affect the Viability and Growth Rate of Influenza A Virus

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It is known that different amino acid sequences of influenza viral proteins contribute to different phenotypes. However, the diversity and its impact on non-coding regions or splice sites are not yet intensively studied. This report focuses on the sequences at the boundary of alternative 5′ splice site of M1 mRNA which is transcribed from segment 7. We introduced six different mutations at the splice site junction by reverse genetics (A/WSN/33 as backbone) and examined the viability and growth rate for those mutants. G12C (G to C mutation at position 12 which is the first nucleotide of intron for mRNA3 5′ ss), C51G (exon boundary for M2 mRNA 5′ss) and G146 C (first nucleotide of intron for mRNA4) are lethal mutations. G11C (exon boundary for mRNA3), G52C (first nucleotide of intron for M2 mRNA) and G145A (exon boundary for mRNA4) were rescued, however, showing significantly lower growth rates. These results suggest that mutations at the splice site junctions can affect influenza A virus production. Notably, G11C, G12C, G52C, and G145A mutations do not alter amino acid residue in the encoded M1 or M2 proteins; however, these mutations cause either lethality or lower the growth rate for the recombinant viruses. We further examined precursor (M1 mRNA) and the spliced products (M2 mRNA, mRNA3, and mRNA4) from transfected cells. M1 mRNA and all the spliced mRNAs were detected in wild-type as well as in G11C mutant; yet barely detected for G12C mutant. It is likely that G to C mutation at position 12 may destroy the promoter in vRNA since we also found significant reduction in vRNA synthesis. On the other hand, mRNA3 and mRNA4 were significantly lower for C51G mutation and the production of M2 mRNA was impaired for G52 mutations. In addition, mRNA4 levels were significantly reduced when substitution of G145A and G146C. We furthermore found that mRNA levels didn't correlate to the protein expression levels. Notably and surprisingly, M2 protein in C51G transfected cells was not expressed, despite of its M2 mRNA was produced efficiently, suggesting that C at position 51 is important for M2 protein production.

Abstract O29

Differential Polymerase Activity in Avian and Mammalian Cells Determines Host Range of Influenza Virus

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We have recently shown that mutations in the polymerase genes causing increased polymerase activity in mammalian cells are responsible for adaptation of the avian influenza virus SC35 (H7N7) to mice (Gabriel et al., Proc Natl Acad Sci USA 102, 18590-5). To find out whether these mutations enhance the polymerase activity of the mouse-adapted variant SC35M only in mammalian cells or also in avian cells and whether transcription or replication were affected, we compared now mRNA, cRNA, and vRNA synthesis in avian and mammalian cells. Enhanced transcription and replication activity of SC35M was dependent on mammalian cells. In avian cells, replication and transcription of SC35M were reduced, whereas both activities were increased with SC35. The differences in the polymerase activity of both viruses in avian cells were reflected by a reduced pathogenicity of SC35M for chick embryos. Analysis of individual SC35M mutations revealed host-dependent as well as host-independent effects on polymerase activities. Thus, PA mutation K615N enhanced only replication activity in mammalian cells. PB2 mutations D701N and S714R as well as NP mutation N319K increased transcription and replication in mammalian cells. PB1 mutation S678N enhanced both processes in mammalian and avian cells, as well. The PB2 and NP mutations of SC35M enhanced also the polymerase activities of reconstituted ribonucleoprotein complexes of an unrelated avian strain in mammalian cells, indicating that they play an important role in host adaptation of influenza-A-viruses, in general. Taken together, these results indicate that conversion of SC35 to SC35M is linked to a decrease of polymerase activity in avian cells and a concomitant increase in mammalian cells. Thus, the efficiency of the viral polymerase activity is a determinant of host specificity.

Abstract O30

Functional Comparisons Between Human and Avian Influenza Virus PB2 Proteins

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The first recorded cases of humans infected with an H5N1 avian influenza virus occurred in 1997 and since then more than 200 human infections have been confirmed – more than 50% of which were fatal. The understanding as to how these avian viruses were able to infect humans and be highly pathogenic remains unknown. One factor which has been determined to play a role in the viruses' ability to overcome host range restriction and be highly lethal in animal models is the viral PB2 polymerase subunit. However, the mechanisms by which this protein overcomes host range restriction are not known. Here we demonstrate that the activity of influenza viral polymerases with either avian or human influenza virus PB2 proteins do not correlate with virulence in humans. However, the PB2 proteins from avian and human influenza viruses differ in their ability to localize to the mitochondria of the host cell.

Abstract O31

The Cytoplasmic Domain of Influenza B Virus BM2 Protein Plays Critical Roles for the Production of Infectious Virus

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Influenza B virus BM2 encoded by RNA segment 7 together with matrix M1 protein is a type III integral membrane protein with the CinNout orientation in the envelope. By analysis of BM2 knockout mutant, BM2 has been suggested to be a necessary component for a capture of M1-viral ribonucleoprotein (vRNP) complex at the plasma membrane and for an incorporation of vRNP complex into virion in assembly process. The BM2 consists of 109 amino acid (aa) residues and possesses the longest cytoplasmic tail than that of other three integral membrane proteins, hemagglutinin, neuraminidase and NB. To explore whether the cytoplasmic tail of BM2 is important for infectious virus production, a series of BM2 deletion mutants lacking 3 to 9 aa residues at the carboxyl (C)-terminus, BM2Δ107-109, BM2Δ104-109 and BM2Δ101-109, were generated by reverse genetics. Intracellular transport and incorporation into virions of the truncated BM2 proteins were indistinguishable from those of wild type BM2. The BM2Δ107-109 mutant produced similar level of infectious virus to wild type virus and revealed spherical morphology. By contrast, the BM2Δ104-109 and BM2Δ101-109 mutants produced noninfectious viruses containing dramatically reduced vRNP complex like BM2 knockout mutant and formed enlarged and irregularly shaped virions. Moreover, the BM2 mutants with three consecutive alanine substitution in the 98-109 region produced comparable level of infectious viruses to wild type virus. These results suggest that for proper packaging of vRNP complex in virus assembly and morphogenesis of virus particles, there is a length requirement but not a specific sequence requirement in the C-terminus of the BM2 cytoplasmic tail.

Abstract O32

Rescue of Influenza C Virus From Recombinant DNA

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The rescue of influenza viruses by reverse genetics has been described only for the influenza A and B viruses. Based on a similar approach we developped a reverse genetics system that allows the production of influenza C viruses entirely from cloned cDNA. For this purpose, human embryonic kidney cells (293T) were transfected simultaneously with seven plasmids that direct the synthesis of each of the seven viral RNA segments of the C/JHB/1/66 virus under the control of the human RNA polymerase I promoter and with four plasmids encoding the viral nucleoprotein and the PB2, PB1 and P3 proteins of the viral polymerase complex. This strategy yielded between 10^3 and 10^4 plaque forming units of virus per ml of supernatant at 8-10 days post-transfection. Using this method influenza C viruses harboring mutations at positions 284 (Thr-->Ala) and/or 465 (Thr-->Ile) in the hemagglutinin esterase fusion (HEF) protein were engineered and successfully rescued. The viruses were found to be genetically stable upon five consecutive passages on SK 93/2 cells. Growth kinetics were performed on MDCK II cells pretreated with bovine brain gangliosides (BBG) in order to provide the Neu5,9Ac2 receptor used by influenza C virus which is lacking on these cells. Under those conditions, the Thr284Ala virus grew to titers similar to the wild-type (wt) virus whereas the Thr465Ile virus or the double mutant grew to 10-fold higher titers. Furthermore, as previously described by Szepansky et al. (1992 Virology 188:85-92), the Thr284Ala substitution resulted in a change of receptor specificity of the virus which was found to be able to grow to high titers on untreated MDCK II cells in the presence of a high concentration of trypsin TPCK. We thus demonstrated that the reverse genetics system decribed here provides a means to study the effects of viable point mutations introduced into the genome of influenza C virus.

Workshop Topic #5

Developments in Diagnostic and Serologic Techniques

Oral Abstracts

Abstract O33

Human Influenza Virus Detection in Stool Specimens From Children Presenting With Acute Respiratory Illness and Diarrhea in Indonesia

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Background: Although primarily an acute respiratory disease with systemic symptoms, influenza is associated with diarrhea in young children. Whether human influenza virus is present in diarrheal specimens of influenza patients is unknown. Detection of avian influenza A (H5N1) virus RNA and virus isolation has been reported in rectal swab specimens from fatal human H5N1 cases with diarrhea. We conducted a study to: 1) determine if influenza virus was detectable in the stool of pediatric patients; 2) determine the prevalence of influenza virus in stool; and 3) compare the detection of influenza viruses in stool and upper respiratory tract specimens. Methods: Following informed parental consent, we enrolled children <6 years old with acute influenza-like illness (ILI; temperature >38°C and either cough or sore throat) and diarrhea who presented to 10 outpatient clinics and hospitals in Indonesia from October 2005 through July 2006. Throat and stool specimens were collected from each participant. RNA was extracted using QIAmp viral RNA mini kit (Qiagen, Hilden, Germany) and a multiplex nested reverse-transcriptase polymerase chain reaction (RT-PCR) was used to detect influenza A and B viral RNA. Results: A total of 466 participants submitted matched throat and stool specimens. The median age was 12 months (range 0 - 66 months), and 56% were male. Overall, influenza viruses were detected in 10.1% of throat specimens (5.4% had influenza A; 4.7% had influenza B), compared to 8.6% of the stool specimens (2.1% were influenza A; 6.4% were influenza B). However, a low concordance was noted when comparing subject's matched specimens, with an overall concordance of 2.6% (1.9% for influenza A; 0.6% for influenza B). Conclusions: Human influenza A and B viruses were detected in stool from young children with influenza-like illness and diarrhea, including in patients without evidence of influenza in throat specimens. Further studies are needed to understand whether human influenza viruses infect the gastrointestinal tract, and the role of diarrheal stool in influenza virus transmission.

Abstract O34

A Semi-automated and Cost Effective Workflow for High Throughput Avian Influenza Detection by Quantitative RT-PCR

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Since 2003, the avian influenza A (AI) H5N1 has spread in many countries, killing hundreds of people and millions of fowl. It is predicted that H5N1 will evolve and cause an influenza pandemic. The USDA has adopted qRT-PCR for AI detection after RNA extraction using a single tube format; however this format cannot handle the sheer number of samples that will need to be processed. We've developed a magnetic bead-based technology for viral RNA isolation. It processes 96 samples simultaneously without cross-contamination, and has passed the equivalency test at USDA/NVSL. It uses only 50 µL sample, leaving remaining sample enough for confirmative tests such as virus isolation or sequencing. We've also developed a one-step qRT-PCR kit for AI detection that targets the AI matrix gene. An artificial RNA is included for monitoring inhibition, sometimes seen in wild bird fecal samples. Using a complete kit instead of homebrew reagents ensures consistent testing and makes cross-lab data comparison possible. Here we present a semi-automated solution for high throughput sample processing and AI detection. RNA isolation is performed on a KingFisher Magnetic Bead Processor (Thermo Electron) with the MagMAX™-96 AI/ND Viral RNA Isolation Kit (Ambion). A Biomek 2000 (Beckman Coulter) assembles reagent plates. An Applied Biosystems 7900HT Real-Time PCR System is used for detection in 384-well format. This semi-automated approach drastically reduces cost (~$200,000) for setting up a high throughput testing facility. RNA isolation takes 15 min; detection requires 2 hours; over 1500 samples can be processed within one day.

Abstract O35

A Rapid and Sensitive Method for Detection of Drug Resistant Influenza A Virus in Quasi-Species

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Antiviral therapy using neuraminidase inhibitors (NIs) is likely to be the first line of defence in strategies to control human to human spread of H5N1 influenza virus, by reducing transmission and severity of illness. Influenza virus mutants resistant to NIs can be present at low levels, as viral quasi-species, after drug treatment or in screening of surveillance isolates. As most NI resistant mutants described to date have exhibited reductions in fitness, classic methods of detection and characterisation including culture and plaque purification or sequencing of viral isolates, may underestimate the detection of viral quasi species and select for viruses with a fitness advantage through lack of suitable detection systems. Rapid molecular screening for detection and quantification of viral mixtures in clinical material is required to support clinical decision making, particularly in NI treated patients and would deliver enhanced sensitivity to surveillance testing for antiviral susceptibility. We have developed a rapid and sensitive method to detect and quantify viral quasi-species containing NI resistant mutants using pyrosequencing technology, with particular emphasis to H5N1. Our approach has the benefit of enabling detection of low levels of resistant virus from direct genotypic analysis of clinical material without the need for culture amplification, a significant problem for highly pathogenic viruses such as H5N1. The mixture analysis pyrosequencing technique has been applied to isolates and clinical material from immunocompromised patients infected with influenza and treated with NI and adamantine drugs. Application of this approach directly to clinical material provides insight into the kinetics of the emergence of viral resistance during prolonged viral shedding of influenza, and is compared with the evaluation of drug susceptibility using cultured isolates.

Abstract O36

Strategies and Procedures for Influenza Diagnostic Testing Using Real-Time RT-PCR for Rapid Detection and Characterization of Season Human and Potential Pandemic Influenza Viruses

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Seasonal human influenza viruses cause significant morbidity and mortality, especially in the very young and elderly. Also, recent increased circulation of H5N1 highly pathogenic avian influenza (HPAI) in avian populations together with sporadic human infections of H5N1 HPAI has raised concerns about the pandemic threat of these viruses. Thus, timely detection of infections due to human influenza or H5N1 HPAI is essential for implementation of an appropriate public health response. Procedures for detection and characterization of influenza viruses using real-time RT-PCR (rRT-PCR) was developed at the Centers for Disease Control and Prevention (CDC), U.S.A. and implemented for rapid testing to identify individuals infected with seasonal human influenza as well as test specimens from suspect H5N1 human cases. Protocols were designed for universal detection of all influenza type A and type B viruses as well as identification of the HA genes of influenza A viruses of human health significance including contemporary human H1 and H3, as well as Asian avian H5, Eurasian H7, North American H7 and Asian H9 viruses. Assays were optimized to perform comparably on a number of different platforms using reaction master mix chemistries from several commercial manufacturers. Detailed protocols including primer and probe sequence information have been made available to public health laboratories (PHLs) within the United States as well as international organizations. Following approval of Asian H5 HPAI specific primer/probe sets for clinical testing by the United Stated Food and Drug Administration, these reagents were made available to laboratories belonging to the CDC Laboratory Response Network in order to further enhance domestic testing capacity for rapid diagnosis of individuals suspected to be infected with H5N1 HPAI. In order to further assist domestic and international PHLs establish confident testing procedures for influenza, strategies for deployment of standardized reagents, control materials, and proficiency testing panels have been implemented.

Abstract O37

Human Influenza A (H5N1) Detection by a Novel Multiplex PCR Typing Method

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We report the development and evaluation of ResPlex™III for genotyping influenza A viruses. The ResPlex™ system incorporates a novel multiplex PCR technology, Tem-PCR (target enriched multiplex PCR), to simultaneously amplify and detect multiple molecular targets in one test. The ResPlex™III assay targets the H1, H2, H3, H5, H7, H9, N1, and N2 genes from the influenza A virus, as well as the NS genes from influenza A (NSA) and B (NSB) viruses, providing detection and genotyping of influenza A and B. The analytical sensitivity for detecting the H5, N1 and NSA genes were 1, 10-1, and 10 TCID50/200µl/reaction, respectively. A total of 217 sequential clinical samples were tested by the ResPlex™III, including 14 samples with human H5N1 infections. The results were compared to a reference standard combined by results of viral culture, conventional reverse transcriptase and real-time PCR. The clinical sensitivity and specificity for detecting H5N1 were 93.3% and 100%, respectively, indicating that different subtypes of influenza A can be quickly and correctly identified using the ResPlex™III genotyping approach. Combined with other existing ResPlex™ panels, which detecting more than 20 respiratory viral and bacterial pathogens, the tem-PCR technology is becoming a powerful surveillance tool to monitor the outbreaks of respiratory diseases.

Abstract O38

Use of Reverse Transcription Loop-Mediated Isothermal Amplification for Molecular Detection of Highly Pathogenic H5N1 Influenza A Viruses

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Reverse transcription loop-mediated isothermal amplification (LAMP) is a novel diagnostic method that employs the amplification of target nucleic acids using reverse transcriptase, DNA polymerase and six target sites in an isothermal reaction. LAMP has a comparable detection limit to WHO recommended PCR methods. The LAMP primer set was designed to target the HA gene of HP H5N1 viruses. 596 HP H5N1 sequences were recruited and aligned, and a highly conserved region was used as the target. Specific amplification and detection of highly pathogenic (HP) H5N1 virus was established in a 1-2 hour assay. Furthermore, results were validated with a panel of genetically diverse HP H5N1 viruses that have been isolated over the last 10 years as well as clinical samples. The assay was also tested against all other HA subtypes, H1-H16, for confirmation of specificity to H5N1. The threat of a pandemic emerging from a HP H5N1 derived virus has made it imminently important for effectual surveillance and diagnostics. LAMP provides an option that is relatively inexpensive when compared to RT-PCR; sophisticated equipment is not required, only water bath or incubator. The single closed tube application also reduces the possibility of contamination. Another benefit to the LAMP assay is that positive reactions generate a precipitate that can be confirmed by visual inspection. In situations where resources are limited, the application of the LAMP assay might prove to be useful, especially for the field surveillance of H5N1.

Abstract O39

Pandemic Influenza Preparedness: New Molecular Tools for Evaluation of Influenza Vaccines and Identification of Serological Epitopes for Avian Influenza Diagnostic Assays

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Current trivalent inactivated influenza vaccines (TIV) provide limited protection against seasonal drift variants. Of even greater concern is the recent spread of avian influenza viruses (AIV) among domestic poultry and transmission to humans that can result in a global pandemic. Concerted efforts are underway to improve seasonal influenza vaccines and to generate stockpiles of effective pre-pandemic vaccines against AIVs in order to increase rates of immune response in all high-risk populations, and for broad heterosubtypic protection. In parallel with the intensive vaccine development efforts, there is an urgent need to improve the analytical tools for comparing immune responses generated by different vaccine

candidates against both human and avian influenza viruses. To address this need, we generated whole-genome-phage display libraries expressing all the open reading frames of two human influenza (H1N1, H3N2) and three avian influenza (H7N7 and two H5N1 clades) viruses. Each of these libraries contain 10^9-10^{10} phages, expressing influenza derived sequences ranging between 30-200 aa as fusion proteins with the bacteriophage pIII coat protein. These FLU-libraries are being used to map antibodies generated by: a) new seasonal influenza vaccines; b) Avian influenza vaccines. They are also being analyzed with convalescent sera from individuals exposed to avian influenza and human influenza, and for mapping the epitopes of broadly neutralizing H5N1 monoclonal antibodies. Thus far, the data using FLU-Gene-fragment libraries with serum samples from a vaccine trial shows the promise of this approach in identification of novel epitopes following vaccination or infection. More diverse epitope profile was observed following vaccination with live-attenuated cold adapted virus (LAIV) compared with inactivated vaccine (TIV). Higher reactivity of selected phages was observed with serum samples collected post-LAIV compared to post-TIV. Antigenic mapping of the hemagglutinin (HA) following vaccination and infection may predict protective epitopes for the seasonal and avian influenza. These epitopes will be used in the development of new assays to evaluate vaccine responses and potency. Hopefully, this approach will identify conserved sequences involved in broad cross- protection that could be incorporated into future vaccines. Epitopes recognized by convalescent individuals may be useful for new serodiagnostic assays to monitor new outbreaks of avian influenza.

Abstract O40

A Novel, Highly Sensitive Neutralization Assay for Influenza H5N1

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Background: The WHO has called for development of improved serological assays for influenza given that HI has been found to be insensitive for measuring responses to avian H5N1 viruses. Furthermore, current neutralization assays are laborious and require specialist facilities and expertise not always available at the front line of an outbreak. Our objective was to develop a widely applicable assay, with greater sensitivity and specificity, and with adaptation to micro-quantities of serum samples, compared to HI and microneutralization. Influenza HA pseudotypes can be used as safe, surrogate viruses for serological assays which can be carried out in BSL2 containment. Methods: Retroviral pseudotypes encode marker genes (GFP/luciferase/β-gal) and bear foreign viral envelopes of interest, in this case influenza H5N1 HA. The transfer of marker genes to target cells depends on the function of the envelope protein; therefore, the titer of neutralizing antibodies against the envelope can be measured by a reduction in marker gene transfer. Pseudotyped viruses were produced by four plasmid transfection of HA, NA, retroviral gag-pol and luciferase/GFP vector plasmids. Results: Functional retroviral influenza H5N1 HA pseudotypes have been constructed and used to develop a high-throughput *in vitro* microneutralization assay that is both highly sensitive and specific for influenza H5N1 neutralizing antibodies. Pseudotype NAb titers correlate strongly with titers obtained by horse HI assay ($r^2 = 0.93$) and microneutralization ($r^2 = 0.78$). Conclusion: This assay does not require the handling of live influenza H5N1 virus; it is a useful tool to determine neutralizing titers during natural infection and for the evaluation of candidate vaccines and therapies. This influenza HA pseudotype NAb assay, with its easily interchangeable HA glycoproteins from different influenza strains and subtypes make it a powerful tool for use in surveillance and seroprevalence studies. Our results suggest that this assay is more sensitive than HI and microneutralization for the detection of antibodies to H5N1. This assay is more widely applicable and is available in a high-throughput format.

Workshop Topic #6

Animal Influenza Ecology

Oral
Abstracts

Abstract O41

High Pathogenic Avian Flu (HPAI) H5N1: Causes and Consequences Virus Penetrations Into Northern Eurasia

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35 years ago we started the monitoring of virus circulation in key points of main migratory roots of birds in Northern Eurasia: Russian plain in European part, West-Siberian lowland, East-Siberian Baikal-lake region, South-Pacific lowland. Complex investigation included: virus isolation and identification, study of genomic structure, receptor activity, pathogenicity, serological investigations of wild and domestic animals and humans, detection of virus RNA by RT-PCR, and bio-chips, action of antivirals, evaluation of candidates for actual vaccine strains. In different regions were isolated all 16 known Influenza A viruses, including H5. We determined peculiarities their circulation for different ecosystems. Phylogenetic study of low pathogenic (LPAI) (Siberia, 1991-2001) and HPAI (Siberia – July 2006, Volga river delta – November 2005, Ubsu Nur lake, June 2006) strains from population of poultry and wild birds showed: 1) LPAI could introduced in 1992-2002 years by autumn migrations of wild bird into S.E. Asian poultry and transformed into HPAI; 2) HPAI with wild birds in spring 2005 penetrated from Qinghai lake (N.W. China) through "Dzhugarian gate" into Siberia and outspread into the territory about 10 mln km^2; 3) Wild birds in autumn 2005 fly back to overwintering places through Russia and other countries again carry HPAI of Qinghai-Siberian genetic clide and polluted water reservoirs in places in routs of flights and overwintering species from different ecosystems could have close contact in this time and conditions for virus exchanges and outspreading of panzooty. Full genome sequence showed: all isolated in 2005-2006 strains belong to one Qinghai-Siberian clide: 1) multiple basic amino acid motif P_{337}QGERRRKKRGLF$_{349}$ in the cleavage site of the HA (HPAI), 2) NA_{49-68} deletion (Z genotype), 3) Glu_{92} in NS1 (resistence to interferon), 4) Ser_{31} in M2 (sensitive to remantadine), 5) Lys_{627} in PB_2 (virulence to mammalian). Biological properties correlated with genome peculiarities. Virus replicated in cell lines of mammalian origin without trypsine. Virus deposited into All-Russian State Collection of viruses (Aug. 08.2005) and sequencing data – to Genebank (Sept. 05.2005) and used for large-scale production of veterinary vaccine in Russia. Migration roots of North Eurasian birds are discussed. Determination of transformation period of HPAI on wild birds to LPAI has in high priority for prognosis of events in future.

Abstract O42

SCHOLARSHIP Promising Junior Investigator Scholarship

The Role of Terrestrial Wild Birds in the Ecology of H5N1 Influenza A Virus

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House sparrows, European starlings and Carneux pigeons were inoculated with four H5N1 influenza A viruses isolated from different avian species. We monitored viral replication, mortality following infection, and transmission to uninfected contact birds of the same species. Sparrows were susceptible to severe infection, with 66-100% of birds dying within 3-6 days. High levels of virus were detected in oral-tracheal and cloacal swabs and in organs of deceased sparrows. Inoculation of starlings caused no mortality, despite high levels of virus shedding evident in oral-tracheal swabs. Least susceptible were pigeons, which showed no mortality and very low levels of virus in both oral-tracheal and cloacal swabs. Transmission to contact birds did not occur frequently: only A/Common Magpie/Hong Kong/645/06 virus was shown to transmit to one starling. In summary, recent H5N1 viruses are pathogenic for small terrestrial bird species and the rate of intra-species transmission in these hosts is very low.

Abstract O43

Susceptibility of Canada Geese (*Branta canadensis*) to H5N1 Highly Pathogenic Avian Influenza

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The H5N1 HPAI outbreak which began in Southeast Asia in late 2003 was preceeded a year earlier by an outbreak of highly pathogenic H5N1 in waterfowl and wild birds in Hong Kong indicating that this virus had already began circulating in wild birds. In the spring of 2005 a mass mortality of wild birds occurred at Qinghai Lake, China, an important migratory bird

breeding site. This event has been viewed as heralding the beginning of the long-range spread, by migratory birds, of H5N1 HPAI from Asia to Europe. Identifying the species of wild birds involved in this spread is not only of academic interest but of practical importance to surveillance activities since there is concern that migratory birds could also introduce H5N1 to the western hemisphere. The susceptibility of a number of indigenous North American duck and gull species has been addressed by experimental studies and has shown that wood ducks (*Aix sponsa*) and laughing gulls (*Larus atricilla*) are fully susceptible and may serve as good indicator species in a dead bird surveillance program. In this study we have examined the susceptibility of juvenile and adult Canada geese (*Branta Canadensis*) to H5N1 HPAI. Large populations of this species can be found in both peri-agricultural and peri-urban environments, a fact that may be of potential epidemiological significance if H5N1 were to establish itself in free-living North American bird populations. Here we demonstrate that immunologically naïve juvenile geese are fully susceptible to H5N1 with death occurring within 5 days of experimental challenge. Systemic infection was apparent with most significant involvement of the CNS. Juvenile geese pre-infected with a North American H5N2 virus were protected from a virulent H5N1 challenge. Interestingly, the adult geese used in this study had evidence of prior avian influenza virus exposure. Both adult geese pre-infected with H5N2 and those that were not showed greater resistance to H5N1 challenge than naïve juveniles. The roles that cross-protective antibody and cell-mediated immunity might play in this resistance will be discussed.

Abstract O44

Establishment of Influenza A Virus (H6N1) in Minor Poultry in Southern China

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Influenza A virus (H6N1) is one of the most frequently detected subtypes in aquatic birds in southern China. A H6N1 virus, represented by Teal/Hong Kong/W312/1997 (W312-like), was isolated during the H5N1 'bird flu' incident in Hong Kong in 1997. Genetic analysis suggested that this virus may have been involved the generation of HK/156/97-like virus as seven of eight gene segments shared high homology. Continuing surveillance in live-poultry markets of Hong Kong showed this W312-like H6N1 virus had became prevalent in quail and pheasant. However, the question of whether this virus was the progenitor for HK/156/97-like virus remained unknown. In this study we conducted further systematic market surveillance in different provinces of southern China from 2000 to 2005. This surveillance revealed that H6N1 viruses were also prevalent in minor poultry, especially in quail, chukka and pheasant, but not in chicken throughout this region. Furthermore, those viruses were predominantly isolated from tracheal swab suggesting this was the major site of virus replication in these hosts. Phylogenetic analysis of 77 H6N1 viruses revealed that all isolates had W312-like virus HA and NA genes. Phylogenetic analysis of the internal genes indicated that there had been extensive reassortment between those H6N1 viruses with either H5N1 or the two lineages of H9N2 viruses (Ck/Beijing/1/1994-like and Qa/Hong Kong/G1/1997-like). Those H5N1 and H9N2 viruses are broadly endemic in land based poultry in southern China. Those reassortant events have generated multiple genotypes of H6N1 viruses, and a similar situation has also been observed for H9N2 viruses in quail. The results of the phylogenetic analysis also suggest that W312-like H6N1 viruses were not the precursor to HK/156/97-like viruses, but were derived from reassortment between HK/156/97-like virus, H9N2 and another H6 subtype virus. These results demonstrate that the continued co-circulation of H5N1, H9N2 and H6N1 influenza subtype viruses leads to frequent reassortment, particularly in minor poultry such as quail, which greatly increases genetic diversity of influenza viruses in this region. These outcomes highlight the importance of systematic influenza surveillance of poultry in pandemic preparedness.

Abstract O45

Pathogenicity and Increased Virulence of Italian H7N1 HPAI Virus in the Mouse Model

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The mechanisms behind interspecies transmission of avian influenza viruses are still to be fully understood, although certain mutations, particularly in the PB2 gene, appear to play a major role in the infection of mammals. Among these, PB2 627

E to K and 701 D to N in mice experiments appear to increase virulence and enable more extensive replication respectively. We conducted a study to investigate the ability of selected Italian H7N1 HPAI viruses to infect and cause pathology in mice. We chose A/ostrich/It/2332/00/H7N1 with a lysine in position 627 of the PB2 protein and A/chicken/It/5093/99/H7N1 with glutamic acid in position 627 of the PB2 protein. Both isolates have aspartic acid (D) in 701 of PB2, which is in-keeping with their avian origin. Female Balb-C mice were infected intranasally with 10^5 of each virus. Clinical signs were recorded and mice were weighed every day for ten days after infection. Two mice from each group were sacrificed on day 3, 5, 7 and 10 post-infection and selected organs were collected (brain, trachea-lung, spleen, liver, kidney and intestine). Samples collected were processed for RRT-PCR, virus isolation, histopathological and immunohistochemical analysis, and full genomes were sequenced; pre and post-infection. MLD_{50} of pre and post-infection isolates were also determined. Both viruses were pneumo-and neuro-pathogenic in mice. In detail, the strain originally possessing PB2-627K (OS/2332/00) spread more rapidly and caused more severe lesions than the other. The MLD_{50} for this virus was $10^{1.75}$ EID_{50}. Chicken isolate CK/5093/99 was only mildly pathogenic and all mice survived infection. However, sequencing of one of the CK/5093/99 isolates recovered from the brain of one mouse on day 10 pi revealed that a mutation to the PB2-627K variant had occurred. The MLD_{50} of the original strain was > $10^{5.6}$ EID_{50} while the post-infection strain value was $10^{3.4}$ $EID_{50,}$ indicating an increase in virulence. The results of this investigation indicate that Italian avian H7N1 isolates may naturally possess or acquire the PB2 627K mutation, and that this results in increased virulence and infectivity for mice. In our experiment the D to N mutation in position 701 of PB2 did not occur, and therefore such mutation does not appear to be essential for efficient replication of H7N1 viruses in mice. The findings reported herein support the general consensus that avian influenza viruses of other subtypes may represent a threat to human health and for this reason these infections should be eradicated rather than contained in the animal reservoir.

Abstract O46

Human-Swine Reassortant H1 Influenza Virus Infections in US Pigs

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Human-swine reassortant H1N2 and H1N1 influenza viruses are currently circulating in pigs throughout the United States. We isolated the first human-swine reassortant H1N2 virus from 4-week-old pigs with respiratory disease in October 2003. The virus was initially detected by a TaqMan™ RT-PCR test for nucleoprotein gene on nasal swabs. Hemagglutination inhibition tests with antisera against A/Sw/IA/1973 H1N1 and A/Sw/TX/1998 H3N2 were negative. The isolate was identified as H1 by nucleotide sequencing. Phylogenetic analysis demonstrated high similarity (>90%) to H1 human influenza virus A/Poitiers/2168/2003. The NA gene was determined to be N2 of swine origin by sequencing and multiplex NA typing RT-PCR. In June 2005, we isolated an H1N1 virus with human-like HA genotype from several groups of pigs within the same geographic region and from sites having both related and unrelated pig flow. The 2005 human-like H1 viruses share >98% similarity to H1 human influenza virus A/New York/3/2003 and <75% similarity with swine influenza reference strains. They share >97% similarity to each other but <95% similarity with the 2003 human/swine H1N2 reassortant viruses, suggesting they are the result of separate reassortment events between human and swine viruses. Phylogenetic analyses support that assumption. To date, over 150 influenza viruses of human-like H1 genotype have been isolated from pigs in eleven states, suggesting that the virus is adapted to swine, spreads readily to pigs in other geographic regions, and is capable of causing respiratory disease. These findings suggest that transmission between humans and pigs occurs but may go undetected unless genetic characterization is undertaken. Reassortant viruses pose additional threats to swine and human health if the acquired changes result in antigenic variation such that existing subtypic immunity is ineffective.

Abstract O47

Newcastle Disease Virus-based Live Attenuated Vaccine Completely Protects Chickens and Mice From Lethal Challenge of Homologous and Heterologous H5N1 Avian Influenza Viruses

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H5N1 highly pathogenic avian influenza virus (HPAIV) has continued to spread and poses a significant threat to both animal and human health. Current influenza vaccine strategies have limitations that prevent their effective use for widespread inoculation of animals in the field. Vaccine strains of Newcastle disease virus (NDV), however, have been used successfully to easily vaccinate large numbers of animals. In this study, we used reverse genetics to construct a NDV that expressed an

H5 subtype avian influenza virus (AIV) hemagglutinin (HA). Both a wild-type and a mutated HA open reading frame (ORF) from the HPAIV wild bird isolate, A/Bar-headed goose/Qinghai/3/2005 (H5N1), were inserted into the intergenic region between the P and M genes of the LaSota NDV vaccine strain. The recombinant viruses stably expressing the wild-type and mutant HA genes were found to be innocuous after intracerebral inoculation of 1-day-old chickens. A single dose of the recombinant viruses in chickens induced both NDV- and AIV H5-specific antibodies and completely protected chickens from challenge with a lethal dose of both velogenic NDV and homologous and heterologous H5N1 HPAIV. In addition, BALB/c mice immunized with the recombinant NDV-based vaccine produced H5 AIV-specific antibodies and were completely protected from homologous and heterologous lethal virus challenge. Our results indicate that recombinant NDV is suitable as a bivalent live attenuated vaccine against both NDV and AIV infection in poultry. The recombinant NDV vaccine may also have potential use in high-risk human individuals to control the pandemic spread of lethal avian influenza.

Abstract O48

Vaccine Efficacy Against Challenge With HPAI H5N1 Virus Isolates From Vietnam

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Multiple isolates of H5N1 were isolated from northern Vietnam in December of 2005. All the viruses characterized were clade 2 viruses, but phylogenetically they formed two separate sub-lineages. The isolation of clade 2 viruses was unexpected, since previous isolations from both northern and southern Vietnam were clade 1 viruses. Viruses from both lineages killed chickens within two days in experimental inoculations, and were also highly virulent for 2-week old Peking ducks, killing them within four days of challenge. Two representative viruses from each lineage were selected for further antigenic and vaccine efficacy studies. Comparison of cross hemagglutination inhibition titers showed marked differences in titer when using homologous and heterologous antigen. For the vaccination efficacy study, 2-week old white leghorn chickens and 1-week old Peking ducks were vaccinated with one of four oil emulsion vaccines, two of which are commercial vaccines currently used in Vietnam. Three weeks later, these birds were challenged intrachoanally (intranasally) with 10^6 EID_{50} of one of two viruses each belonging to a different lineage. All four vaccines provided clinical protection for most vaccinated birds, but differences in antibody titer were seen between vaccines. Oropharyngeal virus shedding was compared by real-time RT-PCR to evaluate shedding among the different groups. All sham vaccinated birds died within 2 days. By compiling the data from these various analyses of the recently isolated Vietnam highly pathogenic H5N1 viruses, we will gain knowledge which can be applied to selecting vaccine seed strain viruses in the future.

Workshop Topic #7

Clinical Vaccine Evaluation

Oral Abstracts

Abstract O49

Influenza Vaccine Effectiveness Among 6-59-Month Old Children During the 2003-04 Season in Three US Communities

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Background: Influenza vaccination is recommended for US children aged 6-59 months. Few studies have assessed the vaccine effectiveness (VE) of the trivalent inactivated vaccine (TIV) in preventing laboratory-confirmed influenza in this age group. Objectives: 1) Assess TIV VE among young children; 2) Compare VE using three study designs. Methods: The New Vaccine Surveillance Network conducted three VE studies during the 2003-04 season in three US counties. Influenza cases aged 6-59 months were identified through active prospective surveillance in hospitals, emergency departments, and clinics in children presenting with fever or acute respiratory illness (ARI). Nasal/throat swabs were tested for influenza by culture and RTPCR. The three studies used the same cases but different control/cohort groups and included: 1) a case-control study with concurrently enrolled influenza-negative ARI surveillance controls; 2) a practice-based case-cohort study with a randomly sampled cohort from a random sample of outpatient practices; and 3) a case-cohort study with a cohort obtained by random digit dial (RDD) phone survey of households. Influenza vaccination data were obtained from healthcare providers and coded as fully, partially (one current year dose and no prior doses), or unvaccinated. VE was calculated as 1-hazard ratio from models adjusting for covariates, including insurance and high-risk status. Results: For the 2003-04 season, 231 influenza cases were identified with 3% fully and 10% partially vaccinated. There were 7% fully and 16% partially vaccinated among 848 influenza-negative children; 9% fully and 11% partially vaccinated among 4291 practice-based cohort children; and 13% fully and 11% partially vaccinated among 932 RDD cohort children. Preliminary estimates of VE (95% CIs) for fully vaccinated were 54% (-12,81), 43% (-28,75) and 44% (-15,73) for studies 1, 2, and 3, respectively. VE for partially vaccinated was substantially lower. Results for the 2004-05 season and subgroup analyses will also be presented. Conclusions: Three VE studies using the same cases found VE of 43-54% during 2003-04 despite circulation of a drifted strain.

Abstract O50

Influenza Vaccination in Community-Dwelling Elderly: 10-year Data Pooling and Quantification of Residual Confounding Project

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Background: Seasonal influenza and estimates of vaccine effectiveness in the elderly can vary from year to year. Most previous vaccine effectiveness studies have been short term and may therefore present an incomplete picture of the benefits of vaccination. Concerns have also surfaced about whether unmeasured residual confounding may have biased study results. This study provides a long term assessment of influenza vaccine effectiveness in the elderly and addresses the impact of potential unmeasured residual confounding. Methods: Data for 18 cohorts of community-dwelling elderly members from 1 (1990-91 through 1995-96) and 3 (1996-97 through 1999-2000) US HMOs were pooled. Logistic regression was used to estimate vaccine effectiveness for preventing hospitalization for pneumonia and influenza and death during influenza seasons after controlling for age, gender, co-existing medical conditions, previous health care use, site and year. Additional analyses explored for evidence of residual confounding. We also conducted a sensitivity analysis to quantify the effects of a hypothetical unmeasured confounder on our estimates of vaccine effectiveness. Results: There were 713,872 person-seasons of observation. Influenza vaccination was associated with a significant reduction in hospitalizations for pneumonia and influenza (adjusted OR .73, 95% CI .68 to .77) and death (adjusted OR .52, 95% CI .50 to .55). These estimates were generally stable across age and risk strata, and we found no evidence for survivor or healthy vaccinee bias. In the sensitivity analysis of the hypothetical unmeasured confounder vaccination was still associated with significant–though lower–reductions in death in all of the considered scenarios and in hospitalizations in all but the more extreme scenarios. Conclusion: Influenza vaccination over the long term, on average significantly reduces hospitalizations for pneumonia and influenza and death during influenza seasons among community dwelling elderly. Even with adjustment for a hypothetical unmeasured confounder substantial benefits persist. Efforts should continue to improve vaccine delivery to this high priority group.

Abstract O51

Efficacy of Influenza Vaccines During Seasons With Antigenically Drifted and Antigenically Matched Circulating Viruses

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We examined absolute and relative efficacies of the inactivated (TIV) and live-attenuated (LAIV) influenza vaccines in preventing laboratory-confirmed symptomatic influenza during the 2004-05 and 2005-06 influenza seasons. Healthy adult (age 18-48 years) men and women were randomly assigned to receive TIV by intramuscular injection, LAIV by intranasal spray, or placebo by injection or spray. Surveillance was carried out each year from November – April with collection of throat swab specimens from participants with influenza-like illness for identification of influenza virus. Relative risk (RR) estimates compared the proportion of cases of laboratory-confirmed influenza in each vaccine group to the placebo (or other vaccine) group; vaccine efficacies (percent relative reduction) were calculated as (1-RR) X 100. 1,247 participants were vaccinated in Fall 2004. During the 2004-05 season antigenically drifted type A (H3N2) virus and type B viruses representing both vaccine-like and variant lineages circulated. Absolute efficacies against both types A and B was 75% (95% CI, 42 to 90) for TIV and 48% (95% CI, -7 to 74) for LAIV as measured by isolation of virus in cell culture or identification using real time PCR. 2,058 participants were vaccinated in Fall 2005. The 2005-06 season was late and of low intensity, with circulation of primarily type A (H3N2) that was antigenically similar to the H3N2 vaccine component. Absolute efficacies of TIV (37%, 95% CI: -51 to 73) and LAIV (36%, 95% CI: -54 to 73) as measured by virus isolation or PCR were low, in part because the attack rate in the placebo group was only 2.4%. When virus isolation and rise in antibody titer were used, absolute efficacies were 53% (95% CI: 5 to 76) for TIV and 55% (95% CI: 8 to 78) for LAIV. Given the vagaries of seasonal influenza activity (both strain and intensity differences), annual evaluation of vaccine efficacy with laboratory endpoints is necessary.

Abstract O52

Incidence of Influenza-Like Illnesses Among US Military Service Members Receiving Live Attenuated Influenza Vaccine Versus Trivalent Inactivated Influenza Vaccine: 2005-2006 Influenza Season

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During the 2005-2006 influenza season, the Department of Defense administered live attenuated influenza vaccine (LAIV) or trivalent inactivated influenza vaccine (TIV) to eligible active duty (AD) personnel. Although LAIV has been found to be more efficacious at preventing influenza among children as compared to TIV, limited data is available comparing the two vaccines in adults. This analysis was conducted to compare the incidence of influenza-like illness (ILI) following immunization among US Military AD personnel receiving LAIV as compared to TIV. The Defense Medical Surveillance System was used to identify the cohort of non-deployed, non-recruit, 17-49 year old, AD military personnel who received either one dose of LAIV or TIV during the 2005-2006 influenza season (September 1, 2005–April 30, 2006). Person-time was calculated as 14 days after vaccination to the end of the flu season or censoring event. A case of ILI was defined as having an outpatient or inpatient visit occurring 14 days post immunization with selected ILI ICD-9 codes. A 30-day window was used to differentiate multiple ILI events. Poisson regression was used and adjustment was made for confounders. A total of 127,427 people received the LAIV and 533,691 people received the TIV, accumulating 48,229 and 185,831 person-years, respectively. Service members receiving LAIV had a significantly higher adjusted ILI incidence rate compared to those receiving TIV (Incidence Rate Ratio = 1.15, $p<0.0001$). This increased risk remained even after restricting the analysis to weeks of intense influenza activity (based on CDC US national data). The increased incidence of ILI among LAIV recipients as compared to TIV recipients is not fully understood. Possible reasons include interference of pre-existing antibody with the LAIV or decreased effectiveness of the LAIV against the circulating influenza strains. Our findings are limited by a lack of culture-confirmed influenza cases; however, when the analysis was restricted to periods of intense influenza activity we found similar results. Regardless of the cause, ILIs occurred more frequently among service members receiving LAIV compared to TIV in this population. Consideration of these findings should be made when developing influenza vaccination policies for adults.

Abstract O53

Live Attenuated Cold-Adapted Influenza Vaccine in Russia: Advantages, Further Research and Development

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The live cold-adapted influenza vaccine (LIV) has been used extensively in Russia and shown to be safe, immunogenic and highly protective among children, adults and the elderly and to provide a significant level of community (herd) immunity. Data are presented demonstrates LIV protection against drifted variants of influenza, strong correlations between high levels of post-vaccination IgA, IFN-γ, cell-immunity, and antibody functional activity and lowered incidence of influenza diseases, decreased level of post-influenza complications. The LIV has been grown efficiently in eggs and in mammalian cell culture. The avian H5N2 reassortant vaccine candidate based on the A/Leningrad/134/17/57 cold-adapted Master Donor Strain has displayed high levels of protection in mice challenged with HP H5N1 viruses. Clinical trials in humans confirmed safety, immunogenicity and genetic stability of LIV from avian strain. Recent evidence has demonstrated that LIV has the additional benefit of providing protection shortly after administration prior to the development of the adaptive immune response. In this study will be discuss data of the interaction of ca viruses with wt viruses, including avian strains, which is the main concern about biosafety of LIV in the case of pre-pandemic and pandemic. Experimental and clinical data of using LIV among patients with allergy and immunodeficiency will be present.

Abstract O54

Adjuvanted H5N1 Pandemic Candidate Vaccine Showed a Positive Safety Profile in Adults Aged 18 Years and Older Within a Phase III Safety Trial

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Background: The use of a pre-pandemic influenza vaccine just before or just after the onset of an influenza pandemic is considered to be one of the most effective measures capable of substantially reducing the overall infection rate and consequent morbidity/mortality within the population. Preliminary analyses have demonstrated that GlaxoSmithKline Biologicals' candidate pandemic influenza split virus vaccine adjuvanted with a novel proprietary oil-in-water based adjuvant system induced a high level of immunity directed against the vaccine strain but also induced cross-immunity against a heterologous strain from clade 2. A 3.8µg antigen adjuvanted formulation was selected from this dose-ranging study for the development of a pandemic influenza vaccine for adults. In parallel we conducted the present study to rapidly collect safety and reactogenicity data from a much larger cohort of adult subjects, including people older than 60 years. We compared the safety of a 15µg antigen adjuvanted H5N1 candidate vaccine with the safety profile of the seasonal influenza vaccine Fluarix™ marketed worldwide by GlaxoSmithKline Biologicals. Methods: This phase III study (e-track:107064/107217; NCT:00319098) was conducted in 2006 in 7 European countries in a total of 5071 subjects (≥18 years of age) who received a monovalent split virus (H5N1) influenza vaccine containing 15µg of adjuvanted HA (N=3802) according to a two-dose regimen (21 days apart), or a first dose of Fluarix™ (day 0) and a second dose of placebo on day 21 (N=1269). Local symptoms and general adverse events (AE) were solicited during a 7-day (Day 0-6) follow up period after vaccination. In addition, any analgesics and/or antipyretics taken by the subject to correct the symptoms (local and/or general) during the 7-day follow-up period after each vaccination were recorded by the investigator. Unsolicited

symptoms were recorded within 21 days following the first dose (Day 0-20) and within 30 days (Day 21-54) following the second dose. Serious adverse events (SAEs) were recorded during the entire study period up until 180 days after the first dose. Results: Considering the entire follow-up and the 18 to 60 years-old adult subjects, pain at the injection site was the most frequent solicited local AE in both groups (90.8% and 66.6% in the adjuvanted H5N1 and control Fluarix™ group). Following administration of the adjuvanted H5N1 vaccine, redness, swelling and induration were reported with significantly higher frequencies in the H5N1 group as compared to control (38.2% vs 27.8%, 33.1% vs 17.5% and 34.4% vs 19.1% respectively), while the incidence of ecchymosis was 9.6% vs 5.8. However, incidences of grade 3 solicited local symptoms were low in both groups and ranged from 0.3% (echimosis) to 7.5% (redness) with the adjuvanted H5N1 vaccine, versus 0.1% to 1.5% in the control group. The 2 vaccines were better tolerated in subjects over 60 years old, with however more solicited local-AE in H5N1 group. Considering the entire follow-up and the 18 to 60 years-old adult subjects, incidences of all solicited general symptoms were significantly higher in the adjuvanted H5N1 vaccine group as compared to the control group., Fatigue, myalgia and headache were the most common AE in both groups (51.9%, 50.0%, 46.9% in the adjuvanted H5N1 vaccine group, versus 30.8%, 24.6% and 31.2% in the control group). Grade 3 solicited general AE were observed with low frequencies, ranging from 0.5% (Fever) to 4.6% (Fatigue) and from 0.0% (Fever) to 1.2% (Headhache) in the two groups respectively. No related SAEs were reported during the study period. Conclusion: Significantly higher incidences of any grade of solicited AE were observed with the adjuvanted H5N1 vaccine containing 15µg HA as compared to the control. However, as incidences of grade 3 solicited symptoms were low, the reactogenicity of the adjuvanted H5N1 vaccine was considered to be medically acceptable. In conclusion, the candidate adjuvanted H5N1 vaccine was associated with a good safety profile and had acceptable reactogenicity in adults aged 18 years and above.

Abstract O55

Rapid Cellular Immune Response to Influenza A/Vietnam/1203/2004 (H5N1) Vaccine After Priming With an Antigenic Variant

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One strategy for pandemic control would be priming prior to a pandemic, allowing boosting with a single dose when a specific pandemic virus emerges. We modeled this strategy by administering a single dose of influenza A/Vietnam/1203/2004 (H5N1, clade 1) vaccine to subjects who had received two doses of influenza A/HK/156/1997 (H5N1, clade 3) vaccine in 1998. Prior serological assays revealed that this strategy was highly successful compared to repeated high dose (90µg) homologous vaccination. To investigate the cellular basis of this improved response, we conducted B cell ELISPOT assays on peripheral blood collected on days 0, 7, 28 and 56 after vaccination. Antigens included in the assay were recombinant hemagglutinins corresponding to H5/HK/156/97, H5/VN/1203/04, H1/New Caledonia/99, H3/WY/03, and H6/Teal/HK/W312/97, or KLH. Robust increases ($P < 0.001$) in the frequency of H5 HA specific B cells were observed as early as 7 days after single (90 µg) dose of A/Vietnam/1203/2004 (H5N1, clade 1) vaccine. The B cell responses against both H5/HK/156/97 and H5/VN/1203/04 were similar in kinetics and magnitude. No significant changes were measured against H1 or H3 derived HA antigens, and responses to control H6 and KLH antigens were negligible. These data suggest that vaccination after priming with an antigenic variant of H5 is a successful and specific strategy for recalling amnestic B cell responses to avian influenza vaccines. This work is supported by NIH AI25460 and AI050020.

Abstract O56

A Phase I Clinical Trial of the Safety and Immunogenicity of a PER.C6® Cell Grown Influenza H7N1 Virus Vaccine

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The ongoing threat posed by avian influenza highlights the need for global preparedness. Influenza H5 and H7 subtypes cause highly pathogenic outbreaks in domestic poultry, and both these subtypes have caused human fatalities. Thus, there is a need for a better understanding and increased knowledge of these subtypes as vaccine candidates to ensure an appropriate vaccine could be rapidly produced in the event of a pandemic. The FLUPAN consortium has developed the first human vaccine against an avian influenza H7N1 virus. A highly pathogenic avian influenza A/Chicken/Italy/13474/99 (H7N1) virus, from a lethal outbreak in Italy 1999, was selected as a candidate virus. The vaccine strain (RD-3) was produced by reverse genetics on the PER.C6® human cell line and an inactivated split virus vaccine was prepared. A phase I clinical trial was conducted to evaluate the safety, tolerability and immunogenicity of this vaccine. Sixty healthy young volunteers (21 males, 39 females) were randomly assigned to 4 equally sized groups. They received two doses of the H7N1 virus vaccine containing 12µg or 24µg HA without adjuvant or formulated at the point-of-use with aluminium hydroxide adjuvant (300µg or 600µg Al, respectively). Five volunteers received only one dose of vaccine. Blood and oral fluid samples were collected to evaluate the kinetics of the influenza specific cellular and serum and local antibody responses. The vaccine was well tolerated in all groups, there were no serious adverse events and 25% of the volunteers reported no side reactions. Almost half of the volunteers reported mild transient local (e.g. pain, swelling or redness at the injection site) or systemic reactions (e.g. headache, tiredness, upper respiratory tract symptoms or myalgia), which usually resolved within three days. Our preliminary findings are that influenza H7N1 specific antibody secreting cells were detected after vaccination, particularly at 7 days after each vaccine dose. Low serum antibody titres were detected 21 days after the second dose of vaccine by haemagglutination inhibition test using horse red blood cells or microneutralisation assay. These antibody responses were mainly detected in the high dose adjuvanted group (8/13 subjects vaccinated with adjuvanted 24µg HA). Further analyses are ongoing.of them became established in this host. Those Ck/Bei-like reassortants generated in quail have then been introduced to other poultry. These complex interactions form a two-way transmission system between quail and other types of poultry. The identification of HA and NP genes with high homology to Ty/WI/1/66 in some H9N2 viruses isolated from quail in 2001 suggested that those viruses had not evolved naturally. The present study provides evidence that H9N2 and H5N1 subtype viruses have also exchanged gene segments to generate currently circulating reassortants of both subtypes that have pandemic potential. Continuing influenza surveillance in poultry is critical to understanding the genesis and emergence of potentially pandemic strains in this region.

Workshop Topic #8

Genetic and Antigenic Evolution

Oral Abstracts

Abstract O57

Promising Junior Investigator Scholarship

Global Patterns in the Evolution and Epidemiology of Influenza A(H3N2) Virus From 2002 to 2007

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Influenza epidemics have been documented to regularly occur in every country in which there has been surveillance. However, long-standing questions remain about disease persistence and the mechanisms that facilitate the year-to-year reoccurrence of epidemics. For example, influenza epidemics occur during the winter in temperate regions, but it is unclear whether influenza viruses continue to circulate at low levels between epidemics and seed the epidemic in the following year, or whether subsequent epidemics are seeded from other regions, or some combination of both. If the epidemics are seeded from other regions?then from where? The primary hypotheses about seeding are that external seeding is due to strains alternately causing epidemics in the northern and southern hemispheres, or that seeding happens from the tropics where influenza circulates year-round, or that seeding happens from Asia, or from China. Although much good work has been done on these questions over an extended period of time, these hypotheses have been difficult to test primarily due to the global nature of the epidemiology confounded by the continual evolution of the virus. Here we describe the evolution of influenza virus A(H3N2) from 2002 to 2007 based on antigenic, genetic, and epidemiological analyses of ~14,000 viruses with a nearly global distribution. Antigenic cartography reveals patterns of substantial antigenic heterogeneity within and between countries. In contrast to previously documented patterns of antigenic cluster jumps followed by periods of relative antigenic stasis, the global data over the last 4 years shows patterns of steady, large-scale antigenic drift. We also see that influenza occurs in seasonal epidemics for almost all countries for which we have data, including 4 out of 5 countries in tropical Asia. A combination of antigenic cartography and phylogenetic analyses were used to test whether the regular isolation of viruses between epidemics in temperate regions is a product of local influenza persistence or a product of external seeding events. We further explore the source of seeding events, and the role of regions in which influenza appears to be circulating continuously via networks of temporally overlapping epidemics.

Abstract O58

Evolution of Influenza B

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Reassortment has long been known to occur both within and between the Victoria and Yamagata lineages of influenza B. However, the extent to which reassortment is reflected in subsequent epidemiological events in influenza B, either alone, or in addition to antigenic drift via amino acid replacements in the hemagglutinin and neuraminidase, has been unclear. The most likely case for reassortment being selectively advantageous in influenza B is the 2001 acquisition of a Yamagata lineage neuraminidase by a virus in the Victoria lineage (Shaw, 2002). Descendents of that reassortment event have since become dominant among lineages carrying a Victoria hemagglutinin allele. However, recent reports suggest that neuraminidase is not the only Yamagata-lineage gene to have reassorted to the Victoria lineage. Whole-genome analyses by Matsuzaki et al. (2004) and by McCullers et al.(2004) both reported extensive segment reassortment between the Yamagata and Victoria lineages. For instance, McCullers et al. (2004) found 47 of the 56 theoretically possible theoretical pairings between each Yamagata segment and the seven other segments of Victoria origin. This complexity has made it difficult to discern the number of reassortment events or the order in which they occurred, much less any role those reassortments might have played in natural selection. We present a new analysis of data to examine the simultaneous roles of reassortment and positive selection as selective forces driving the evolution of influenza B, and show the effects of positive selection in the divergence of the Yamagata and Victoria strains.

Abstract O59

Characterization of Swine-like Triple Reassortant Influenza A (H1N1) Viruses Isolated from Humans in United States

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Since 1998, a total of 10 cases of human infections with swine influenza A (H1N1) viruses have been identified by the Influenza Division at CDC. In most of the cases, contact with pigs prior to the illness was documented and there was limited evidence of person-to-person spread. Pigs are uniquely susceptible to infections with swine, human, and avian influenza viruses, and if co-infected, may serve as potential "mixing vessels" to facilitate the emergence of new genotypes. Since 1998, reassortant swine influenza viruses incorporating gene segments of different origins have been isolated from multiple outbreaks of respiratory disease in U.S. swine herds. In 1998, a swine-like influenza A (H1N1) virus was isolated from a Wisconsin resident. Antigenically the virus, A/Wisconsin/10/98 was similar to classical swine (H1N1) viruses. Between 2005 and 2006, three additional human cases were identified from Wisconsin, Missouri, and Iowa. Sequence analysis of their gene segments revealed that they were triple reassortant genotypes composed of swine, human, and avian lineage genes. The NS, M, NA, NP, and HA genes of those isolates are of classical swine H1N1 virus origin; the PB1 gene was derived from a human influenza A virus, and the PA and PB2 genes were most closely related to those of avian influenza viruses. Phylogenetic analyses suggested that such triple reassortant viruses might arise by reassortment of a swine H1N1 virus and one of previously described reassortant H3N2 and H1N2 viruses currently circulating among pigs in the United States. Such triple reassortant viruses have become the most prevalent genotype of H1N1 virus isolated from North-American pigs. The prevalence of these triple reassortant viruses in swine and the susceptibility of pigs to avian influenza infections facilitate genetic reassortments resulting in further mixing of swine, avian, and human influenza genes, which may generate a virus with pandemic potential.

Abstract O60

Genetic and Antigenic Characterization of Avian Influenza A (H5N1) Viruses Isolated From Humans in Mainland China

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Twenty cases of H5N1 influenza virus infection in humans that resulted in 13 deaths were identified in China between November 2005 and July 2006. In this study, we performed genetic and antigenic analyses on the 16 H5N1 virus strains isolated from these infected persons. Sequence data obtained in our study indicates that isolates from southern and northern China each form unique sublineages among H5N1 viruses, with the isolates from southern China clustering in a novel clade. Antibodies raised against the H5N1 virus A/Vietnam/1194/2004, a candidate for current human pandemic vaccine production and clinical trials, had very limited cross-reactivity with the Chinese H5N1 isolates. Our study contains valuable information for global pandemic vaccine selection by providing evidence for the need to develop vaccine candidates selected from the H5N1 viruses isolated from these more recent human cases from China.

Abstract O61

Characterization of Low Pathogenic H5 Subtype Influenza Viruses From Eurasia: Implications for the Origin of Highly Pathogenic H5N1 Viruses

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Highly pathogenic avian influenza (HPAI) H5N1 viruses are now endemic in many Asian countries, resulting in repeated outbreaks in poultry and increased cases of human infection. The immediate precursor of these HPAI viruses is believed to be A/Goose/Guangdong/1/96 (Gs/Gd)-like H5N1 HPAI viruses first detected in Guangdong in 1996. From 2001 onwards, many novel reassortant H5N1 influenza viruses, or genotypes, have emerged in southern China. However, precursors of the Gs/GD-like viruses and their subsequent reassortants have not been fully determined. Here we characterize low pathogenic avian influenza (LPAI) H5 subtype viruses isolated from poultry and migratory birds in southern China and Europe from the 1970s to the 2000s. Phylogenetic analyses revealed that Gs/GD-like virus was likely derived from an LPAI H5 virus in migratory birds. However, its variants arose from multiple reassortment between Gs/GD-like virus and viruses from migratory birds, or with those Eurasian viruses isolated in the 1970s. It is of note that unlike HPAI H5N1 viruses, those recent LPAI H5 viruses have not become established in aquatic or terrestrial poultry. Phylogenetic analyses revealed the dynamic nature of the influenza gene pool in Eurasia with repeated transmissions between the eastern and western extremities of the continent. The data also shows reassortment between influenza viruses from domestic and migratory birds in this region that has contributed to the expanded diversity of the influenza gene pool in poultry Eurasia.

Abstract O62

Ecology and Evolution of Influenza A (H5N1) Virus in Asia: Evidence From Systematic Influenza Surveillance

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The development of highly pathogenic avian influenza A (H5N1) viruses in poultry in Eurasia accompanied with the increase in human infection in 2006 and the recent reemergence of poultry outbreaks throughout the region, suggests that the virus has not been effectively contained and that the pandemic threat persists. Virological and epidemiological findings from our surveillance in live-poultry markets conducted over the last seven years in southern China has provided a comprehensive view of the ecology and evolution of H5N1 influenza viruses and revealed that they are endemic in different types of market poultry. Genetic and antigenic analyses have also demonstrated the dynamic evolution of these viruses in the region, with repeated introductions from southern China to neighboring regions, including Cambodia, Indonesia, Malaysia, Thailand, and Vietnam. A further dramatic spread of the virus westwards throughout Central and Southern Asia, Europe and Africa was also seen after detection of an initial outbreak in wild birds at Qinghai Lake in Central China, in line with our predictions. Genetic analyses revealed that after the H5N1 influenza viruses had established and been endemic several years, they developed into regionally distinct sublineages that allowed us to further trace their transmission pathways. However, recent surveillance data has revealed the emergence and predominance of a single H5N1 virus sublineage in poultry since late 2005 that has gradually replaced those previously detected multiple regional distinct sublineages in China. These viruses have already transmitted to neighboring regions thereby resulting in a new transmission and outbreak wave in Southeast Asia. The persistence of H5N1 virus since it first caused human disease in Hong Kong 11 years ago, as demonstrated by its endemicity over a large geographical region, along with repeated disease outbreaks in both poultry and humans indicates that it will be a long-term and difficult task to bring this virus under control in the absence of well developed disease control systems.

Abstract O63

Antigenic Profiling of Avian H5N1 Influenza Virus With a Panel of H5-Specific Monoclonal Antibodies

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Continued circulation of H5N1 viruses in poultry has led to the establishment of multiple sub-lineages with diverse antigenic features in Asia, which causes difficulties in the selection of vaccine strains. To improve our understanding of the antigenic determinants and variations among different strains of H5N1 virus, we investigated antigenic profiles and mapped antigenic determinants using a panel of monoclonal antibodies (mAb) raised against strains of H5N1 influenza viruses representing different genetic lineages. More than 300 mAb clones have been produced and confirmed by haemagglutination inhibition test (HI) and cell-based neutralisation test (NT). H5N1 virus strains representing different phylogenetic lineages, isolated from different regions and at different times were selected to characterize these monoclonal antibodies. Based on both HI and NT profiles, these mAb were assigned to 3 major groups. Group I consists of mAb that have broad reactivity to all the selected H5N1 virus strains, whereas group II and III represent mAb with more narrow spectra of reactivity. H5N1 viruses representing current genetic lineages were then grouped according to their reactivity to different groups of mAb. Group A viruses react to all three groups of mAb. Group B and C viruses show different antigenic profiles, with reactivity to all group I mAb but not to some group II and III mAb, respectively. Group D viruses only react with group I mAb. The relationship between the antigenic profiles of H5N1 viruses and their corresponding genetic variations was also investigated; clusters of genetic polymorphisms associated with the differential antigenic patterns of these representative H5N1 strains were identified and characterized. This classification system will facilitate recognition of the antigenic features of newly emerged H5N1 virus strains, and aid in selection of vaccine strains.

Abstract O64

Evolution of H9N2 Influenza A Viruses in Quail From Southern China

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H9N2 influenza A viruses have become established and maintain long-term endemicity in terrestrial poultry in Asian countries. Occasionally these viruses transmit to other mammals including humans. Increasing epidemiological and laboratory findings suggest that quail may be an important host as they are susceptible to different subtypes of influenza viruses. To better understand the role of quail in influenza ecology and evolution, H9N2 viruses isolated from quail during 2000 to 2005 were antigenically and genetically characterized. Our results showed that only three subtypes of influenza viruses (H5, H6, H9) could be detected in quail in southern China. Furthermore, H9N2 viruses are prevalent year-round in southern China with higher isolation rates observed in the winter and that those viruses mainly replicate asymptomatically in the respiratory tract of quail. Antigenic and genetic analysis revealed that both the G1-like (genotype A series) and Ck/Bei-like H9N2 lineages (genotype B series) were co-circulating in quail since 2000. Phylogenetic analyses demonstrated that most of the isolates tested were double or multiple reassortant variants, with four G1-like (A0-A3) and 16 Ck/Bei-like genotypes (B1-16) recognized. Non-reassortants of Ck/Bei-like viruses were not detected in quail. A novel genotype of G1-like virus, designated as Genotype A3, had become predominant in quail since 2003, while multiple Ck/Bei-like genotypes were introduced to quail wherein they incorporated G1-like gene segments, but none of them became established in this host. Those Ck/Bei-like reassortants generated in quail have then been introduced to other poultry. These complex interactions form a two-way transmission system between quail and other types of poultry. The identification of HA and NP genes with high homology to Ty/WI/1/66 in some H9N2 viruses isolated from quail in 2001 suggested that those viruses had not evolved naturally. The present study provides evidence that H9N2 and H5N1 subtype viruses have also exchanged gene segments to generate currently circulating reassortants of both subtypes that have pandemic potential. Continuing influenza surveillance in poultry is critical to understanding the genesis and emergence of potentially pandemic strains in this region.

Workshop Topic #9

Antivirals and Resistance

Oral
Abstracts

Abstract O65

Comparison of the Effectiveness of Oseltamivir and Zanamivir against Influenza A Infection in Children

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Background: In Japan, most patients with an influenza-like illness are tested with rapid diagnostic tests, and if the results are positive, they are treated with oseltamivir. However, since zanamivir has recently been approved for the treatment of influenza in children, we compared clinical effectiveness and viral shedding in an oseltamivir-treated group and a zanamivir-treated group, by children with influenza A. Methods: We used oseltamivir to treat 47 children with influenza A (mean age: 7.0 years) in the pediatric outpatient clinic of our hospital, and used zanamivir to treat 25 children with influenza A (mean age: 8.1 years). All children were tested with rapid diagnostic tests and diagnosed with influenza A before the start of therapy. Oseltamivir was prescribed in weight-based unit doses twice daily for 5 days. Zanamivir (10 mg) was inhaled twice daily for 5 days. Results: Duration of fever after the start of neuraminidase inhibitor therapy was 1.5 ± 0.9 days in the oseltamivir group and 1.5 ± 0.7 days in the zanamivir group. In the oseltamivir group, the survival rate of influenza A (H3N2) virus in the throat was 80% on the 3rd day of treatment, and 47% on the 5th day of treatment, whereas in the zanamivir group, it was 54% on the 3rd day of treatment, but dropped to 8% on the 5th day of treatment, a significantly lower rate. Conclusion: Our results suggest that oseltamivir and zanamivir are equally effective in reducing the febrile period of influenza caused by influenza A subtypes H1 and H3. Comparison of the virus survival rate in the throat after the start of neuraminidase inhibitors revealed a striking difference between the groups. Zanamivir decreased viral shedding more rapidly in the influenza A patients. In the patients treated with oseltamivir, on the other hand, influenza viruses with high infection titers survived longer in the throat, probably inducing oseltamivir-resistant mutants.

Abstract O66

Monitoring of Influenza Virus Susceptibility to Neuraminidase Inhibitors (NAIs)

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Oseltamivir and zanamivir are currently approved NAIs for the control of influenza infections; a third drug, peramivir, is undergoing clinical evaluation. NAI-susceptibility is commonly assessed through an enzyme activity inhibition assay with either chemiluminogenic or fluorogenic substrate. Standardization of assay conditions is essential for reliable drug resistance monitoring. As a part of continued influenza strain surveillance, we utilized a recently developed NAStar kit for the detection of NAI-resistant mutants by means of chemiluminescence. Zanamivir and oseltamivir were used to determine IC_{50} values for 133 A(H1N1), 186 A(H3N2), and 118 B viruses collected in 2005-2007. All viruses appeared sensitive to both drugs with one exception. An influenza B virus, with a R371K substitution at the enzyme active site, exhibited elevated IC_{50} values when tested with both zanamivir (~150nM) and oseltamivir (~1,000 nM), which indicates drug resistance. Another influenza B virus, although sensitive to both drugs, contained substitution H274Y in the NA active site, which was previously shown to confer in-vitro resistance to peramivir. The two newly identified variants and a panel of well-characterized oseltamivir- or zanamivir-selected mutants and their respective wild types (WT) were then tested with four NAIs (oseltamivir, zanamivir, peramivir, and A-315675). When tested with oseltamivir, the oseltamivir-selected mutants [e.g., H274Y(N1)] exhibited 10-fold or greater IC_{50} values than their WT. In contrast, a majority of the zanamivir-resistant viruses [e.g., E119G(N2)] had <10-fold increase in IC_{50} values compared to their WT, when tested with zanamivir. Nevertheless, when tested with oseltamivir, two of those mutants [R152K(B) and R292K(N2)] exhibited >10-fold increase in their IC_{50} values compared to their WT viruses. The H274Y(B) variant exhibited a 4-fold increase in IC_{50} value for peramivir compared to that of the control virus. When the same variant was tested in fluorescent assay, increase in IC_{50} values was greater for both peramivir (~10-fold) and oseltamivir (~5-fold). The R371K variant exhibited cross-resistance to all four NAIs in both assays (≥10 fold). A majority of NAI-resistant mutants were detected with NAStar kit; thus, it could be useful for drug resistance surveillance. To ensure dependable detection of every zanamivir- and peramivir-resistant mutant, improvements to the kit should be considered.

Abstract O67

Novel Mutations in the '150-Cavity' of N1 Neuraminidase Confer Reduced Sensitivity to the Neuraminidase Inhibitors

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The neuraminidase (NA) inhibitors are a specifically designed class of antiviral drugs that bind to the active site of the NA surface glycoprotein of newly formed influenza virus particles and prevent their efficient release from the host cell. Although resistance to these inhibitors is relatively rare, investigation of circulating strains for changes in susceptibility remains important. Analysis of recent influenza isolates received at the WHO Collaborating Centre for Influenza in Melbourne has revealed four A(H1N1) strains with increased IC_{50} (50% inhibitory concentration) values to either zanamivir or oseltamivir carboxylate. These isolates were received from locations where little or no NA inhibitors were in use and are therefore assumed to be derived from patients not being treated with the NA inhibitors. Two of the strains, which demonstrated a 250-fold increase in zanamivir IC_{50} but no change in oseltamivir carboxylate IC_{50}, were found to have a Q136K mutation in the NA gene, while a third strain which had a 30-fold increase in zanamivir IC_{50} and no change in oseltamivir carboxylate IC_{50} was found to have a K150T mutation in the NA gene. The fourth A(H1N1) virus identified to confer reduced sensitivity to the NA inhibitors, had a 30-fold increase in zanamivir IC_{50} and a 10-fold change in oseltamivir carboxylate IC_{50} and was found to have a K143R NA gene mutation. Interestingly, all of the four mutants contained substitutions at residues located in and around the recently identified '150-cavity' (Russell et al, 2006), which following x-ray crystallography was shown to be adjacent to the NA active site of group-1 neuraminidases (N1, N4, N5, N8), but is not present in group-2 neuraminidases (N2,N3,N6,N7,N9). None of these sites has previously been identified as conferring NA inhibitor resistance either *in vitro* or in clinical studies. Given that residues in this cavity appear to impact on zanamivir sensitivity, and to a lesser extent oseltamivir sensitivity, it will therefore be important to determine the extent to which both H1N1 and highly pathogenic H5N1 viruses with mutations in this region may be circulating.

Abstract O68

Naturally Occurring Antiviral Drug Resistance in Avian H5N1 Virus

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Mutations conferring resistance to the adamantanes, amantadine and rimantadine, were detected in the majority of H5N1 viruses in Vietnam, Thailand and Cambodia during 2004-2005 but were less prevalent among H5N1 viruses of other genetic lineages. Resistance to the neuraminidase inhibitor, oseltamivir, was found in H5N1 virus isolated from infected patients in Vietnam but no recent avian H5N1 isolates have been reported to have known neuraminidase inhibitor resistance mutations. Our previous study identified a dual mutation motif, Leu26Ile plus Ser31Asn, on the M2 gene of all amantadine-resistant H5N1 viruses isolated in Vietnam, Thailand and Cambodia during 2004-2005. However no isolates carrying the same dual Leu26Ile plus Ser31Asn mutation were detected in other H5N1 affected countries. We found that influenza viruses carrying the dual mutations seem to replicate better at higher temperatures (37°C and 40°C) than otherwise genetically identical viruses without these mutations. This may contribute to the expansion of viruses carrying these mutations in avian which typically have core-body temperatures higher than 37°C. The most common amantadine resistance mutation, Ser31Asn, is observed to be more prevalent in H5N1 than in other avian virus subtypes, H9N2 and H6N1, isolated from Southern China during the same time-period. We suggest that Ser31Asn in avian H5N1 influenza virus is a naturally occurring mutation which may be associated with virus host adaptation. It is still not clear whether the NA1 gene neuraminidase mutation, His274Tyr, detected in H5N1 infected human cases originated during disease treatment or came from the avian virus source. A 2003 HK chicken H5N1 isolate was found to contain the His274Tyr mutation on NA1 and resistance to oseltamivir was confirmed using a cell-based assay. To investigate if His274Tyr might naturally occur at low levels mixed with wild type in H5N1 poultry infections we examined isolates from different hosts and geographical locations using a differential RT-PCR assay. The His274Ty quasi species was more frequently recognized in isolates from infected chickens than in isolates from ducks and geese, but no geographical difference was observed. Passage His274Tyr mutant virus in mammalian and avian cell backgrounds also indicated that the His274Tyr mutant might be subject to host selection.

Abstract O69

Indonesian H5N1 Isolates Demonstrate Decreased Sensitivity to Oseltamivir

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Two different strains of highly pathogenic H5N1 avian influenza have been circulating since 2003. Clade 1 has been found in Vietnam, Thailand, Cambodia, Laos and Malaysia. Clade 2 subsequently emerged and spread from China to Indonesia, Europe and Africa in 2004-2005. Due to its systemic availability oseltamivir is the drug of choice for treating infected humans. We tested the drug sensitivity of NAs from eight H5N1 viruses from 2004 from Vietnam, two from 2004 from Malaysia, six from Cambodia from 2004, four from 2005 and six clade 2 2005 viruses from Indonesia. Viruses were isolated from chickens, ducks, geese and quail. In the absence of a validated cell culture assay the IC_{50} measured in the MUNANA based NA enzyme inhibition assay was used for measuring drug sensitivity. All clade 1 and clade 2 viruses had a similar sensitivity to zanamivir as the reference human H1N1 NA. Sensitivities of the NAs to oseltamivir fell into three groups compared to the human H1N1 strain. The clade 1 isolates from 2004 were all more sensitive to oseltamivir than the human H1N1 control. However the NAs of the 2005 Cambodian viruses showed a 6-7 fold decrease specifically in oseltamivir sensitivity compared to the 2004 Cambodian isolates. These 2005 isolates came from the same area as one of the more sensitive 2004 isolates, suggesting regional evolution had occurred. Of more concern was the third group. The NAs from all the clade 2 2005 Indonesian viruses demonstrated a 25-30-fold decrease in sensitivity specifically to oseltamivir compared to the clade 1 viruses. This decrease in sensitivity is even greater than that conferred by the N294S recently detected in Egypt, known to be selected for by oseltamivir treatment. There was no altered sensitivity to a third inhibitor, 4-AminoNeu5Ac2en, which shares the 4-amino substitution with oseltamivir, but has the original glycerol side chain at the 6' position. This indicates that the altered binding of oseltamivir was due to altered interaction with its hydrophobic pentyl ether group, thus explaining why no altered binding to zanamivir was seen.

Abstract O70

SCHOLARSHIP International Scholarship

Catalytic Nucleic Acids Mediated Gene Silencing of Influenza A Virus

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Background: Influenza virus infects thousands of people each year, causing epidemics with high mortality. Moreover, there is an increasing concern about a potential influenza pandemic, as highly virulent avian influenza strains are spreading from South-East Asia, with a high risk to cross species-specific barriers. Recent advances in antisense technology have emerged as a ray of hope against many pathogens.The aim of this study to use the catalytic nucleic acids *viz* Ribozymes and DNAzymes as a candidate to have a control on the spread of the influenza virus in the host cells. Methodology: The M1 and PB1 gene of A/PR/8/34 (H1N1) and A/Udorn/307/72 (H3N2) strains were cloned in pcDNA. The computer based secondary structures of RNA were analyzed to design the DNAzymes with 10-23 catalytic motifs and the hammerhead Ribozymes. These DNAzymes and Ribozymes were used for *in vitro* cleavage of RNA of the corresponding M1 and PB1 gene in the presence of different concentration of $MgCl_2$. The cleaved product were separated by PAGE and analyzed by autoradiography. The DNAzymes and Ribozymes were also used in combination. Result: The DNAzymes were able to cleave the M1 RNA at 137 nt position whereas Ribozymes targeted at163 nt position in the same target. These catalytic nucleic acids were highly efficient under the simulated physiological conditions. When DNAzymes and Ribozymes were used in combination, the cleavage was enhanced as compared to when they were used alone. Conclusion: This combinatorial strategy can be used to design multi target DNAzymes and Ribozymes to delay the appearance of escape mutants because of the low probability of simultaneous mutations in both the target RNA sites. This antiviral approach suppresses the production of influenza virus in host cell.

Abstract O71

A Novel Sialidase Fusion Protein DAS181 (Fludase®) Protects Mice Against Lethal H5N1 Avian Influenza Infection

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Effective antiviral drugs are a major component of public health countermeasures for the control of seasonal and pandemic influenza. The recent emergence of human H3N2 and H1N1, as well as avian H5N1 influenza viruses, that are resistant to the adamantanes, as well as isolation of quasispecies that include oseltamivir resistant variants from H5N1-virus-infected patients, highlights the need to develop alternate approaches for the prevention and/or treatment of influenza. DAS181 (Fludase®) is a sialidase fusion protein which enzymatically removes sialic acids on respiratory epithelium, therefore rendering cells resistant to infection of a wide array of influenza A and B viruses. Previous studies determined that DAS181 exhibited antiviral activity in a dose-dependent manner *in vitro,* and reduced replication of H1N1 viruses in mice and ferrets. Here, we use a mouse model to evaluate the efficacy of DAS181 treatment against a highly pathogenic avian influenza (HPAI) H5N1 virus. When used to treat mice daily beginning one day before infection with A/Vietnam/1203/2004 [(H5N1); VN/1203] virus, DAS181 treatment at 1 mg/kg/day protected 100% of mice from fatal disease, prevented viral dissemination to the brain, and effectively blocked infection in 70% of mice. DAS181 at 1 mg/kg/day was also effective therapeutically, conferring enhanced survival of H5N1 virus-challenged mice when treatment began 72 hrs post-infection. This notable antiviral activity against a highly virulent H5N1 virus underscores the potential utility of DAS181 as a new class of drug effective against influenza viruses with pandemic potential.

Abstract O72

The Antiviral Activity of Oseltamivir Against H5N1 Human A/Vietnam/1203/04 and A/Turkey/15/06 Influenza Viruses in Ferrets

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Although stockpiling of antiviral drugs is an essential component of global influenza pandemic preparedness, there is too little information about their efficacy against potential H5N1 pandemic viruses. Therefore, we evaluated the use of the neuraminidase (NA) inhibitor oseltamivir for early post-exposure prophylaxis and for treatment in ferrets exposed to representatives of two clades of H5N1 virus which have markedly different pathogenicity to ferrets. Ferrets given oseltamivir 5 mg/kg/day 4h after virus inoculation were protected from lethal infection with the A/Vietnam/1203/04 (H5N1) virus but higher daily doses (25 mg/kg) were required for treatment when initiated 24 h after virus inoculation. For ferrets inoculated with the less pathogenic A/Turkey/15/06 (H5N1) virus, a treatment of 10 mg/kg/day of oseltamivir was sufficient to reduce the lethargy of the animals, inhibit inflammation in the upper respiratory tract, and block virus spread to the internal organs. Importantly, all ferrets that survived the initial infection were re-challenged with homologous virus after 21 days and were completely protected from infection. Direct sequencing of the NA or HA1 gene segments in both H5N1 viruses isolated from treated ferrets showed no amino acid substitutions known to cause drug resistance in conserved residues. Analysis of individual clones detected NA amino acid changes in 1 of 10/20 clones (V116A; H274Y) sequenced from samples of ferret inoculated with A/Vietnam/1203/04 (H5N1) virus and treated with 10 mg/kg/day and in 1 of 10 clones (H274R; E277Q) sequenced from sample of ferret treated with 25 mg/kg/day of oseltamivir. However, no change in oseltamivir carboxylate susceptibility in vitro was found. These studies demonstrated that oseltamivir was effective against infection by two different clades of H5N1 influenza viruses in ferrets. Early oseltamivir treatment is crucial for protection against highly pathogenic H5N1 viruses and that higher dose may be needed for the treatment of more virulent viruses.

Workshop Topic #10

Innate and Adaptive Immunity

Oral Abstracts

Abstract O73

Influenza-Specific CD8+ T Cells Require Interactions With Dendritic Cells in the Lungs in Order to Mediate Viral Clearance and Recovery From Infection

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Our recent studies have demonstrated that while respiratory dendritic cell (rDC) migration from the lungs to the lymph nodes is limited to the first 48 hours post influenza virus infections, DC accumulation and migration into the lungs from the blood and bone-marrow continues for at least 6 days post infection. Since these recruited DC do not subsequently traffic from the lungs to the lymph nodes and CD8 T cells are thought to follow a programmed expansion following encounter with antigen bearing DC in the lymph nodes, it was unclear what role the newly recruited lung DC might play in influenza immune responses, particularly influenza-specific CD8 T cell responses. Our results show that depletion of rDC from the lungs at 48 hours post infection has a profound effect on the outcome and course of influenza infections. rDC depleted mice show increased and sustained pulmonary viral titers, morbidity and mortality. Further, our results suggest that this increase in influenza-associated disease may relate to a significant decrease in influenza-specific CD8 T cells in the lungs. This reduced pulmonary T cell response does not appear to be attributable to altered or inappropriate activation of influenza-specific CD8 T cells in the lymph nodes or to altered migration of the recently activated T cells, as T cell activation and division in the LN and seeding of other organs occurs at normal rates. Rather the reduction in influenza-specific CD8 T cell effectors in the lungs appears to instead relate to an incomplete expansion and proliferation of the T cells once they enter the lungs. Our in vivo and in vitro results show that the continued expansion of T cells from rDC-depleted animals requires that the T cells engage MHC class I bearing DC. Further only pDC, CD8α+ DC or iDC, but not aDC, purified from the lungs of infected hosts are able to rescue the T cell response to levels achieved in rDC competent hosts. Together our results suggest that the influenza-specific CD8 T cell response is not fully programmed in the lymph nodes during the initial encounter of naïve T cells with DC and instead requires subsequent T cell interaction with antigen bearing DC in the lungs in order to achieve the full influenza-specific effector response.

Abstract O74

Analysis of the Cellular Components of the Inflammatory Response in the Mouse Lung Following Infection With Highly Virulent Influenza H5N1 Viruses and the Reconstructed 1918 Influenza Pandemic Virus

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To better understand the immune mechanisms involved in influenza virus infection, clearance and their role in pathogenesis, it is necessary to assess the cell types responding to infection. An over-zealous cellular infiltration may impair lung function directly e.g. by interfering with gas exchange, or indirectly through the release of soluble immune mediators. Human or murine lungs infected with highly-pathogenic (HP) influenza viruses such as the H5N1 subtype exhibit severe lung pathology with heavy infiltrations of inflammatory cells. In an effort to characterize the cellular components in the lung following a primary HP H5N1 or 1918 virus infection, female BALB/c mice were infected intranasally with a sub-lethal dose of either HP virus or a low-pathogenic (LP) avian or human influenza virus and inflammatory cell populations in the lungs were examined by flow cytometry over the course of infection. Elevated numbers of macrophages were observed during infection with both LP and HP viruses compared to mock-infected animals. However, significantly higher numbers of macrophages were detected in the lungs of mice infected with HP versus LP viruses beginning 2 days post-infection (p.i). Neutrophil numbers were higher in mice infected with any of the strains tested compared with those in mock-infected mice by 1 day p.i. although the numbers of neutrophils in the lungs of HP virus infected mice were significantly higher than those in LP virus-infected mice throughout the course of infection. In contrast, dendritic cell populations declined during the course of infection with HP viruses, but increased in numbers during LP virus infections. Patterns of T cell migration during HP infection are under investigation. Identification of key immune cell populations and their association with enhanced virulence of influenza virus infections may suggest ways to modify these host responses in patients with severe influenza.

Abstract O75

TRAIL Deficiency Increases the Severity of Influenza Virus Infections

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Clearance of primary influenza virus infections is largely thought to be mediated through influenza-specific CD8 T cells, which promote recovery from infection by killing infected cells through perforin and FasL-mediated mechanisms. Interestingly, recent studies have suggested that CD8 T cells can also trigger the apoptosis of virus-infected cells during many virus infections through the expression of TNF-related apoptosis-inducing ligand (TRAIL). Thus, we examined what role TRAIL might play in immunity to influenza virus infections. Our data show that both TRAIL and DR5 (TRAIL receptor) are significantly upregulated in the lungs of wildtype mice following influenza virus infections. This expression correlates with the influx and expansion of TRAIL+IFN©+ influenza-specific CD8 T cells and the subsequent elimination of virus from the lungs, suggesting that TRAIL/DR5 interactions (i.e. CD8 T cell/infected cell interactions) could be an integral part of productive immunity to influenza virus. In this connection, TRAIL deficiency increases influenza-associated morbidity and disease severity. The increase in disease severity also correlates with increased influenza virus titers and a decreased level of influenza-specific cytotoxicity, which occurs despite the induction of equivalent numbers of pulmonary IFN©+ influenza-specific CD8 T cells in both TRAIL competent and deficient mice. Together these results suggests that TRAIL expression is an important component of immunity to influenza virus infections and that TRAIL deficiency may alter CD8 T cell-mediated cytotoxicity thereby increasing the severity of influenza virus infections.

Abstract O76

Promising Junior Investigator Scholarship

Aging-related Impairments in IFN-α Production by Plasmacytoid Dendritic Cells and IFN-γ Production by T Cells Contribute to Down-regulated Natural Killer (NK) Cell IFN-γ production in Elderly Subjects in Response to Influenza Virus

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Introduction: Influenza-caused mortality and morbidity increase with advanced aging. Understanding how aging influences immune response to influenza is important for developing more effective prophylactic and therapeutic regimens for elderly people. NK cells and T cells secrete significant amount of IFN-γ, an important Th1 cytokine, in response to influenza infection. NK cell IFN-γ production is mediated by IFN-α and IFN-γ. How aging influences NK cell IFN-γ production and how aging-related changes in IFN-α and IFN-γ production contribute to this process have been addressed in our study. Methods: Ex vivo stimulation of peripheral blood mononuclear cells (PBMC) with influenza A virus followed by intracellular cytokine staining and flowcytometry were used to determine the frequency of IFN-γ producing cells and intensity of IFN-γ in a per-cell basis. ELISA was used to examine cytokine level in supernatant. Results: 1) Influenza virus-induced NK cell IFN-γ production determined as the frequency of IFN-γ-secreting NK cells and intensity of IFN-γ in a per-NK cell basis in PBMC are down-regulated in elderly subjects compared to young subjects. 2) Addition of anti-IFN-α, -IFN-α/β receptor antibody or removal of pDC from PBMC decreases the frequency of IFN-γ-producing NK cells in response to influenza virus. 3) T cell preceded NK cell in activation, and the presence of rIFN-γ, which mimics IFN-γ secretion from activated T cells, induces IFN-γ production by purified NK cells without the presence of T cells in response to influenza virus. 4) Plasmacytoid dendritic cells (pDC) from elderly subjects display lower frequency in resting PBMC and purified pDC from elderly subjects secrete less IFN-α upon influenza virus stimulation than those from young subjects. 5) Lower level of IFN-α and lower frequency of IFN-γ-producing T cells are found in parallel with lower frequency of IFN-γ-producing NK cells in PBMC from elderly subjects than young subjects in response to influenza virus. Conclusion: IFN-α and IFN-γ are important mediators for influenza-induced NK cell IFN-γ production. Aging-related impairments in IFN-α production by pDC and IFN-γ production by T cells contribute to the down-regulated human NK Cell IFN-γ production in elderly subjects compared to young subjects in response to influenza virus.

Abstract O77

T-Lymphocyte Epitope Based Vaccine for Pandemic Influenza

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The emergence of subtypes of influenza with potential adaptation towards more efficient infection and replication within human cells is just one example of the complexity of predicting which influenza virus strains might initiate the next pandemic. To optimally address this issue of viral variation, a vaccine product capable of inducing efficacious immune responses against many viral variants must be designed and manufactured prior to a pandemic. Our working hypothesis is that vaccines, based on conserved T-lymphocyte (HTL and CTL) influenza-specific epitopes capable of inducing T-lymphocyte immune memory responses, can contribute to control of viral infection. Memory immune responses are predicted to support the expansion of viral specific immune responses induced immediately following infection. Our group developed a highly effective process to identify conserved HTL and CTL epitopes, with predictable levels of immunogenicity and vaccine design parameters, to produce products with unbiased human population coverage. We applied this process to HTL epitopes derived from influenza viral isolates from avian, porcine and human sources which are potential components of pandemic and interpandemic influenza viruses. In our initial studies, 53 highly conserved HTL epitopes were identified. Immune response studies, using IFN-γ production by T-lymphocytes as the primary assay readout, were completed using human PBMC. The data demonstrated that 9 of these epitopes were recognized as a consequence of natural infection and recovery. To assess the contribution of these immune responses to control of viral infection, HLA-DR4 transgenic mice were immunized with 1 μg of peptide representing these epitopes and challenged with the PR8 mouse adapted influenza strain. Immunized mice were protected whereas non-immunized mice were not. These preliminary studies document the critical importance of responses to conserved HTL epitopes as a component in immune response to influenza virus and demonstrate the potential utility of epitope-based vaccines.

Abstract O78

The Human Monoclonal Anti-Influenza Response

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Influenza infection causes 36,000 deaths and 200,000 hospitalizations each year in the United States alone, and pandemics caused by antigenic shift can cause massive mortality worldwide. At seven days after many types of immunization, a burst of antibody secreting cells (ASC's) appears in the peripheral blood. After influenza immunization, these ASC's are present in large numbers and we have shown them to be highly specific for influenza. These ASC's, therefore, represent a potential source of human monoclonal anti-influenza antibodies. Here we show that we can indeed make human anti-influenza antibodies which have both high affinity and are protective. We find that seven days post immunization, as many as 70% of the IgG producing ASC's in the peripheral blood produce high affinity anti-influenza antibodies. By single-cell sorting these ASC's and then cloning and expressing their antibody variable region genes, we can produce large numbers of protective anti-influenza antibodies with affinities up to $7x10^{-11}$ M. We have produced over 50 antibodies to influenza A and B strains from both the 2005/6 and 2006/7 vaccines. In studying these antibodies from various donors, we find that persons who receive regular immunizations show a more "tuned" antibody response than those receiving the immunization for the first time. Significantly, those that have limited immunization histories produce a largely polyreactive response whereas those with immunization histories are highly specific to the virus strains. Our results show a means by which B cell memory can counter influenza's antigenic drift or shift by massive clonal expansions in conjunction with excessive variable gene somatic mutation. This process leads to a specific but polyreactive B cell repertoire that can provide early protection against new influenza strains. Overall, human anti-influenza antibodies cloned and expressed from ASC's provide a means for studying the influenza immune response in detail, as well as being a potential source of therapeutic pharmaceuticals.

Abstract O79

Protection From Avian Influenza? Broadly Cross-Reactive T-Cell Responses to Influenza A in Healthy Individuals in the UK and VietNam

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The threat of H5N1 avian influenza infection in humans is an increasing public health concern. Although few people possess antibodies to the H5 protein, memory T-cell responses to seasonal influenza A infection (H3N2) could potentially provide protective immunity if there was sufficient cross-recognition of H5N1 strains. We therefore performed a comprehensive analysis of memory T-cell responses to the influenza A proteome by using an interferon-gamma ELISPOT assay stimulating fresh PBMC with overlapping peptides (representing the whole H5N1 and H3N2 virus proteome), set out in a 3-dimensional matrix, in 92 healthy individuals recruited in the UK and Viet Nam and determined the extent of cross-reactivity against corresponding proteins from H5N1. More than 80% of study subjects exhibited detectable influenza-specific CD4+ and CD8+ T-cell responses, of which 58% (UK cohort) - 74% (Viet Nam donors) showed cross-recognition of H5N1 peptides. Matrix (M) and Nucleo-protein (NP) were the most commonly recognised proteins, by both CD4+ and CD8+ T cells, while hemagglutinin (HA) was predominantly targeted by CD4+ T cells. Considerable variation in peptide recognition patterns was observed between individuals and 139 18mer peptides containing potential influenza specific T cell epitopes were identified. Of these, 28 were absolutely conserved and a further 47 showed cross-reactivity between H3N2 and H5N1 sequences. These studies provide the basis for evaluating the role of influenza specific T-cells in protection from (or indeed contribution to) the pathogenesis of human infection with H5N1 influenza. In addition, these studies also provides important evidence that the design of universal T cell based vaccines, effective against all influenza virus strains, may be possible.

Abstract O80

The Adjuvants MF59 and Alum Induce Monocyte Chemoattractants and Enhance Monocyte Differentiation Towards Dendritic Cells

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The use of vaccines is one of the most cost-effective public health measures with a huge impact on lowering disease burden and increasing life expectancy. Adjuvants are crucial elements of vaccine formulations as they increase the immunogenicity of the antigen. While a vast number of substances are currently under investigation of their effectiveness as adjuvants, the range of currently approved adjuvants comprised for a long time only two compounds, namely aluminium hydroxide (alum) or -phosphate and the oil-in water emulsion MF59, with the recent addition of a detoxified form of LPS, MPL. MF59 is a widely used, safe and effective adjuvant, yet its mechanism of action is unclear. We assessed effects on human immune cells and found that monocytes and granulocytes are activated by MF59. These target cell types are induced to secrete chemokines such as CCL2 (MCP-1), CCL4 (MIP-1ß), and CXCL8 (IL-8), all reported to be crucial for recruitment of immune cells from the blood into peripheral tissue. On monocytes, MF59 leads to increased endocytosis, enhanced surface expression of MHC class II and CD86 and down-regulation of the monocyte lineage marker CD14, phenotypic changes consistent with a differentiation towards dendritic cells (DCs). In experimental conditions where monocytes differentiate into DCs by addition of GM-CSF and IL-4, the presence of MF59 enhances the acquisition of a mature DC phenotype as monitored by the expression of MHC II, CD86, and CD71. On maturing DCs, presence of MF59 leads to an earlier and overall higher expression of the maturation marker CD83 and the chemokine receptor CCR7 which has a central role in DC homing from peripheral tissue into lymph nodes. Several, but not all of these effects, were also induced by alum, another widely used adjuvant, and not by LPS, suggesting a common adjuvant mechanism that is different from that mediated by danger signals. This is the first identification of the human target cell types of MF59 action, and of the effects that MF59 has on these cells. We conclude from our data that during vaccination, adjuvants like MF59 augment the immune response at a range of intervention points: Through induction of chemokines, they increase recruitment of immune cells to the injection site, they augment Ag uptake by monocytes at the injection site, and they enhance differentiation of monocytes into DCs which represent the gold-standard cell type for priming naïve T cells. A particularly important feature of MF59 is that it strongly induces the homing receptor CCR7 on maturing DCs, thus facilitating their migration into draining lymph nodes where they can trigger off the adaptive immune response specific to the vaccine Ag.

Workshop Topic #11

Mathematical Modeling

Oral
Abstracts

Abstract O81

Impact of Influenza Control Strategies on Seasonal Mortality in the Japanese Senior Population, 1995-2006

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Background: In Japan mass vaccination of schoolchildren from 1962 to 1994 was associated with mortality reductions in persons over 65 (seniors) despite no vaccination program in this population. Since the mid-1990's, vaccination of schoolchildren ended and vaccination of seniors has slowly increased. Since 2001, oseltamivir treatment increased to over 9 million treatment courses sold in 2005-06. We investigated influenza-related mortality in Japanese seniors over this time period. Methods: We used a cyclical regression model to generate seasonal excess mortality estimates of influenza-related mortality (pneumonia and influenza (P&I) and all-cause) among seniors aged 65-89 years during 1995-2006. We compared mortality in seniors in A/H3N2-dominated seasons in the period after 2001 (high vaccine coverage, oseltamivir) to 1995-2001 (low vaccine coverage, no oseltamivir), and mortality from the period when seniors were presumed to be only indirectly protected from vaccinated schoolchildren. Results: In A/H3N2-dominated seasons, crude excess mortality rates among Japanese seniors were 3-fold lower after 2001 than during 1995-2001. Age-adjusted rates showed a similar decline, dropping from 87 to 26 all-cause deaths/100,000 (p=0.04) and from 29 to 10 P&I excess deaths/100,000 (p=0.11). The lack of significance for the P&I trend is likely due to insufficient statistical power. As a comparison, age-adjusted mortality rates in seniors during the peak of the childhood vaccination program (1982-1992) was approximately 2-fold lower than the 1995-2001 period when little to no vaccine was used. Conclusions: Japanese trends data allows for a unique comparison of vaccination and antiviral strategies on a national level. This is a preliminary assessment of the recently changed Japanese influenza control strategy for seniors. While trend studies in the US and Italy have not shown reductions in influenza-related mortality among seniors during periods of increased vaccine uptake, the recent trend in Japan differs markedly. While trends in mortality from the 6 seasons consistently demonstrate a reduction compared to 1995-2001, this assessment is based on relatively few years. Attribution of the relative contribution of vaccination and treatment cannot be readily discriminated. Eventually, a comparison of Japanese trends and trends from other countries may disentangle the relative benefits of antivirals and vaccination.

Abstract O82

Economic Evaluation of Influenza Pandemic Mitigation Strategies in the US Using A Stochastic Microsimulation Influenza Model

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Background: The occurrence of the next influenza pandemic is unpredictable, inevitable and possibly imminent. Recent modeling work highlights the importance of targeted antiviral porphylaxis (TAP) and social distancing measures. However, an important missing component is a realistic cost effectiveness analysis of the potential pandemic influenza mitigation strategies. Our objective is to project the potential impact of pandemic influenza mitigation strategies on health outcome, cost, and cost-effectiveness from a societal perspective in the US. Methods: We use a stochastic agent-based model to simulate pandemic influenza in the community. We compare 17 strategies, focusing on TAP with oseltamivir (treatment of identified index cases and prophylaxis of exposed people) alone and in combination with school closure. We also consider pre-vaccination of the population. We use the human capital approach to estimate productivity loss. Outcomes include number of cases, deaths, QALYs, direct and indirect costs, and incremental cost-effectiveness ratios (ICERs) expressed as costs per QALY gained. Results: In the absence of intervention, we predict a 50% attack rate with an economic impact of $187 per capita. TAP+school closure and pre- vaccination+school closure (preventing 94-96% of cases at $2,730 per capita)) are comparable in terms of QALY gain and total costs. The ICER compared to TAP alone (the most effective single strategy) is about $51,000/QALY for either strategy. The most effective single strategy is TAP alone (prophylaxis of 60% of close contacts of index cases) which effectively prevents 54% of cases at a cost of $124 per capita. If vaccine is available and administered before the onset of the pandemic, then pre-vaccinating 70% of the population with a partially effective vaccine prevents 48% of cases and is the least costly alternative ($99 per capita). Sensitivity analysis on key variables does not change the ranking of strategies. Conclusion: Targeted antiviral prophylaxis is an effective and cost-saving measure for mitigating pandemic influenza. Adding school closure provides greater benefit and is likely to be an attractive strategy if mortality is high.

Abstract O83

Epidemiologic Characterization of the Summer Wave of the 1918 Pandemic in Copenhagen: Implications for Pandemic Control Strategies

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The A/H1N1 Spanish influenza pandemic killed ~50 Million people worldwide in two major waves between September 1918 and May 1919. Sporadic influenza activity occurred throughout Europe in the summer of 1918, but quantitative studies have been missing. We set out to quantify the Copenhagen summer wave in terms of transmissibility and impact on morbidity and mortality. We obtained surveillance data compiled weekly in Copenhagen, Denmark, for 1910-1919; records included medically attended influenza-like illness (ILI), hospitalizations and deaths. We used a Serfling regression model to quantify excess morbidity and mortality, and estimated the reproductive number (R) for each wave. An intense influenza outbreak occurred in Copenhagen in summer, 1918. Approximately 29-34% of the total ILIs and hospitalizations recorded in 1918 occurred during the summer wave, and the peak ILI incidence was ~200-fold higher than any previous summer since 1910. The case fatality in summer (0.3%) was many-fold lower than in fall (2.3%); however, the associated age distribution of summer excess deaths was similar to that observed in the fall wave. The reproductive number R was substantially higher in summer (2.9-4.7) than in fall (1.4-1.7). Overall, about 0.4% of the Copenhagen population died of pandemic influenza during 1918-1919. The summer pandemic wave in Copenhagen may have been caused by an early version of the A/H1N1 pandemic virus that was transmitted efficiently but had not yet acquired its high lethality. The widespread summer outbreak may have provided Copenhagen's population with partial immunity against the lethal fall wave, which would explain the relatively low overall pandemic mortality. Therefore, the R measured in the summer wave is likely a better approximation of the intrinsic transmission in a fully susceptible population (Ro) and substantially higher than ~2 found in previous US studies measured in the fall mortality wave. In the context of pandemic planning, the Copenhagen 1918 summer wave experience is immensely interesting because our upwards reassessment of the transmissibility suggests that mitigating the impact of a future 1918-like pandemic would be more difficult than current modeling efforts would suggest.

Abstract O84

The 1918-19 Influenza Pandemic in England and Wales: Spatial Patterns in Transmissibility and Mortality Impact

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Introduction: There is a growing body of literature on the mortality impact of the devastating 1918-19 influenza pandemic, but spatial heterogeneity in influenza transmissibility and death rates have not been quantified at a refined spatial scale. Methods: We analyzed spatial heterogeneity in weekly influenza-specific deaths during the fall and winter waves of the 1918-19 pandemic in England and Wales, focusing on two levels of spatial resolution (333 administrative units and 62 counties). Transmissibility was estimated through the reproduction number, R, using a sequential Bayesian approach combined with a SEIR (susceptible-exposed-infectious-removed) model. Death rate and R estimates were then compared against historical census variables. Results: The mean R estimate across administrative units was 2.02 (95% CI: 1.97, 2.07) for the fall wave and 1.58 (1.53, 1.62) for the winter wave, in agreement with county-level estimates. There was significant heterogeneity in R across counties during the fall and winter waves (ANOVA, $P<0.02$), partly explained by differences in population density and size (correlation coefficient with R, rho=0.30, $P<0.001$). Transmissibility and death rates declined between the fall and winter wave in most areas of England and Wales, suggesting cross-immunity between the influenza viruses circulating in the two waves – in line with anecdotal evidence. Lorenz curves revealed substantial heterogeneity in death rates as well, both at the unit and county level. The cumulative number of influenza deaths varied as a power law of population size with an exponent significantly less than one (~0.85), driven by the more rural and sparsely populated areas. Conclusion: Our findings, based on refined spatio-temporal disease data, indicate the presence of spatial heterogeneity in the transmissibility and mortality rates of the 1918-19 influenza pandemic. While transmissibility tends to be higher in highly and densely populated areas, death rates are negatively correlated with population size, suggesting that rural and less densely populated areas experience a disproportionately large mortality burden. Further research should clarify whether these patterns can be generalized to other pandemics and seasonal epidemics and evaluate their implications for influenza control.

Abstract O85

Modelling Preventive Measures During an Influenza Pandemic in Italy: A Real Time Simulation Strategy

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We have developed a stochastic individual-based discrete-time model to assess the impact of pandemic influenza containing strategies. We randomly allocated the Italian population (57 millions of inhabitants) to households and schools/workplaces, to match the 2001 census data. The importation of infection was modelled by coupling a global SEIR model with data on arrival and departures in the 44 Italian international airports. The spread of influenza was then modelled with specific levels for households, schools/workplaces, and random contacts. We considered different R_0 values (1.4; 1.7; 2), evaluating the impact of combined preventive measures (vaccination, antiviral prophylaxis [AVP], border restrictions and increased social distancing). For each measure, various scenarios were considered, assuming different target populations, timing and duration of interventions. The administration of two vaccine doses was considered, assuming that the first one would be available 4-6 months after isolation of the pandemic viral strain. Different values for vaccine efficacy according to age were also considered (59% in children and adolescents, 70% in adults and 40% in elderly). The model took about 10 minutes to run on a standard computer (Pentium D 3.20GHz, 4 GB RAM). With no interventions, importation would occur 50-133 days after the first world case, depending on R_0 value. Border restrictions and social distancing measures would delay the spread of the pandemic by a few weeks. With R_0 values 1.4-1.7, the use of combined measures would reduce attack rates (AR) from 34-56% to 4-10%. Assuming $R_0 = 2$, AR can be reduced from 69% to 20% only if vaccination starts 4 months after isolation of the pandemic strain, in combination with 99% reduction of airport traffic, closure of schools/workplaces for 4 weeks and AVP of close contacts of clinical cases. The results show that this individual-based model is a convenient tool allowing simulation of influenza spread in a specific, structured population, and estimation of the impact of control measures aimed at mitigating a future pandemic with little computational effort. It would thus be suitable also for real-time evaluation of actions to be undertaken if a new pandemic influenza virus would emerge.

Abstract O86

A Model for Testing the Effects of Pharmaceutical and Non-pharmaceutical Interventions During a Simulated Influenza Pandemic

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Background: US pandemic response plans recommend both pharmaceutical and non-pharmaceutical interventions. We used Susceptible-Exposed-Infected-Removed (SEIR) models to estimate the effects of school closings, voluntary confinements of ill persons and their household contacts, reductions in contacts among long-term care facility (LTCF) residents, and allocation of limited vaccine supply on illnesses, hospitalizations, and deaths. Methods: We simulated an influenza outbreak in a small urban US community based on data from the 1957-58 pandemic and from studies of seasonal influenza excess morbidity and mortality. Age and household size data were based on the 2000 US Census. Persons belonged to one of five strata: preschool children (aged 0-4 years), school children (aged 5-18 years), adults (aged 19-64 years), seniors (aged 65+ years) living at home or seniors living in LTCFs. Each person belonged to one or more mixing group: households, daycare centers, schools, work places, LTCFs, and the community. Results: Our results suggest that short term school closings of 1-3 weeks would not substantially affect outcomes unless additional measures were taken to reduce out-of-school contacts. If schools were closed for 7 days when the proportion of ill children exceeded 10%, the effectiveness of the intervention in reducing illness was 10.3% (95% CI 7.5%-0.13.1%). If persons who develop symptoms and their household contacts were encouraged to withdraw to their homes, with a delay of two days and 60% compliance, the effectiveness of these interventions on illness rates was 33% for confining the ill only and 80% for confining ill persons and their household members. Preventing ill LTCF residents from making contacts with other residents would reduce morbidity and mortality in this group by ~60%. Finally, with pandemic vaccine available for 20% of the population, vaccination of school children first was more effective than vaccination of the elderly, reducing illnesses by 78% and 40%, respectively. Conclusions: These models highlight benefits of both pharmaceutical and non-pharmaceutical interventions during a pandemic. Early isolation and quarantine even with moderate compliance is a viable method for reducing outcomes associated with a pandemic. If vaccine is available, vaccinating school-aged children first likely will be most effective in reducing pandemic outcomes.

Abstract O87

Viral Resistance May Limit the Benefit of Neuraminidase Inhibitors Therapy During an Influenza Pandemic

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With an influenza pandemic seemingly imminent, most industrialized countries have stockpiled large quantities of neuraminidase inhibitors (NIs) to use for treatment or contact prophylaxis. De novo resistance to these drugs is rare and resistant viruses appear to be compromised in transmissibility, explaining the low prevalence of resistant strains in the seasonal context. However, during a pandemic, the impact of a huge use of NIs on the selection of resistant strains is unknown. Using an individual-centered simulation model [1], we explored various scenarios combining use of one (or two) NIs for treatment or contact prophylaxis. Data on real pandemics were used to calibrate the model. Results presented below focused on the use of a single NI for treating infected people consulting a physician (70% within the first two days of illness). The reference scenario considered a basic reproductive ratio (R0) of 2, a decrease of infectivity of treated patients by 80%, no other pharmaceutical or non pharmaceutical interventions. We performed a sensitivity analysis on the impact of de novo resistance (0 to 5%, 0.2% in the ref. scenario), relative transmissibility of the resistant strains as regards the sensitive strains (0 to 100%, 100% in the ref scenario) and treatment coverage with NIs (0% to 100%, 80% in the ref scenario). Without any treatment, 47.2% of the population would be affected. A treatment coverage of 80%, without any resistance would limit the size of the epidemic to 33.9% of the population. In the reference scenario, the size of the epidemic would not be modified and the proportion of cases infected with a resistant strain would remain low (median value: 0.18%). These values would increase when the rate of the de novo resistance would increase to 1%, 3%, or 5%, with a size of 34.4%, 35.5%, 36.2% and a proportion of cases infected with a resistant strain of 1.4%, 5.4%, 9.6%, respectively. A decrease in the relative transmission fitness of the resistant strain would decrease the impact of the resistance, even when the resistance rate would be high: with a rate of 3%, the epidemic size and the proportion of cases infected with a resistant strain would be 34.3%, 1.8%; or 34.7%, 2.4% for a relative transmissibility of 15%, 50%, respectively. Finally, an increase of the treatment coverage from 80 to 100% would not affect the size of the epidemic, as the decrease of infections with a sensitive strain would be counterbalanced by the growing number of infections with a resistant strain. With a resistance rate of 3%, the proportion of cases infected with a resistant strain would increase up to 18%, without modifying the size of the epidemic. Further analyses are now being conducted to study the potential benefits of using two different antiviral drugs and their most efficient combinations for mitigating the burden of an influenza pandemic. [1] Carrat F, Luong J, Lao H, Salle AV, Lajaunie C, Wackernagel H. A 'small-world-like' model for comparing interventions aimed at preventing and controlling influenza pandemics. BMC Med. 2006 Oct 23;4:26.

Abstract O88

Mathematical Model for Interferon Response on High-virulent Influenza A Infection

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Recent animal studies on 1918 H1N1 or high virulent H5N1 influenza viruses suggest that atypical host innate immune responses may contribute to lethality of the virus. As an effort to understand innate immune responses, we modeled the viral dynamics of primary human influenza infection, especially focusing on the interferon response. Type I interferons can reduce viral production from infected cells or set up an antiviral state in which cells may be made resistant to infection. However, the influenza A NS1 protein can provide the virus with some resistance to the effects of type I interferons, thus making the effects of interferon difficult to predict. The model we developed is useful in this regard and suggests that abnormal response of type I interferon can induce persistence of viral load.

Workshop Topic #12

Virus Host Interaction/Pathogenesis

Oral Abstracts

Abstract O89

Promising Junior Investigator Scholarship

The Influenza A Virus PB1-F2 Protein Contributes to the Pathogenesis of Viral and Secondary Bacterial Pneumonia

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Despite the worldwide devastation resulting from the 1918 Influenza A virus (IAV) pandemic, the contribution of specific viral virulence factors has yet to be fully elucidated. Secondary bacterial pneumonia following IAV infection was an important contributor to excess mortality during this pandemic, and the mechanisms by which the 1918 pandemic strain facilitated bacterial superinfections are of interest. Following the recent discovery of the IAV accessory protein PB1-F2, we decided to assess whether PB1-F2 contributes to IAV virulence and hence may one of the factors involved in the severity of the 1918 pandemic. Initially we generated a mutant IAV engineered by reverse-genetics which did not express PB1-F2, yet was otherwise isogenic to the parent virus (A/Puerto Rico/8/34). Using our influenza-bacteria synergism animal model, we demonstrated that the PB1-F2^{null} virus had an equivalent lung viral load and was equally virulent in mice (Balb/c) when compared to the wild-type virus, but primed less efficiently for secondary bacterial pneumonia. Additionally, mice with pneumococcal pneumonia after infection with wild-type influenza had higher numbers of white blood cells present in their bronchoalveolar lavage (BAL) fluid than mice initially infected with the PB1-F2^{null} virus. Flow cytometry used to further characterize the cellular response to infection showed an increased induction of neutrophils, T cells and monocytes within the BAL fluid of wild-type infected mice, compared to mice infected with the PB1-F2^{null} virus. We then engineered the A/PR/8 virus to express the 1918-PB1-F2 on an otherwise isogenic backbone. The IAV expressing the 1918 PB1-F2 exhibited enhanced growth characteristics in vitro, was more virulent in mice, induced more pulmonary immunopathology and more efficiently primed for severe secondary bacterial pneumonia. These findings may help explain both the unparalleled virulence of the 1918 strain and the high incidence of fatal pneumonia during the pandemic.

Abstract O90

The C-Terminus of the NS1 Protein From Highly Pathogenic Avian Influenza Viruses is a Potential PDZ Binding Domain That Contributes to Increased Virulence in Mice

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PDZ domains are protein-protein recognition modules that mediate the assembly of multi-protein complexes involved in diverse cell signaling pathways. PDZ domains specifically recognize short peptide motifs of 4-5 amino acids found at the C-terminus of the PDZ ligand (PL). The C-terminal four amino acids of the influenza virus NS1 protein from highly pathogenic avian strains, EPEV and ESEV, fit the consensus sequence for a PL. It has been proposed that binding of the avian NS1 protein to PDZ domain-containing proteins within mammalian cells may disrupt certain cellular signaling pathways and may be responsible for the increase in virulence of H5N1 viruses isolated from humans. To this hypothesis a number of potential PL sequences were engineered into the A/WSN/33 virus NS1 protein by reverse genetics. Viruses were generated containing the C-terminal NS1 sequences of the A/Brevig Mission/1/18 virus (KSEV), two H5N1 influenza strains isolated from humans (EPEV and ESEV), a contemporary human H3N2 strain (RSKV), the avian ESEV sequence with a seven amino acid extension found in a number of human H1N1 and H3N2 strains (ESEVRRDKMAD) and an NS1 protein containing a four amino acid truncation thereby lacking a potential PL sequence. In tissue culture the viruses containing the NS1 truncation and the seven amino acid extension were attenuated compared to the other viruses. However when inoculated into mice a significant increase in virulence over the wild type A/WSN/33 virus was observed for the two viruses containing the "avian-like" PL sequences. Differences in MLD_{50}, lung titers, histopathology of tissue samples and the binding of expressed NS1 proteins to PDZ protein arrays will also be discussed.

Abstract O91

SCHOLARSHIP Promising Junior Investigator Scholarship

Seasonal Factors Affecting Influenza Virus Transmission

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In temperate regions of both the northern and southern hemispheres, influenza activity is tightly associated with the winter months. Although this marked seasonality is a familiar feature of influenza epidemiology, the underlying reasons for it are not clear. Theories to explain the winter-time spread of influenza abound. Indoor crowding during cold weather, seasonal fluctuations in host immune responses, and environmental factors including relative humidity, temperature and UV radiation have all been suggested to account for this phenomenon, but none of these hypotheses has been tested directly. Using the guinea pig model of influenza virus transmission, we have evaluated the effects of ambient temperature and relative humidity on influenza virus spread. By housing infected and naïve guinea pigs together in an environmental chamber, we were able to carry out transmission experiments under conditions of controlled temperature and humidity. Our data show that transmission of the human isolate, influenza A/Panama/2007/99 virus (H3N2), varies with relative humidity. Low relative humidities of 20-35% were most favorable, while transmission was completely blocked at a high relative humidity of 80%. Temperature was also found to have an effect: when guinea pigs were kept at 5°C, transmission occurred with greater frequency than at 20°C; while at 30°C, no transmission was detected. Our data implicate low relative humidities produced by indoor heating and cold temperatures as features of winter which favor influenza virus spread. In addition, our findings suggest a simple means to control epidemic influenza: in order to minimize spread, the indoor air of hospitals, schools and nursing homes should be maintained at warm temperatures and intermediate or high relative humidities.

Abstract O92

SCHOLARSHIP Promising Junior Investigator Scholarship

Contribution of Viral Polymerase Complex Genes to the High Pathogenicity of A/Vietnam/1203/2004 (H5N1) Influenza Virus

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H5N1 influenza viruses transmitted from birds to humans in Asia, Europe and Africa cause high mortality. Viral and host genes involved in H5N1 pathogenesis must be identified to determine which factors may be therapeutic targets. We utilized reverse genetics to generate H5N1 reassortants combining genes of lethal A/Vietnam/1203/04 (VN1203), a fatal human case isolate, and non-lethal A/chicken/Vietnam/C58/04 (CH58). In vivo pathogenicity of these H5N1 viruses was tested using mice and ferrets. Exchange of the VN1203 polymerase genes with those of CH58 resulted in attenuation of virulence in vivo and reduced polymerase activity. Interestingly, substituting CH58 NS gene partially attenuated VN1203 in ferrets but not in mice. The role of host cytokine responses to these reassortant viruses was investigated using mice deficient in the hallmark inflammatory cytokines. Overall, our results suggest that the high virulence of avian H5N1 in mammalian species may be due to adaptive changes in viral polymerase complex genes.

Abstract O93

Highly Pathogenic Avian Influenza H5N1 Viruses Elicit an Attenuated Type I Interferon Response in Polarized Human Bronchial Epithelial Cells

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The unparallelled spread of highly pathogenic avian influenza A (HPAI) H5N1 viruses has resulted in devastating outbreaks in domestic poultry and sporadic human infections with a high fatality rate. To better understand the mechanism(s) of H5N1 virus pathogenesis and host response in humans, we used a polarized human bronchial epithelial cell model that expressed both avian alpha-2,3 and human alpha-2,6 linked sialic acid receptors on the apical surface to investigate the relative replication efficiency and ability to trigger type I interferon (IFN) responses by avian H5N1 and a human H3N2 viruses. Two 2004 H5N1 viruses isolated from humans that exhibited a high or low pathogenicity (HP and LP) phenotype in small animal models were selected for analysis. Both avian H5N1 viruses and the human H3N2 virus replicated efficiently in the human bronchial epithelial cells, the latter doing so in the absence of exogeneous trypsin. However, the HP H5N1 virus replicated more rapidly and to higher titers than the LP H5N1 virus, consistent with their relative phenotypes in animals. H5N1 viruses, especially the HP H5N1, induced a significantly delayed and reduced production of IFNβ compared with the H3N2 virus. Furthermore, phosphorylation of STAT2 and induction of interferon-stimulated genes (ISGs), such as MX1, ISG15, IRF7 and RIG-I, were substantially delayed and reduced in cells infected with either H5N1 virus. These data suggest that H5N1 viruses, especially the HP H5N1, possess greater capability to attenuate the type I IFN reponse compared with the human H3N2 virus. These differences may, in part, contribute to the relative virulence of H5N1 viruses in humans.

Abstract O94

Amino Acid 226 in the Hemagglutinin of H9N2 Influenza Viruses Determines Cell Tropism and Replication in Human Airway Epithelial Cells

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Influenza A viruses of H9N2 subtype are endemic in poultry of many countries and have occasionally caused clinical respiratory disease in humans. While some avian H9N2 viruses have glutamine (Q) at amino acid position 226 of the hemagglutinin (HA) receptor-binding site, an increasing number of isolates have leucine (L) at this position, which has been associated with the establishment of stable lineages of H2 and H3 subtype of viruses in humans. Little is known about the importance of this molecular trait for the ability of H9N2 viruses to infect humans. We show here that during the course of a single cycle of infection in human airway epithelial (HAE) cells cultured in vitro, the L-226 containing H9N2 viruses display human virus-like cell tropism (preferentially infecting nonciliated cells). In contrast, Q-226 containing poultry isolates infect both ciliated and nonciliated cells at ratios of 1:1 to 3:2 and other waterfowl viruses (which preferentially infect ciliated cells). During multiple cycle replication in HAE cultures, L-226 containing H9N2 isolates grew more efficiently and reached approximately 100-fold higher peak titers than those containing Q-226,. Our results suggest that the variation in residue 226 in the HAs affects both cell tropism and replication of H9N2 viruses in HAE cells, and may have implications for the ability of these viruses to infect humans.

Abstract O95

Pathogenesis of the Asian Lineage HPAI H5N1 A/turkey/Turkey/05 Virus for Experimentally Infected Pekin Ducks

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An infection timecourse experiment was conducted where 3-week-old Pekin ducklings were infected via the intranasal and intraocular routes with HPAI H5N1 Asian lineage A/turkey/Turkey/05 virus. Days one to five post infection all live birds were swabbed by the cloacal and buccal routes and three animals, selected at random, were euthanased and necropsied. Tissues were processed for M gene real-time RT/PCR (RRT/PCR) analysis, histopathology and immunohistochemistry. Good correlation was obtained between RRT/PCR and immunohi stochemistry. Swabs were tested by RRT/PCR. Clinical signs were observed in some animals 4 days post infection (dpi) and included depression, reluctance to feed, in-coordination and torticollis. At 5 dpi all remaining animals were severely sick and would not have survived the infection. All inoculated animals became infected, as shown by positive results by both RRT/PCR and immunohistochemistry for lung tissues on all dpi. Immunohistochemical detection of avian influenza nucleoprotein showed immunolabelled cells from 1 dpi in nasal cavity, lungs and spleen. The organs where immunolabelled cells were more numerous and consistently present throughout the infection were lung, heart, brain and pancreas. No immunolabelled enterocytes were observed, and only a few tracheal epithelial cells were labelled in some birds at 3 and 4 dpi. Viral load in the lungs peaked at 2 dpi and only low levels of virus was detected by 5dpi. In contrast, high viral loads were observed in the heart and brain of animals from 2- 5dpi. Virus was detected in breast muscle as early as 1 dpi in some animals. Cloacal swabs were not a useful indicator of infection since only two animals shed virus via the cloaca and only on days 4 or 5 post infection. Virus shedding via the buccal route was detected in all animals with one exception at one dpi. However, virus was not detected by this route on every dpi for every animal. A virus dose that was lethal to 100% of 3-week-old Pekin ducks, infected 100% of 8-week and 12-week-old animals but caused no deaths. One animal showed neurological signs but recovered, demonstrating that older ducks can become systemically infected with this virus and survive.

Abstract O96

Detailed Pathogenesis of Swine Influenza Virus in the Natural Host

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Introduction: Swine influenza virus (SIV) is a major cause of acute respiratory disease in pigs [1]. Three SIV subtypes – H1N1, H1N2 and H3N2 – are enzootic in swine populations worldwide, and they do not seem to differ in virulence or pathogenicity. SIV has been shown to replicate in the nasal mucosa, tonsils, trachea, tracheobronchial lymph nodes and lungs of pigs [2]. In the lungs, viral antigen and/or viral RNA have been detected in epithelial cells of the bronchi, bronchioli and alveoli, and in alveolar macrophages [3]. Viremia or virus replication in extra-respiratory sites, in contrast, has only rarely been found [2, 4]. Still, the detailed pathogenesis of SIV remains unexplored. It is unknown, for example, whether the virus replicates better in the upper or lower respiratory tract and which are the major target cells. In addition, the possibility of virus replication outside the respiratory tract has rarely been examined. This study aims to investigate and elucidate these issues. Materials and Methods: Seven 4-week-old conventional pigs were bought on a SIV negative farm and were housed in isolation units with positive-pressure ventilation. The individual pigs were inoculated intranasally with 10^7 50% egg infectious doses (EID_{50}) of the A/swine/Belgium/1/98 (H1N1) isolate in 3 ml of phosphate-buffered saline. The pigs were monitored clinically and one pig per day was euthanized from 1 to 7 days post inoculation (dPI). Nasal and oropharyngeal swabs were collected daily from 0 to 7 dPI or until euthanasia. Samples from the nasal mucosa (olfactory and respiratory part), nasopharynx, tonsils, trachea, four different parts of the lung (left apical and cardiac lobes, left diaphragmatic lobe, right apical and cardiac lobes, and right diaphragmatic lobe), retropharyngeal and tracheobronchial lymph nodes, spleen, ileum, colon, brain stem and conjunctiva were collected for virus titration and immunofluorescence (IF). Serum for virus titration was also collected at euthanasia. Virus titration was performed in Madin-Darby canine kidney (MDCK) cells. IF staining against influenza-A nucleoprotein was performed on all tissues from which SIV was isolated. A scoring system was used to quantify the extent of IF in the epithelium of upper respiratory tract samples, trachea, bronchi, bronchioli and alveoli. Results: Clinical signs, if present, were very mild and lasted for only 1 day. Sneezing was occasionally observed in some pigs at 1-2 dPI. Nasal and oropharyngeal swabs from all pigs were positive by virus titration on days 1-5 PI except for oropharyngeal swabs in 2 pigs on 5 dPI. Virus was isolated from nasal mucosa (olfactory and respiratory part), nasopharynx, tonsils, trachea, lungs, retropharyngeal and tracheobronchial lymph nodes, but not from serum, internal

organs or the intestinal tract. Highest titers were found in the lower respiratory tract. The trachea and all four lung tissue samples tested were positive from 1 throughout 5 dPI, whereas 2/4 lung samples were positive in the pig killed at 6 dPI. Virus titers in the lungs and trachea usually exceeded 6.0 $\log_{10}$ $TCID_{50}$ between 2-4 dPI. Samples from the upper respiratory tract were positive from 1-4 (olfactory part of nasal mucosa and tonsils) or from 1-5 (respiratory part of nasal mucosa and nasopharynx) dPI. Virus titers in these samples were around 4-5 $\log_{10}$ $TCID_{50}$ and clearly lower than those in the lower respiratory tract at all times except 1 dPI. Retropharyngeal and tracheobronchial lymph nodes were positive on days 2-4 and 2-5 respectively, though titers were still lower (2.3-4 $\log_{10}$ $TCID_{50}$) than in the upper respiratory tract. Virus was also isolated from the brain stem and conjunctiva between 2-4 dPI, and virus titers were between 2.0-4.38 $\log_{10}$ $TCID_{50}$ and 2.5-2.67 $\log_{10}$ $TCID_{50}$ respectively. The immunofluorescence stainings confirmed massive virus replication in the lungs. Positive cells were found between 2-6 dPI, mainly in bronchioli and to a lesser extent in bronchi and alveolar tissue. In the trachea virus was found from 2 to 5 dPI in single or groups of cells in the mucosa, and often in fluorescing cellular debris in tracheal lumen. Only single positive cells were found in the nasal mucosa, nasopharynx, tonsils and lymph nodes. Identification of virus-positive cells remains to be performed. IF stainings of the conjunctiva and the brain stem were negative. Discussion: Though it is clear that mammalian influenza viruses cause an acute infection of the respiratory tract the detailed pathogenesis of such viruses remain obscure. It is still unclear, for example, as to whether human influenza viruses replicate mainly in ciliated or non-ciliated cells of the respiratory tract. Our data indicate that SIV has a stronger tropism for the lower respiratory tract of pigs, bronchioli in particular, than for the upper respiratory tract. We will continue to determine the exact cell tropism of SIV in the respiratory tract. The single extra respiratory tissues from which SIV could be isolated were the conjunctiva and brain stem. Virus titers in the brain stem correlated with those in the olfactory part of the nasal mucosa, and we found no evidence for systemic spread of SIV. Both findings suggest that SIV may spread to the brain by olfactory nerves, as has been described in mice with the mouse-neuroadapted WSN/33 influenza virus. In humans, influenza has been occasionally associated with encephalitis and the virus has been isolated from the brain of fatal influenza cases [5]. We have previously suggested that pigs are valuable animal models for research on human influenza [6]. The present study will allow us to further compare the pathogenesis of influenza in pigs with that in other influenza models like mice and ferrets. References: 1. Olsen C.W., Brown I.H., Easterday B.C., Van Reeth K. Swine influenza, in Straw B.E., Zimmerman J.J., d'Allaire S., Taylor D.J. (Ed.), Diseases of swine, Blackwell Publishing, Oxford, UK, 2006, pp 469-482; 2. Brown, I. et al., 1993. Vet Rec. 132: 598-602; 3. Jung, T. et al., 2002. Vet Pathol. 39: 10-16; 4. Kawaoka, Y. et al., 1987. Arch Virol. 93: 303-308; 5. Frankova, V. et al.,1977. Arch Virol. 53: 265-268; 6. Van Reeth, K. et al., 1998. J Infect Dis. 177: 1076-1079.

Workshop Topic #13

Clinical Guidance and Policies

Oral
Abstracts

Abstract O97

Clinical and Epidemiological Characteristics of H5N1 Cases in China, October 2005-February 2007

Hongjie Yu[1], Zhancheng Gao[2], Zijian Feng[1], Yuelong Shu[3], Zhongjie Li[1], Nijuan Xiang[1], Lei Zhou[1], Yang Huai[1,4], Zhibin Peng[1], Cuiling Xu[3], Maowu Wang[1], Pingping He[5], Shui Shan Lee[6], Timothy M Uyeki[7], Weizhong Yang[1], Yu Wang[1]

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Background: While human cases of highly pathogenic avian influenza A (H5N1) virus infection continue to increase globally, available clinical data on H5N1 cases are limited. Whether there are age-related differences in outcomes is unclear. Methods: We conducted a retrospective observational study of all confirmed human H5N1 cases identified through surveillance in China from October 2005 through February 2007. Data were collected from field epidemiological investigations and hospital medical records of H5N1 cases and analyzed. Findings: Twenty-two confirmed H5N1 cases (case fatality 59%) were identified in 12 provinces. Five cases occurred in children younger than 15 years old in rural areas. All 22 cases except one adult had a history of exposure to sick or dead poultry, or had visited live poultry markets prior to illness onset. At admission, all H5N1 cases had fever and cough, most had sputum production and tachypnea. Clinical complications included liver inflammation (86%), renal dysfunction (14%), ARDS (64%), and cardiac failure (45%). Cases treated with antiviral medications had a significantly higher proportion of survivors than those not treated with antivirals (73% VS. 9%, p=0.008). Compared to children, adult cases appeared to have a shorter median time from onset to death (11 days vs. 23.5 days, p=0.064). Interpretations: Human illness with H5N1 virus infection in China was attributed to poultry contact or visiting live poultry markets. Clinical findings were not significantly different between adult and pediatric H5N1 cases except for a trend toward more rapidly fatal outcomes in adults than children.

Abstract O98

Early Oseltamivir Initiation Produces Marked Reductions in Illness Duration, Symptom Severity and Secondary Complications in Children With Influenza

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Children bear a disproportionately high burden of influenza–symptoms are often severe and secondary complications such as otitis media are common. Oseltamivir is an orally-administered neuraminidase inhibitor that significantly reduces the duration and severity of symptoms and risk of associated complications in adults and children when started within 48 hours of symptom onset. In adults, earlier treatment initiation is associated with more marked treatment effects.[1,2] In a prior study, we established the efficacy of oseltamivir in children with influenza when treatment was initiated within 48 hours of symptom onset.[3] Here, we report the findings of a retrospective analysis that explored the impact of time to treatment initiation on effectiveness. A total of 452 children (aged 1–12 years) were randomized into the original study. Inclusion was based on the presence of fever (≥38°C) and cough or coryza of <48 hours duration. Participants received either oral oseltamivir (2mg/kg/dose, n=217) or placebo (n=235) twice daily for 5 days. In the current analysis, the impact of time to treatment initiation (<24 hours or ≥24 hours from symptom onset) on the following parameters was evaluated: time to freedom from illness; duration of fever; symptom score; time to return to normal health and activity; and the incidence of influenza-associated complications. The beneficial effects of oseltamivir treatment over placebo were observed in all analyses ($p<0.05$ for 5/6 and 4/6 analyses in the <24 hours and ≥24 hours groups, respectively). Versus placebo, early initiation (<24 hours) was associated with more marked treatment effects when compared with later initiation (≥24 hours) for the following parameters: reduction in time to freedom from illness, 34.5% (3.75 vs 5.73 days) versus 19.3% (4.61 vs 5.71 days; absolute improvement over later initiation: 78.8%); reduction in duration of fever, 41.5% (1.81 vs 3.10 days) versus 33.1% (1.80 vs 2.70 days; absolute improvement: 25.4%); reduction in symptom score, 52.4% versus 16.5% (absolute improvement: 217.6%); and reduction in the incidence of otitis media, 54.2% versus 35.0% (absolute improvement: 54.9%), respectively. The impact on time to return to normal health (34.2% vs 37.8%, respectively) and the overall incidence of influenza-associated complications (37.9% vs 40.7%, respectively) was less clear. These data confirm that oseltamivir is an effective intervention for the treatment of influenza in children. These findings also confirm that early treatment initiation (within

24 hours of symptom onset) is associated with sizeable and additional clinically meaningful improvements in efficacy. This information provides important new insights into how best to manage symptomatic influenza in children. References: 1. Aoki FY, et al. *J Antimicrob Chemother* 2003;51:123–9; 2. Nicholson KG, et al. *Lancet* 2000;355:1845–50; 3. Whitley RJ, et al. *Pediatr Infect Dis J* 2001;20:127–33.

Abstract O99

Do Statins Reduce the Risk of Influenza and Acute Respiratory Infections?

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The next influenza pandemic is long overdue and may be imminent. The re-emergence of influenza A/H5N1 has reinforced the importance of pandemic preparedness. Antiviral drugs are available, but they are expensive and are unlikely to be available in sufficient quantity. There is therefore an urgent need to identify other therapeutic measures that could offer benefits in terms of the prevention and treatment of influenza. Patients infected with H5N1 appear to suffer an overwhelming inflammatory response, as was probably the case during the 1918 pandemic. Statins decrease levels of pro-inflammatory cytokines and increase levels of anti-inflammatory cytokines. Therefore their potential role for treatment and prophylaxis of pandemic influenza warrants thorough immediate review and exploration. We are currently undertaking a publicly funded project of a retrospective cohort study to examine whether statin use reduces the occurrence of influenza-like illness (ILI) and other acute respiratory infections (ARIs). Using sentinel practices of the Royal College of General Practitioners, we intend to recruit a minimum of 11,000 patients to detect a relative risk of 0.70 with 80% power at 5% significance level. The patients recruited will be aged 45 years and over with chronic conditions such as diabetes and cardiovascular disease, who were registered with an ambulatory care physician (general practitioner) during the period between 01/07/1998 and 30/06/2006. Those with a diagnosis of HIV/AIDS and organ transplantation will be excluded. Episode rates for ILI, ARIs and acute bronchitis between groups with different levels of statin use (user, possible user and non-user) will be compared using appropriate methods of univariable and multivariable analyses. Possible confounding factors will be taken into account including age, sex, co-morbid conditions, smoking history, body mass index, influenza and pneumococcal vaccination, and use of anti-inflammatory drugs. Analysis will be made in each year separately and in three age groups (45-64, 65-74 and 75+ years). The preliminary results will be presented.

Abstract O100

National Vaccination Programs and Trends in Influenza-attributable Mortality in Four Countries

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From 1980-2000 among US elderly, influenza vaccination coverage increased from ~20% to ~65%. Nevertheless, a recent report found that influenza-attributable (excess) mortality did not decline during this period in any elderly age group. This result was unexpected. This study was repeated using data from Italy with a similar result. We decided to provide an even more stringent test of this result. We studied trends in excess mortality data for 1968–2000 from Australia, Canada and France. Canada was chosen as a control for the US, while the impact of influenza was expected to be substantially higher in France and Australia than in northern North America. In each country, vaccination has been directed to defined risk groups with some differences in distribution over time and age group. Excess mortality was found to be largest in Australia, intermediate in France and lowest in Canada. The slope estimates for linear models of 10 yr age groupings were not statistically different from zero for Canada, as for the US. In contrast, in France and Australia for the period, 1980-2000, three age groups in each country were found to have non-zero, negative slopes. The slope estimates for seasons dominated by A(H3N2) viruses and the combination of seasons dominated by A(H1N1) and B viruses (H1&B) were indistinguishable everywhere such comparisons could be made. All age groups in Australia and 6 of 8 for France had statistically significant, non-zero, negative slopes for H1&B seasons over the entire study period, 1968-2000. The 95% confidence intervals for the slope estimates for the six age groups (3 each in France and Australia) for 1980-2000 overlapped those for 1968-2000. Therefore, while there was a general negative trend over time for excess mortality, there was no evidence that these trends were augmented by any of these 5 national vaccination programs. At the population level, national control strategies have not produced observable reductions in influenza-attributable mortality. These programs may require enhancement, since it is likely that this lack of effect is largely due to high mortality, unmodified by vaccination, in a group in frail health, most of whom are elderly.

Abstract O101

Cost-effectiveness of Influenza Vaccination of Adults 50 to 64 Years: A Systematic Review

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Background: Expanding current target groups for annual influenza vaccination may improve the control of seasonal influenza and enhance preparedness for pandemic influenza. Incremental extensions of current age-based target groups represents one such approach. While many countries currently recommend annual vaccination of the elderly, few include all persons 50 to 64 years of age in their national recommendations. Methods: Systematic review of published cost effectiveness studies of influenza vaccination of adults 50 to 64 years of age. Published studies from 1980 to the present were identified through literature reviews. The quality of identified studies was assessed using published criteria; study models, parameter estimates and outcomes were compared using narrative and tabular summaries; and summary results presented using permutation and hierarchical matrices. Results: Four studies were identified: two from the US, one from the UK, and one international model presenting results for Brazil, France, Germany and Italy. The quality of the studies was generally quite high. The studies varied with regard to specifics of model design and parameter estimates used. Net health effects for each of the studies were presented in terms of costs per quality-adjusted life year (QALYs). Each of the studies found that vaccination was likely to be cost-effective. Conclusion: Extension of routine annual influenza vaccination to all persons 50 to 64 years of age appears to be a cost effective intervention that might help to improve the prevention and control of seasonal influenza and promote enhanced pandemic preparedness.

Abstract O102

Cost Effectiveness of Adjuvanted Influenza Vaccination in France

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Mapi Values, SHW Health

Background: This study compares the incremental cost-effectiveness of the MF59 adjuvanted vaccine Fluad®* to non-adjuvanted vaccines in France in situations where the circulating strain matches the strain prepared in the vaccine, and also where there is antigenic drift – where the circulating strain differs slightly from the one included in the vaccine. Methods: A decision analysis model was developed using evidence from clinical studies concerning the relative effectiveness of MF59 adjuvanted vaccine and non-adjuvanted vaccines in the over 65 population. Evidence concerning the impact of MF59 adjuvanted vaccine on hospitalization was taken from an observational study, other resource use parameters and costs from the literature. The analysis was performed from the payer perspective (direct medical costs). Results: Based on the published literature, MF59 adjuvanted vaccine confers superior protection compared to non-adjuvanted vaccines, resulting in a reduction in cases in the community of between 11.8% and 23.8% depending on the circulating influenza strain. Using these estimates, for an attack rate of Influenza-like Illness of 5% and a discount rate of 3%, the incremental cost per life year gained (CLYG) ranges from €3,103 to €23,894. In situations where there is antigenic drift (which occurs every 2-5 years), all vaccines are less effective. Clinical evidence suggests that the efficacy of MF59 adjuvanted vaccine falls by only 2%, whereas that of non-adjuvanted vaccines falls by 22%. With antigenic drift, the cost-effectiveness of MF59 adjuvanted vaccine is enhanced, and depending on the circulating strain, ranges from being cost saving to an incremental CLYG of €5,709. Conclusion: Compared to non-adjuvanted vaccines, MF59 adjuvanted vaccine is cost-effective. The reduction in hospitalisations and mortality justifies the additional cost of the MF59 adjuvanted vaccine. Given that older people are at higher risk of influenza related complications, Fluad® should be considered to be the vaccine of choice in this population. Fluad® is registered under the trade name Gripguard® in France.

Abstract O103

The Macroepidemiology of Influenza Vaccination in 70 Countries: Update for 2004-2005

T Tam

On behalf of the Macroepidemiology of Influenza Vaccination Study Group, Ottawa, Ontario, Canada

In an earlier report, the Macroepidemiology of Influenza Vaccination Study Group (MIVSG) documented influenza vaccine distribution, recommendations and reimbursement in 56 countries for the period 1997-2003. In 2003, the MIVSG countries used 275 million (M) doses, 94% of the 292 M doses distributed worldwide. The MIVSG now includes 70 countries and has updated its findings for 2004 and 2005. In 2005, the 70 MIVSG countries used 331 M doses. In most countries, levels of vaccine use (doses distributed/1000 total population) showed relatively little change from 2003 to 2005, although large differences persisted between individual countries (figure). Six countries increased vaccine use by >60 doses/1000 over the two-year period (Belgium, El Salvador, Japan, Latvia, Malta and Mexico), and Malta's increase (124 to 657 doses/1000) was remarkable. In a few countries, vaccine use decreased, sometimes due to supply shortages. Some form of public reimbursement for vaccination was provided in 60% of countries, and they tended to have higher levels of vaccine use compared with countries with no public reimbursement. Vaccination recommendations showed little change, although the age cut-off levels decreased in several countries and 7 countries adopted policies for vaccinating children 6-23 months in age. Overall, nine vaccine-producing countries used 59% of the doses of vaccine, but had only 12% of the world's population. Influenza vaccine use is increasing more rapidly in countries that do not produce it. This may lead to great difficulties for pandemic vaccine supply unless there is a substantial expansion in global production capacity.

Abstract O104

Prioritization for Vaccination for Influenza Pandemics; Integrating Appropriate Outcomes and Variables for Preparedness Planning

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Background: Influenza control has traditionally focused on immunizing those at highest risk for severe outcomes – the infirm and elderly. This prioritization scheme for allocating a limited vaccine supply does not consider the (highly variable) age-specific pattern of mortality rates associated with the three most recent pandemics, the age-dependence of vaccine efficacy and potential years of life lost. We integrate these factors and demonstrate differences in the optimal strategy to reduce the number of deaths versus years of life lost (YLL). Methods: We applied a seasonal regression model to historical US vital statistics mortality data to estimate age-specific excess death rates associated with the 1918, 1957 and 1968 influenza pandemics. Mortality rates were projected onto the 2000 US population to estimate the age-specific YLL of these pandemics in the current population. We then estimated YLL prevented per dose of administered vaccine incorporating age-specific vaccine efficacy data. Conclusions: The risk of influenza-related death was highest in younger adults for the 1918 pandemic scenario, and highest in the elderly for the 1957 and 1968 pandemic scenarios. The YLL measure shifted the mortality burden towards younger age groups for all 3 scenarios; 56-99% of YLL was projected to occur in persons under 65 years old. The optimal groups for vaccination, which maximized the number of YLL saved per vaccine dose, comprised people under 45 for the 1918 scenario, people 45-64 for the 1968 scenarios, and remained unclear for the 1957 scenario. Measures of YLL rather than mortality rates refocus control strategies for pandemic influenza towards younger populations. The optimal age group for vaccination was different from that previously prioritized in the US pandemic preparedness plans in 2 of the 3 scenarios considered, based on our past experience with pandemics. This study provides further support to prioritize vaccination in younger populations especially in light of mounting evidence of their better age-specific vaccine response, and transmission reduction effects on the overall population. Priorities for receipt of vaccine based on age categories is best determined before the beginning of a public health emergency when there is time to reflect on appropriate decision-making health outcome metric.

Workshop Topic #14

Preclinical Vaccines and Other Intervention Strategies

Oral Abstracts

Abstract O105

Lipopeptide Vaccines Illustrate the Potency of Subtype-Crossreactive T Cell Responses in Protection Against Lethal Influenza Infection

Lorena E Brown, Georgia Deliyannis, Katherine Kedzierska, Yuk Fai Lau, Wy Ching Ng, Bradley Gilbertson, Bock Lim, Weiguang Zeng, Stephen J Turner, David C Jackson

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Large-scale preparation and stockpiling of inactivated vaccines based on current H5N1 strains cannot be justified. The strategy of wait-and-see has therefore been adopted with the hope that if a pandemic occurs, mortality in the period until sufficient vaccine is produced can be minimalised. An alternative strategy is to implement vaccines that boost heterosubtypic $CD8^+$ T-cell immunity to conserved internal viral proteins, which will be effective without prior knowledge of the emerging strain. These will not prevent infection but may lessen its severity and protect against death in this interim period. To test this, we have used lipopeptide vaccines that induce potent $CD8^+$ T-cell responses and characterised the phenotype, function, location and longevity of these cells. We show that lipopeptides containing $CD4^+$ and $CD8^+$ T-cell epitopes and the lipid Pam2Cys, interact with dendritic cells (DC) via TLR2 and this triggers simultaneous uptake of the peptide cargo and signalling via the NFκB pathway for DC maturation, resulting in efficient T-cell priming. $CD8^+$ T cells induced by a single lipopeptide vaccination preferentially establish a memory population equivalent in magnitude to that induced by virus infection. These cells show enhanced longevity and sufficient potency on recall to protect against death from lethal PR8 virus infection in the mouse model. We conclude that boosting of heterosubtypic $CD8^+$ T cells by lipopeptide vaccination of the population would provide a strategy that may lessen the morbidity and mortality that would be expected immediately after a pandemic occurs. This is similar to the use of antiviral drugs but with the advantage of single shot efficacy.

Abstract O106

The Immunogenicity of Influenza Vaccines Reflects Their Effects on Dendritic Cells In Vitro

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In order to facilitate the rational design of future influenza vaccines we compared the immune response induced by current influenza vaccine formulations in vivo with their effects on gene expression profiles of dendritic cells (DCs) in vitro. Balb/c mice and C57BL/6 mice were immunized intramuscularly with whole inactivated virus (WIV), virosomes, subunit vaccine (SU) or active virus. These experiments revealed that WIV induce higher hemagglutination inhibition titers and higher IgG titers than the other vaccine formulations. Moreover, the response to WIV was found to be of a TH1 type with high IgG2a and and a high number of IFNγ-producing T cells, thus resembling the response found after virus infection. In contrast, the response to virosomes and subunit vaccine was dominated by IgG1 and IL4-producing T helper cells indicative of a TH2 response. Subsequently, we elucidated the effects of the different vaccines on plasmacytoid and myeloid DCs in vitro. DCs are the most important antigen-presenting cells and of crucial importance in the induction of immune responses to infections and to vaccination. The quality of the immune response observed in the in vivo experiments correlated with in vitro results. Exposure of plasmacytoid DCs to active virus or WIV resulted in substantial production of IFNα while exposure to subunit did not induce IFNα synthesis. Myeloid DCs reacted to active virus and WIV by maturation and activation and profound changes in gene expression as revealed by microarray analysis. Interestingly, the expression of many genes was similarly altered by active and inactivated virus. In contrast, subunit vaccine had much less pronounced effects on the maturation and the gene expression pattern of myeloid DCs. Our results indicate that the composition of the vaccine rather than the ability to replicate determine the quantity and the quality of the induced immune response. Given the fact that infection with live virus provides strong protection we conclude that WIV – eliciting a similar response than live virus - is a very promising vaccine candidate.

Abstract O107

A Multi-clade Adenoviral Vector-based Pandemic Influenza Vaccine Confers Protection Against Antigenically Distinct Human H5N1 Strains

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Avian H5N1 influenza viruses that are currently circulating in Southeast Asia may acquire the potential to cause the next influenza pandemic. Development of immunogenic vaccines capable of providing effective and durable protection against H5N1 viruses or other newly emerging strains is critical for our preparedness for the next influenza pandemic. Previously, we described the development of an egg-independent strategy by using a replication-incompetent adenoviral (Ad) vector expressing the hemagglutinin (HA) from A/Hong Kong/156/1997 (H5N1). The Ad-H5HA vaccine (Ad vector containing the HA gene of H5N1 virus) induced both humoral- and cell-mediated HA-specific immune responses and conferred protection against death and primary viral replication of antigentically distinct strains of human H5N1 influenza viruses in the mouse model (Lancet 2006: 367:475-81). As little as $1x10^6$ pfu of vaccine was sufficient to induce protective immune responses against challenge with a highly pathogenic homotypic H5N1 virus. The vaccine-induced humoral and cellular immune responses persisted for at least one year, as did the complete protection against a lethal disease from a homotypic H5N1 virus. Because antigentically distinct avian H5N1 viruses have emerged, an optimal pre-pandemic vaccine would contain the HA from different clades with or without conserved internal proteins to confer protection, cross-reactive antibodies and cell-mediated immune responses against future H5N1 viruses. Hence, we have developed an Ad vector vaccine containing the HA from a clade 1 and a clade 2 virus with or without the highly conserved nucleoprotein (NP) from A/Vietnam/1203/2004. Preliminary analyses of these vaccines indicate that the inclusion of both HAs generated HA-specific antibody responses against both clades and the inclusion of NP broadened the protective response. We will present and discuss our recent findings from pre-clinical studies.

Abstract O108

Recombinant Modified Vaccinia Virus Ankara (MVA)-Based Vaccine Induces Protective Immunity in Mice Against Infection With Influenza Virus H5N1

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Since 2003, the number of human cases of infections with highly pathogenic avian influenza viruses of the H5N1-subtype is still increasing and therefore the development of safe and effective vaccines is considered a priority. However, the global production capacity of conventional vaccines is limited and insufficient for a worldwide vaccination campaign. In the present study an alternative H5N1 vaccine candidate, based on the replication deficient modified vaccinia virus Ankara (MVA) was evaluated. C57BL/6J mice were immunized twice with MVA expressing the HA-gene from influenza virus A/Hongkong/156/97 (MVA-HA-HK/97) or A/Vietnam/1194/04 (MVA-HA-VN/04). Subsequently, recombinant MVA-induced protective immunity was assessed after challenge infection with three antigenically distinct strains of H5N1 influenza viruses: A/Hong Kong/156/97, A/Vietnam/1194/04 and A/Indonesia/5/05. Our data suggest that recombinant MVA expressing the HA of influenza virus A/Vietnam/1194/04 is a promising alternative vaccine candidate that could be used for the induction of protective immunity against various H5N1 influenza strains.

Abstract O109

Replication Deficient Intranasal DelNS1 H5N1 Influenza Vaccine

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We developed a novel, replication-deficient, intranasal influenza vaccine with self-adjuvant properties. The principle of this vaccine is based on the deletion of the NS1 gene. The lack of the NS1 gene has two important consequences: (i) when delivered into the respiratory tract, the vaccine

virus infects cells, expresses viral antigens but fails to form viral progeny; (ii) it induces immunostimulatory cytokines that efficiently stimulate mucosal secretory IgA and CTLs, which, in turn, provide cross-reactive immunity. This vaccine approach is applicable for all types of influenza. We used delNS1 technology to obtain several H5N1 vaccine candidates. Importantly, they were able to grow to high titers in Vero cells. The candidates were evaluated in mice, ferrets and macaques. Animal studies confirmed the replication-deficient phenotype and a high immunogenic potential. A single intranasal immunization protected animals against challenge with H5 viruses belonging to different clades. Moreover, despite of avian (Sia2-3Gal type) receptor specificity, the vaccine appeared to be highly immunogenic in macaques after intranasal application. These preclinical findings indicate that delNS1-based pandemic vaccines might be an attractive alternative to conventional approaches.

Abstract O110

Induction of Cross-Clade Anti-H5N1 Immune Responses in Mice and Guinea Pigs by Vero Cell-Derived H5N1 Whole Virus Candidate Vaccines

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The re-emergence of highly pathogenic avian influenzaviruses (HPAI) of subtype H5N1 in South East Asia since 2003, their spread over several African and European countries by migratory birds, and the infection of more than 270 humans with a case-fatality-rate of 60% has increased the awareness of the threat of a new influenza pandemic. This has resulted in pandemic preparedness activities including the development and production of H5N1 vaccines for either stockpiling of currently available candidate vaccines or advanced purchase agreements of vaccines against newly emerging pandemic influenzaviruses. Both approaches have their specific advantages and disadvantages; stockpiling of the vaccine ensures its immediate availability whenever a pandemic starts, but it may be less effective because of antigenic mismatches with the actual pandemic strain. On the other hand, the production of a pandemic vaccine in the scope of advanced purchase agreements can result in a more effective vaccine by using the actual pandemic virus, however, huge quantities of vaccine have to produced within a short timeframe. Baxter has produced H5N1 whole virus candidate vaccines against H5N1 clade 1 (A/Vietnam/1203/2004) and clade 2 (A/Indonesia/05/2005) strains at an industrial scale using its serum protein free Vero cell fermenter technology. These candidate vaccines have been shown to be highly immunogenic in mice and guinea pigs by inducing cross-neutralizing antibodies (which has been confirmed in a clinicical phase I/II study in humans) and showing cross-protectivity against the homologuous strain of the same clade and the heterologuous strains of the other clades in mice. The immunization and protection studies in guinea pigs and mice have been extended by performing heterologuous prime-boost studies i.e. priming with the clade 1 Vietnam 1203 candidate vaccine followed by a booster with the clade 2 Indonesia candidate vaccine or vice versa. These studies have shown that the heterologuous prime-boost strategy resulted in broad anti-H5N1 immune responses with excellent cross-neutralization activities and cross-protectivity against representative H5N1 viruses of clade 1, clade 2, subclades 1 and 2, as well as clade 3 strains. The impact of these results on stockpiling and advanced purchase agreement strategies will be discussed.

Abstract O111

Cross-Protection Against Heterologous H5N1 Challenge in Ferrets With Low Dose Adjuvanted Split H5N1 Vaccine

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Background: The production of pre-pandemic vaccine using viral strain derived from the currently circulating avian H5N1 strain is currently considered as an attractive strategy to allow for early vaccine availability in case of a pandemic. To be successful, such pre-pandemic vaccine must confer cross-protection since pandemic strains might not match exactly the avian antigen in the vaccine. In the present study, adjuvantation has been selected as the strategy of choice to confer cross-immunity because it has been shown to induce cross-protection when combined with seasonal influenza antigens and it is readily available. Aims: To evaluate, in a preclinical challenge model, the cross-protective potential of an adjuvanted clade 1 H5N1/A/Vietnam/1194/04 split vaccine following a challenge with a heterologous clade 2 H5N1/A/Indonesia/5/05 virus. Methods: Four groups of 6 ferrets (Mustela putorius furo) were immunized intramuscularly with 15, 7.5, 3.8 or 1.7 µg of H5N1/A/Vietnam/1194/04 (clade 1; NIBRG-14) vaccine containing a novel proprietary adjuvant system. The two control groups included ferrets immunized with either the adjuvant alone or the non-adjuvanted H5N1/

A/Vietnam/1194/04 vaccine (15 µg). Ferrets were vaccinated on days 0 and 21 and challenged intratracheally on day 49 with 10^5 $TCID_{50}$ (50% Tissue Culture Infective Dose) of H5N1/A/Indonesia/5/05 (clade 2). Lung tissues were collected after the challenge to quantify viral load by culture on MDCK cells. All animals surviving challenge were euthanized on day 54. Results: Challenge of ferrets with H5N1/A/Indonesia/5/05 showed that all control animals receiving adjuvant alone or non-adjuvanted H5N1/A/Vietnam/1194/04 vaccine died or were moribund and needed to be euthanized on days 3 or 4. The majority of animals immunized with adjuvanted H5N1 split vaccine were protected against the lethal challenge with H5N1/A/Indonesia/5/05 and 96% survived to the end of the challenge phase on Day 5. Viral replication was observed in all ferrets immunized with the adjuvant alone or non-adjuvanted H5N1/A/Vietnam/1194/04 vaccine that exhibited a viral load higher than $10^{5.5}$ $TCID_{50}$/g of lung tissue. An antigen-dose dependent decrease in viral load was observed in all ferrets immunized with adjuvanted vaccines: In 70% ferrets immunized with adjuvanted H5N1 vaccines no virus was detected (under the limit of detection of 10^2 $TCID_{50}$/g of lung tissue). Specifically, no virus was detected in 80% and 84% of ferrets in the adjuvanted H5N1 7.5 µg and adjuvanted H5N1 15 µg, respectively. Animals immunized with the adjuvant alone showed a higher incidence of primary atypical pneumonia in the cranial lobe of their lungs suggesting a more severe respiratory distress. Pulmonary oedema and enlarged bronchial lymph nodes were observed in animals from all groups except those immunized with the highest doses (7.5 and 15 µg) of adjuvanted vaccine, suggesting protection from challenge virus mediated pathology. Furthermore, serological assessments showed that the adjuvanted monovalent H5N1 A/Vietnam/1194/04 split formulations induced neutralizing antibody responses against the homologous H5N1 A/Vietnam strain. Adjuvanted H5N1 A/Vietnam vaccine also induced neutralizing antibody responses to the heterologous clade 2 H5N1/A/Indonesia strain while no neutralizing antibody response (< 40) was observed in ferrets immunized with the non-adjuvant A/Vietnam vaccine or the adjuvant alone. Interestingly, all ferrets with anti-H5N1 A/Vietnam neutralizing antibody responses below 40 were not protected in terms of mortality or viral shedding in the lung. Conclusions: We have now identified a candidate H5N1 pandemic influenza vaccine formulated with a novel adjuvant with a good safety profile in humans (Borkowski et al. International Conference on Influenza Vaccines for the World, Vienna, Austria, 18-20 Oct 2006). In the clinic, this vaccine has demonstrated superior antigen-sparing activity and induction of high levels of cross-neutralizing antibodies against drifted H5N1 virus (see Leroux-Roels et al.'s abstract to be presented). Further to this, the cross-protection data against heterologous challenge in ferrets reported in the present abstract will significantly impact the future development of vaccines against H5N1 pandemic influenza and support a pre-pandemic immunization strategy.

Abstract O112

Neutralizing Human Monoclonal Antibodies Against the H5 HA Are Effective in Prevention and Treatment of H5N1 Infection in Mice

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The morbidity and high mortality rate associated with human infections by highly pathogenic avian influenza (HPAI) A H5N1 viruses highlights the need for the development of novel prophylactic and therapeutic interventions. Memory B cells from Vietnamese patients who recovered from HPAI H5N1 infections were immortalized using Epstein Barr Virus. B cell lines that secreted anti-H5N1 neutralizing antibodies were screened, cloned, and monoclonal antibodies were purified. Several human monoclonal antibodies (hMab) with broad cross-neutralizing activity against different strains of H5N1 were identified. hMab FLA3.14, FLA5.10, FLD20.19 and FLD21.140 neutralized clade I viruses in vitro and hMabs FLA3.14 and FLD20.19 exhibited neutralizing activity against clade II H5N1 viruses as well. The prophylactic and therapeutic efficacies of these hMabs were evaluated in BALB/c mice. Prophylaxis with hMabs FLA3.14 and FLA5.10, administered intraperitoneally (ip) 24 h prior to administration of A/VietNam/1203/04 (H5N1) conferred 100% protection from lethality in mice. Prophylaxis with these hMabs also resulted in a statistically significant decrease in pulmonary virus titer, reduced associated inflammation in the lungs, and restricted extrapulmonary dissemination of the challenge virus. The therapeutic efficacy of hMabs were determined by administering them ip at 24, 48 and 72 h post-infection (p.i.) with 5 LD50 of A/VietNam/1203/04. All of the mice that received hMabs FLA5.10 and FLD21.140 survived, 80% of the mice that received either FLA3.14 or FLD20.19 24 h p.i. survived, and 100% of the mice that received these hMabs at 48 and 72 h p.i. survived. hMabs FLA3.14, FLD20.19 and FLD21.140 were also effective against challenge with 5 LD50 of a clade 2 H5N1 virus. These results demonstrate the proof of concept that fully human Mabs with neutralizing activity generated from the peripheral blood of convalescent patients can effectively prevent and treat H5N1 infection in vivo. A panel of neutralizing cross-reactive hMabs may be useful for prophylaxis or adjunctive treatment of human H5N1 infections.

Workshop Abstracts:

POSTER PRESENTATIONS

Workshop Topic #1

Disease Surveillance and Impact

Poster Abstracts

Abstract P101

Influenza Virus Detection During a Fatal Outbreak of Adenovirus Associated With Idiopathic Acute Febrile Syndrome, 2005 Summer, Havana Cuba

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Background: Human influenza is an annual cause of morbidity and mortality world-wide. Infection typically causes an acute febrile respiratory illness. Neurological complications associated with influenza have been reported for more than 100 years. In the late of 1990s in Japan was associated an epidemic of influenza with hundreds of cases of encephalopathy among children. Small numbers of children with neurological complications have been reported sporadically in Europe and Canada. During 2003-2004 season Texas reported some cases associated with a variety of neurological symptoms. At the beginning of the 2005 Cuban summer, an outbreak of idiopathic acute febrile syndrome has occurred characterized by a spectrum of syndromes (viral meningitis, fever, vomiting and diarrheas). Our main objective was to detect the etiologic agent that causes a morbidity increase and 8 deaths. Methods: We used two Nested RT-PCR multiplex systems to detect respiratory viruses. The sample used to detect possible etiologic agent of viral meningitis was cerebrospinal fluids (CSF) with n=22. Extractions of RNA and DNA were realized using the method published by Casas et al in 1995. Results: Between July and August 2005 a total amount of 22 CSF samples from patients suspected of viral meningitis were studied. Mainly observed symptoms were fever (90%), vomit (45%), weakness (45%) and acute disease diarrhoeal (37%). We can detect 2 patient's positive to influenza C and 1 for influenza A. Conclusions: Besides etiologic agent that causes the idiopathic acute febrile syndrome we found interesting results. Obtained results here contribute to raise awareness of the neurological complications of influenza. We cannot forget the variability of RNA viruses, which can change host range and the viral tropism more easily than other viruses. Until now, international papers report that influenza virus is an infrequent cause of viral meningitis, but is true too that recently some researcher groups have powerful tools for the detection of influenza virus in CSF, since the isolation is very difficult.

Abstract P102

Surveillance of Influenza Viruses in Taiwan During the Seasons 1999-2006

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Introduction: Influenza virus is a worldwide important pathogen that can cause severe illness in young children and elderly people, and is one of the most active pathogens in Taiwan. Monitoring influenza activity has been coordinated by the Center for Diseases Control, Taiwan since July 1999. For the mutability of influenza viruses, surveillance is mainly and based on laboratory isolation of influenza viruses and sentinel reports of influenza-like illness. Analyses of epidemic influenza virus isolates have chiefly focused on antigenic and genetic characterization of the hemagglutinin (HA) glycoprotein in order to detect new variants of each epidemic strain and provide analysis results to WHO global influenza surveillance. Materials and methods: All respiratory specimens were collected from sentinel physician networks (300 more sentinel clinics throughout the whole island) and other sources. Specimens were tested for influenza, mainly by virus culture and reverse transcription polymerase chain reaction amplification (RT-PCR). Virus culture and IFA methods were applied to identify influenza viruses. Viruses were characterized by hemagglutination inhibition (HI) assays using the WHO Influenza Diagnostic Kit. Phylogenetic analysis was carried out for genes encoding hemagglutinin (HA) of influenza A and B viruses. Results: From July 1999 to November 2006, 7339 influenza viruses were isolated in Taiwan, including 4682 influenza A viruses and 2657 influenza B viruses. Among type A viruses, 1116 were H1N1 and 2382 were H3N2, and the others remained as unsubtype. The influenza A viruses peaked up during winters of 1999, 2001, 2002, 2003, and 2005. The influenza B viruses peaked up in the winters of 2000 and 2004. However, some isolated peaks of influenza A were found in the summer and fall of 2004 and 2005. An unusual peak of influenza B was observed in the summer of 2002. In the meantime, A/H1N1 was the major circulating strain for 2001-02 season. Subsequently, influenza A/H3N2 became dominant during the following consecutive three seasons. Results of HI assay for the epidemic strains during each season revealed partial mismatch of the epidemic strain versus the vaccine strain was also noted for several seasons. Discussion: During the 7 continuous influenza seasons in Taiwan, some mismatches were observed between the endemic strains and WHO recommended vaccine strains. A 1-year lag phenomenon before vaccine strains recommended by WHO was also seen in Taiwan. Analyzing the activity of influenza in Taiwan, the subtypes of epidemic strains were extremely different from those of the European countries and the US;

they were relatively similar to those of some Asian countries such as Japan and Hong Kong. Preparation for an influenza pandemic is presently a high priority in Taiwan. Laboratory-based surveillance systems must be timely in order to be effective. The results from our data indicate that multiple viruses cocirculate in Taiwan during each flu season, and also indicate the need to establish more sensitive methods for influenza virus diagnosis and surveillance, In addition, our data provide clear information of influenza activities in Taiwan and are helpful for global surveillance of newly emerging influenza viruses.

Abstract P103

Influenza Like Illness Surveillance Among Renal Transplant Patients Presenting With Respiratory Acute Infection in a Outpatient Clinic From 2002 to 2004

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Immunosuppressed patients can present worse clinical outcome after influenza acquisition because a compromized immune response to infection. This growing population is surviving longer due to advances in drug therapy. There is a lack of information about influenza infection among renal transplant patients. Sao Paulo Federal University is one of the largest center of kidney transplant around the world. We investigated influenza infection from 2002 to 2004 in an outpatient renal transplant group. Methods: Two hundred and forty nine patients attending a renal transplant outpatient clinic underwent to epidemiological, clinical and laboratory investigations. Patients were interviewed by our researchers and information included: demographic data, household children contact, history of symptoms, clinical presentation, comorbidities, smoking, influenza vaccination status and post-transplant period. One nasal wash (NW) specimen was collected from each individual presenting with acute respiratory symptoms referred by physicians. All specimens were evaluated on the same day by direct fluorescent assay for Influenza A and B, RSV, Adenovirus, Parainfluenza 1, 2, and 3 (Chemicon International Inc., Temecula, CA). Frozen samples from 2002 and 2003 aliquots were tested by duplex RT-PCR (flu A M-gene and B NS-gene) and MDCK cell culture. Results: Subjects: From 2002 to 2004 a total of 249 subjects were included as follows: 41 in 2002, 28 in 2003 and 180 in 2004. Among them 54.2% female (135), with mean age of 38 years (5-67 years) and 14 months of organ transplant (0.5-144 months). Only 8% related influenza immunization and vaccination was delivered before transplantation. Children household contacts were reported by 29% of the patients. Etiology Results: Among 249 samples tested, 11 (4.4%) resulted positive for Influenza A (6 cases) or B (5 cases). Frequencies of other viral etiology were: 9 cases of RSV (3.6%), 1 case of PIV-1 (0.4%). Samples were collected between 1 and 13 days after onset of symptoms (mean 7 days) with 40% of samples taken within 5 days. Influenza Results: We obtained different peak activity frequencies for each annual season. Positivity on 2002, 2003, and 2004 were 9%, 2%, and 3%, respectively. Influenza A and B co-circulate at same time in 2002 (type B predominance) and 2004 (type A predominance), but in 2003, influenza B was not found. Clinical data: Considering all patients, fever was reported by 19% of patients, cough 76%, coryza 78%, headache 41%, sore throat 33%, myalgia 26%, chills 16%. Fever was reported by 63% of influenza laboratory-confirmed patients and among 17% of influenza negative patients. Headache, myalgia and and chills were respectively reported by 41%, 26% and 16% of the entire study group. These general symptoms reached 63% (headache), 36% (myalgia) and 18% (chills) among Influenza positive renal transplant patients. Influenza positive cases were enrolled with 1-15 days of symptoms (mean 3 days). Children contact risk factors: Analysis of exposed patients showed that no association could be detected among patients with influenza laboratory –confirmed infection and contact with children in a univariated analyse. Influenza vaccine: None of influenza positive cases reported immunization. Discussion: This is first study assessing clinical, epidemiological and virological aspects of respiratory viral infections among renal transplant patients in Brazil. Viral laboratory analyse assessed an etiology in 8.4% of cases, using direct fluorescence assay. This method is the most used in laboratories in Brazil. However, comparison with molecular and culture methods for influenza did not lead to increased detection in this study. Indeed, during the study period there was influenza low activity in Sao Paulo city (official data). Monthly incidence of influenza cases correlated with expected seasonality in southeast that usually starts in April and ends in September. Influenza virus types found during the study period correlated well with those of the influenza isolates from official surveillance in this region: prevalence of Influenza A in 2003 and 2004, but Influenza B in 2002 season. Reappearance of Influenza B Victoria-lineage was replaced by the Yamagata-lineage during 2002, which diverged from the recommended season vaccine, and was most prevalent in relation of influenza type A. Renal transplant patients had an unexpected low influenza laboratory confirmed results considering immunosuppression and lack of Influenza vaccination, in this first investigation. Of note, the influenza positive cases were less symptomatic as usually reported for general community population. Probably, transplanted patients with mild infections did not realize themselves as really sick or were ruled out because physicians misdiagnosis of common cold. In addition, they may have asymptomatic infections that our protocol could have missed. We hypothesized that the use of steroids and others immunosuppressors reduced clinical expression as fever and general symptoms. Differentiating Influenza virus from the

others respiratory viruses is of prime importance because the influenza virus infection is associate with higher morbidity and mortality and can be managed with specific antiviral. Influenza infections cause an extremely varied clinical syndrome, which result in variable case definition for ILI worldwide. However, in this group of immunosuppressed patients, ILI case definition would underestimate the correct impact of infection. We propose a routine surveillance including less symptomatic patients during influenza seasons and a follow-up of hospitalized patients. Immunization programs should also be implemented even in the possibility of poor immune response. In conclusion, Influenza activity varies among different seasons. Clinical expression among transplanted patients may vary from common immunocompetent population. Clinical patterns of infected influenza patients should guide more adequate influenza surveillance for renal transplant patients, particularly in this pre-pandemic time.

Abstract P104

Analysis of the Epidemiological Situation of Influenza during the years 2000-2005 in Pedro Betancourt Municipality, Matanzas, Cuba

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Acute Respiratory Infections (ARI) and specially Influenza viral infection cause a broad array of respiratory illnesses that are responsible for significant morbidity and mortality in children and elderly people as well as in the rest of the population, mainly those with conditions that might predispose them to complications, this is specially important in countries where this group of patients usually lack an adequate medical attention. Influenza might affect between 10 to 20 percent of the world population in a given year and is situated as the 6th place in all causes of death in all ages. Is calculated that more than 4 million of children die yearly as consequence of ARI, most of them in the under developing countries. For Cuba influenza represents a burden as it is for the rest of the world, Influenza appears among the 10 leading causes of death during the past 20 years, being the 5th for those under 5 years of age and in the 6th place for the general population on the year 2003. Studies had shown that influenza and Influenza Like Illnesses (ILI) also represent a great number of consultations per year, approximately 25 to 30% and Cuba as part of the countries belonging to the WHO, has set a group of measures that will hopefully allow us to decrease the impact of Influenza outbreaks. International experience reveals that Health promotion together with other specific measures of prevention and a proper medical attention can have a positive impact on the reduction of morbidity by Influenza as well as the possibility of decreasing the number of complications and deaths occurred by this disease. Methods: The total population that attended any of the Primary Health Facilities in our Municipality during the years 2000 to 2005 constituted the universe of our investigation. The total of patients that attended the Health facilities and were classified as suffering from ARI were considered as the sample in this research. The data was compiled from the Doctor's daily reports received from the clinics and the Emergency Department and were processed and tabulated by the Statistic Department of our municipality using an IBM compatible processor. The Municipal Death Registry provided us all the figures and information regarding the area under discussion. From all these statistics collected, we made a descriptive-retrospective investigation. The marks are displayed in graphic and tables and are expressed in percentage. During the discussion the results are compared to similar investigation carried out in Cuba and other countries. Results: The results of an investigation using a descriptive-retrospective type of study are shown in this research. The population under investigation was the entire residents been attended by Primary Health Care network in Pedro Betancourt Municipality during the years 2000 to 2005. Data was collected by the Municipal Statistic Department from the Doctor's attendance records in the Clinics as well as in the Emergency Department. From a total of 926431 persons attended in that period of time, 108451 were classified as ARI, in accordance with the Integral Program for Attention and Control of ARI cases released by the Ministry of Health in 2000. The incidence of such ailment according to the figures obtained by our Statistic Department was barely 11.70%, which was very distant from the figures accepted internationally as probable for a given period. The months that were more affected by ARI were in decreasing order August with 13.54% of all cases, followed by September and October with 12.86% and 12.73% correspondingly. Of 108451 persons classified as suffering from ARI, sample to determine the specific pathogen was merely obtained in 10129 what represented 9.33% of all patients. We also observed than only 21% of population at risk of contract Influenza and Pneumonia were vaccinated with the Influenza vaccines what definitely put them on a higher risk of suffering complications or even death from Influenza and Pneumonia. Conclusions: 1. The incidence of ARI in the analysed years was 11.70% of all consultations, which is considered low for a given period of time. The months with the higher incidence of cases of ARI, during the years 2000 to 2005 were in declining mode August (13.54% of all consultations), September (12.86% of all consultations) and October (12.73% of all consultations); 2. An entire amount of 108451 patients were classified as ARI during the years 2000 to 2005. From that sum 10129 serum samples to be sent for classification of the pathogen were obtained, what represented a low 9.33% of all cases of ARI; 3. Only 24.29% of the population received the Influenza vaccine during the period of the six years studied. These results are very low compare to the goals set by WHO; 4.

A total of 243 persons died of Influenza and Pneumonia during the years investigated what represented a 16.22% of all deaths on all ages. The biggest amount of deaths by Influenza and Pneumonia were reported during the year 2004 with 50 deaths for these diseases, positioning it as the third leading cause of death for all ages in our municipality on that particular year; 5. The age groups that prevailed as the one with the biggest number of deaths by means of Influenza and Pneumonia were the +75 years of age having a 21.97%, consequently the –1-year of age with 16.66% and the 65 to 74 years of age with 14.82%. The months that were responsible for the greatest quantity of deaths reported were April and July with 19.53% of all deaths by Influenza and Pneumonia each and September with a 19.00%; 6. Influenza and Pneumonia placed itself in the third position among the leading causes of deaths for all ages in our municipality, being in a position even higher than the one it occupied in a country level.

Abstract P105

Practical Aspects of Strengthening Human Cases of Avian Influenza Surveillance in Georgia

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Learning Objectives: 1) to identify key determinants of a functioning human avian influenza surveillance program; 2) to recognize that, in order to make a sustainable improvement, all health system factors that may affect the functioning of surveillance, need to be studied and addressed. Abstract: Recent outbreaks with human fatalities in two neighboring countries—Turkey and Azerbaijan—highlight the critical HPAI threat facing Georgia. To keep the infection at bay, in 2006 the USAID Avian Influenza project began providing assistance to the Government of Georgia to improve surveillance of human cases of avian influenza. On the basis of the system assessment findings the National Surveillance Task Force and project advisors developed a system strengthening package, that included innovative technical standards and procedures (one of the first technical guidelines in the region), job aids, district-level training, and continuous supervision and support. The package was implemented nationwide within the following 6 months. Its effectiveness was assessed through an evaluation using quantitative and qualitative data collected at the district- and facility-levels. The evaluation results provide evidence that the reforms led to improvements in the health workers' knowledge of surveillance and infection control procedures and pandemic influenza preparedness as a whole. However, the impact was limited due to a number constraints related to the surveillance and health systems in general, such as lack of legal and administrative power for centers of public health to carry out effective program management and inadequate public financial resources for the heath sector. These limitations are now being taken into consideration as the Government proceeds with its reorganization of the public health services aimed at optimization of the program structure and funding, and became determined to continue improving the system itself as the foundation for addressing the entire spectrum of public health threats, including but not limited to influenza pandemic.

Abstract P106

Fatal Pneumonia Due To The H1 Influenza Virus Strain, In São Paulo, Brazil, During Influenza Virus Season 2006

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Introduction: Many viruses are responsible for both mild and severe respiratory infections. Of them influenza is responsible for the most severe illnesses in most age groups. Case History: On June 29, 2006 the Respiratory Virus Section of the Institute Adolfo Lutz, São Paulo, Brazil, received a postmortem blood sample from a 03-year–old female child from Ribeirão Preto city, State of São Paulo, Southeast region of Brazil. According to her parents, on June 24, 2006, she presented mild influenza–like symptoms and good health and has died suddenly on June 26. We are requested for investigating adenovirus and enterovirus. Virus isolation attempts: For virus isolation attempts the clinical sample was inoculated in the Hep-2 and MDCK cell cultures. RNA Extraction: RNA was isolated from the blood sample by using the "QIAMP VIRAL RNA MINI KIT" (QIAGEN, Santa Clarita, California, USA) according to the manufacturer's instructions. RT-PCR- Influenza virus detection: Universal Type A, Universal Type B and specific primers to H1, H3 and H5 influenza virus strains were kindly provided by the Centers Disease Control and Prevention (CDC) Georgia. Primers for enterovirus group detection were also including. Phylogenetic analysis: The sequences were assembled, aligned, and edited using DNAStar (Madison, WI) and BioEdit version 5.0.6. Results: Influenza of type A subtype H1 was specifically identified by RT-PCR performed with RNA extracted from the blood specimen. Regarding virus isolation

attempts, no viral replication was observed in the cell cultures. During the autopsy, the main gross finding was the heavy lungs, with marked beefy appearance caused by a diffuse edema. Formalin-fixed paraffin-embedded sections of the lungs showed, at the H&E stain, diffuse alveolar damage by mononuclear cells, and marked fibrinous exudates in the air spaces. Although only postmortem blood sample was under investigation, due to the rapid evolution to death, influenza virus RNA detected in the blood highlights the rare occurrence of acute fatal influenza pneumonia. Discussion and Conclusion: This investigation brought out the first documented case of fatal pneumonia in our experience due to influenza virus infection. Phylogenetic analysis of HA1 gene sequence indicated that this strain is referred to Clade 1 viruses.

Abstract P107

Influenza Activity in Poland in the Epidemic Seasons 2004/2005-2006/2007 According to Data Collected by the SENTINEL Influenza Surveillance System

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Influenza surveillance is essential for obtaining data on virus activity in a given season and population, selection of vaccine strains and warning in the case of epidemic. A significant role of surveillance is highly emphasized, especially since 1997 when the first human infections with avian influenza were confirmed and the risk of pandemic outbreak occurred. Epidemic season 2004/05 was the first season of existence of SENTINEL influenza surveillance in Poland. This study presents the efficacy of this system and data collected in the last three epidemic seasons. Participants are Voivodship Sanitary-Epidemiological Stations (VSESs), family physicians and the National Influenza Center (NIC). Physicians register on a weekly basis number of cases of influenza-like illness in the age groups: 0-4, 5-14, 15-64 and ≥ 65 years and collect specimens. They send epidemiological data and specimens to VSESs, where virological tests are performed. VSESs prepare epidemiological and virological reports for NIC. The latter prepares report with data from the entire country and sends information to the European Influenza Surveillance Scheme and FluNet/WHO. In the season 2004/05 epidemiological reports were received from 8 of 16 VSESs, and virological from 9 VSESs. Number of reporting physicians ranged from 165 to 219. Within SENTINEL system 399 specimens were tested. Respiratory infections (influenza, RS, adenovirus parainfluenza) were confirmed in 84 cases, including 63 influenza cases. Forty-one influenza strains were isolated (23 B, 17 A/H3N2, 1 A/H1N1). In the season 2005/06 since week 45/2005 epidemiological reports were received from 16 VSESs and virological reports from 11 VSESs. Number of reporting physicians ranged from 98 to 949. Number of specimens amounted to 949. Respiratory infections were confirmed in 83 cases, including 47 influenza cases. Thirty-five influenza strains were isolated (27 B, 6 A/H1N1, 2 A/H3N2). In the season 2006/07 since week 40/2006 epidemiological reports were received from 16 VSESs, and virological from 14 VSESs. Until week 7/2007, 650 specimens were collected and respiratory infections were confirmed in 27 cases. Establishment of the SENTINEL influenza surveillance system improved the collection of data on influenza activity, but much work is still required in the area of laboratory testing and its quality.

Abstract P108

Influenza Virus Surveillance Efforts by the US Department of Defense (DoD): A Unique "System of Systems"

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The US DoD Global Emerging Infections Surveillance and Response System (DoD-GEIS) Influenza Surveillance System has three major components consisting of: 1) sentinel surveillance among military and dependent personnel in the US and overseas; 2) population-based surveillance among US military recruits undergoing initial entry training, shipboard personnel deployed to geographically-dispersed regions of the world, and patient groups of Mexican-Americans along the border with Mexico; and, 3) international surveillance that monitors local populations in five geographically dispersed regions outside the United States. This Influenza Surveillance System was initiated in earnest in 1996-97 and has grown tremendously in the past year. Our surveillance system is laboratory-based, consisting of a rather large network of military and civilian health facilities, research laboratories and field sites accruing specimens from over 260 participating sites in 56 countries around the world. Depending on the geographic location of collection of nasopharyngeal wash or swab specimens and associated epidemiologic information are sent either to the US Air Force's reference laboratory in San Antonio, Texas or the US Navy's Respiratory Disease laboratory in San Diego, California. Influenza isolate subtyping and genetic sequencing data are also provided to the Centers for Disease Control and Prevention (CDC) and represents a significant

contribution to the global World Health Organization (WHO)-led efforts in vaccine development. Enhancements in DoD's surveillance and response capabilities have taken place within the past year. Additional field and laboratory-based surge capacity is expected to come on-line in 2007. The strategic location of high-level, BSL-3 laboratories around the world provides the DoD with a unique capability which is leveraged by our global partners, especially in avian influenza endemic regions. This unique "System of Systems" approach plays a key role in facilitating real-time influenza strain monitoring as well as laboratory training and pandemic influenza (PI) preparedness and response activities throughout the world. The contributions by DoD-GEIS have had a large impact on the selection of new vaccine strains for Northern and Southern Hemisphere seasonal influenza vaccines and will also continue to contribute to the future development of PI vaccines.

Abstract P109

Statistical Analysis of Clinical Manifestations in Influenza-associated Encephalopathy

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Objective: To examine the differences by age and by virus type in clinical manifestations in influenza-associated encephalopathy, and to identify the risk factor of this disease. Materials and Methods: Between 1998 and 2004, 848 cases of influenza-associated encephalopathy were reported to the Collaborative Study Group on Influenza-Associated Encephalopathy. For examination of the differences by age, these patients were divided into 3 groups, younger than 1 year, 1-5 years and 6-15 years. Odds ratios and their 95% confidence intervals were estimated with reference to the data of the 1–5 years group. The differences by virus types were evaluated with χ^2 test. For analysis of prognostic factors, the outcome for each patient was classified as survival or death. Predictors of death were identified using a logistic regression analysis. Results: (1) Differences by age; Compared to patients aged 1–5 years, those younger than 1 year had a significantly higher frequency of convulsions as the initial neurological symptom, later onset of neurological signs, lower frequency of hematuria or proteinuria, and higher frequency of hemorrhage as the brain computed tomography (CT) finding on admission. In comparison with those aged 1–5 years, patients aged 6–15 years had a significantly greater incidence of type B infection, lower frequency of convulsions, higher frequency of loss of consciousness and altered consciousness as the initial neurological symptom, later onset of neurological signs, lower serum transaminase levels, and lower incidence of sequelae. (2) Differences by virus type; Less cases caused by type AH1 virus had extremely abnormal findings of blood examination. The cases by type B virus had poorer prognosis than cases by type AH1 virus. (3) There were four major prognostic factors, which were found to be significant by the multivariate analysis ($p<0.05$): elevation of aspartate aminotransferase, hyperglycemia, the presence of hematuria or proteinuria, and use of Diclofenac Na. The following variables tended to be related to a poor prognosis in the multivariate analysis ($0.05 \leq p < 0.20$): hyperthermia; thrombocytopenia; hypoglycemia; abnormal findings of brain CT; and use of mefenamic acid.

Abstract P110

Analysis of the Prevalence of Influenza A and B Viruses in Europe in the Period 1996-2006

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Introduction: The European Influenza Surveillance Scheme (EISS) has accumulated clinical and virological influenza surveillance data for Europe of ten consecutive winter seasons (1996-2006). Through analysis of this data EISS aims to improve the understanding of factors affecting the timing of circulation and the relative prevalence of influenza A and B viruses. Methods: The weekly prevalence of influenza virus types and subtypes was analyzed for Europe as a whole for ten seasons (1996-2006; weeks 40 - 20). Results: The following patterns were observed. Two times, three seasons with increased influenza B virus circulation (mean 25%, range 6% – 36% of total viruses) were followed by one season (i.e. 1999-2000 and 2003-2004) of absence of influenza B virus (≤1% of total viruses). The absence of influenza B virus coincided with the introduction of the A(H3N2) drift variants A/Panama/2007/99 in 1999-2000 and A/Fujian/411/02 in 2003-2004. Reduced circulation of influenza B viruses in the season 1997-1998 (6% of total viruses) could be explained by the introduction of the A(H3N2) drift variant A/Sydney/5/99. Except for the 2000-2001 season in which about 97% of influenza A viruses were of the H1N1 subtype, in all other seasons the H3N2 subtype was most prevalent (mean 90%, range 60% - 99%). During one season only (2005-2006), influenza B viruses were dominant

(58% of total viruses), which might be related to reduced drift potential of the A(H3N2) virus and the replacement of B/Yamagata/16/88 by B/Victoria/2/87 lineage viruses that started during the 2004-2005 season. In the 1996-1997, 1997-1998, 2000-2001 and 2004-2005 seasons, the influenza B viruses peaked 3 - 7 weeks later than the influenza A viruses, and in the 1998-1999, 2001-2002 and 2002-2003 seasons at the same time. In the 2005-2006 season in which influenza B viruses dominated, the B viruses peaked 3 weeks earlier than influenza A viruses. Conclusions: The analyses of virus data for the whole of Europe offer explanations for the relative prevalence of influenza virus types and subtypes over several seasons. Further analyses of data from individual countries will be performed to expand and substantiate these findings and to look for correlations with intensity of clinical influenza.

Abstract P111

Associations Between the Timing of Influenza Seasons in Europe and Weather: A Pilot Study Using European Surveillance Data

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Although it is clear there are climatic influences for seasonal influenza in temperate zones, the actual factors behind them are not known. We conducted a pilot study using European country-scale weekly surveillance data (EISS). Methods: Weather conditions predictive of a peak were identified, after discounting the average seasonal pattern. Peak weeks were defined for each season in each country for each year, as where the maximum virologically confirmed cases was >4 times the median. The timing of peaks (y-variable) was regressed on preceding temperature, humidity and windspeed in the preceding two weeks (x-variables) using Cox's Proportional Hazards regression model. The possibility of slower effect of weather was also examined by also using the preceding four weeks. Parallel models were fit for each country series and a combined model fit stratified by country. The association between weather and occurrence of a peak was summarised as the hazard ratio associated with the derived 10th-90th centile increase in each weather variable, after adjusting for season and averaged across countries. Results: Complete information was available from five countries where several seasons were available, from 1996-97 to 2006 for 38 winter seasons. Two country-years had no peaks. No weather variable association was statistically significant. The stronger of the non-significant associations were: higher temperature in the last two weeks HR 2.41 (95% CI 0.85,6.80) and high windspeed over two weeks HR 1.94 (0.75,5.01); high relative humidity (over two or four weeks) was non significantly associated with reduced risk of peaks HR 0.51 (95% 0.20,1.35) and 0.34 (95% 0.11,1.08) respectively. Discussion: This pilot suggests that it is feasible to examine timing of seasonal influenza peaks in relation to weather. It avoids having to allow for intra-year variations in the numbers of susceptible (ie non-immune) persons in the population. It also avoids the need to know the proportion of total cases reported. The findings themselves, are limited by low power. Despite this, lack of association with preceding cold weather was surprising. The suggestion that high relative humidity may be protective was borderline statistically significant (p=0.07). Inclusion of more countries and travel patterns are next steps.

Abstract P112

Co-morbidities Associated With Influenza-attributed Hospital Admissions and Deaths, 1994-2000, Canada

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Background: We aimed to estimate influenza-attributable mortality rates by age, health status, place of residence (health care institution or community) and place of death (in hospital or other) and influenza-attributed hospitalization rates by age and health status for Canada. Methods: Hospital discharge records of persons admitted due to respiratory conditions were extracted from the national hospitalization database (HDB), Canadian Institute of Health Information, for 1994/95-1999/2000, a period for which admissions were exclusively coded to ICD-9. Stratified, weekly admissions were modelled as a function of viral activity, seasonality, and trend using Poisson regression, providing an estimate of influenza-attributable outcomes. A similar analysis of deaths from the mortality database (Statistics Canada) provided estimates of influenza-attributable deaths. For selected Canadian provinces, hospitalization records extracted from the discharge abstract database (DAD) were further stratified by place of residence. Results: The influenza-attributable mortality rate in Canada was approximately 4,000 deaths per year. Of these only 40% occurred in hospital (n= 1,700) and most (n=1,400) were in patients with chronic heart or respiratory conditions. The role of asthma, without another chronic cardio-respiratory condition, was minimal in all seasons. Approximately 20% of the influenza-attributed hospital deaths occurred among persons transferred from a long-term care facility. For persons over 85, mortality rates were similar for institutional and community dwelling elderly. The influenza-attributed

mortality rate exceeded 1/100 population for persons ≥85 years with Chronic Obstructive Pulmonary Disease (COPD). Of the 14,000 annual influenza-attributed admissions among adults (≥ 20 years) 48% were in persons with COPD, 11% with chronic heart disease, 8% with asthma, and another 6% in persons with other conditions considered risk factors of influenza complications. Only 13% of admissions were in persons without any co-morbidity other than acute respiratory or heart conditions. All rates increased with each 5 year age group beginning at age 50. Conclusions: Our estimated rates are consistent with current vaccination recommendations. In identifying sub-groups at highest risk, rates by health status and age may be used to guide vaccine use in times of scarce resources and other influenza prevention and treatment strategies.

Abstract P113

Geo-temporal Spread of Influenza A, Canada and United States

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Background: As Canada and the United States share a common border, we sought to map the geo-temporal spread of annual laboratory-confirmed influenza by Canadian province and US region. Methods: Provincial laboratory confirmations for influenza were obtained from the Respiratory Virus Detection Surveillance System, Public Health Agency of Canada for the period of 1999/2000 to 2005/2006. Laboratory confirmations for influenza tested by U.S. World Health Organization (WHO) and National Respiratory and Enteric Virus Surveillance System (NREVSS) collaborating laboratories were downloaded by U.S. regions. For each region and influenza season, the midpoint of the regional influenza A season was identified (median week of laboratory confirmation for influenza A). The region reaching its midpoint first was identified as the reference region, and other regions coloured on a map of Canada and the United States according to their delay. Results: Results show general spatial agreement with contiguous areas. For the 1999/2000 influenza season, Alberta reached its epidemic midpoint first, in week 50 of 1999, followed closely by the US mountain region directly to the south. Within 6 weeks, all other regions had reached their midpoint. In 2003/04 and 2004/05, spread between the two populous and adjacent provinces, Ontario and Quebec, was delayed by 4 to 5 weeks. For the 2003/2004 season, Quebec lagged Alberta by a total of 10 weeks, while Ontario and the New England region were synchronized. For the 2004/2005 season, Quebec and the New England region were synchronized, while Ontario and the South East lagged by 6 weeks. The 2005/2006 epidemic had distinct regional patterns, with a total 10 weeks between the US west and Canadian regions north and east of Ontario. Conclusions: Geo-temporal spread of an annual influenza epidemic varied each year, with spatial correlations observed between adjacent regions in Canada and the United States. This variation in geographic patterns of spread for different seasons of annual influenza is a viable data source for influenza modelling. Laboratory confirmations for finer geographic detail would provide further insight into the spatial spread of annual influenza and may show some additional delay in geographic spread.

Abstract P114

Using Ontario's Telehealth Helpline as an Early-Warning Influenza Surveillance System

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The Ontario Teleheath System (henceforth referred to as "Telehealth") is a tollfree telephone helpline provided by the Ontario Ministry of Health and Long-term Care, and is available to all residents of Ontario. Our aim is to evaluate Telehealth as a syndromic surveillance system as an early warning system for influenza. Telehealth operates 24 hours a day, 7 days a week and all calls are answered by registered nurses who have at least three years of recent clinical experience. Between June 2004 and June 2006, there were a total of more than 2 million calls with 83.8% of calls for medical advice (approximately 2.28 calls per 1000 population per year). For all medical calls the call nurse follows through a decision tree, based on the algorithm that the nurses assesses as best describing the caller's initial complaint. The 440 algorithms available to the call nurse were classified by an emergency department physician into 6 revised RODS prodromes (Respiratory, Dermatologic, Neurologic, Gastrointestinal, Fever?ILI, Other). 11.3% of the calls were classified in either the respiratory or Fever?ILI prodromes. Using these data, we will apply various statistical abberation detection techniques (including CUSUMS and RLS with different SD levels) to estimate Telehealth's usefulness at identifying influenza-related outbreak activity. The next step will be to examine graphically whether Telehealth is successful at identifying influenza-related outbreaks events prior to other data sources and, if so, by how long. Using correlelograms, which measure the time lag and relationships between multiple datasets simultaneously with several time lags, we will compare the Telehealth data to Ontario National Ambulatory Care Reporting System data and Ontario laboratory reports. We are hopeful that Telehealth will be successful at predicting

influenza outbreaks in a timely manner, thus potentially providing an additional level of preparedness for pandemic influenza in Ontario, Canada.

Abstract P115

Predicting Pneumonia and Influenza Mortality From Morbidity Data

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Background: The mortality burden of influenza epidemics is highly variable from year to year. While influenza-related morbidity is monitored in most European countries, reactive surveillance of influenza mortality is conducted in few of these. We developed a simple model based on Poisson seasonal regression to predict excess pneumonia and influenza (P&I) mortality during influenza epidemics in subjects older than 65 years, based on morbidity data and the types/subtypes of circulating viruses. Methods: Between 1984 and 2004, a seasonal regression model was applied to French influenza-like illness (ILI) morbidity data obtained from the French nationwide network of general practitioners Sentinelles, in order to identify epidemic periods and to calculate excess ILI morbidity. A similar strategy was applied to P&I mortality data obtained from death certificates: excess P&I mortality was calculated as the difference between observed and baseline seasonal P&I mortality, during epidemic weeks. A Poisson regression model predicting the weekly excess P&I mortality from the weekly excess ILI morbidity, the weekly proportion of ILI cases in subjects older than 65 years and the dominant influenza virus types/subtypes (A/H3N2, A/H1N1, B), was fitted on 14 influenza seasons and was validated on six subsequent influenza seasons. Observed and predicted weekly mortality data were cumulated to yield the mortality burden of epidemics, classified in 'low', 'moderate', and 'high'. Results: The seasonal regression model applied to ILI morbidity data identified 147 epidemic weeks that were used to estimate the Poisson regression predictive model, and 66 subsequent weeks used for validation. The average mortality burden of epidemics was 22.6 per 100,000 inhabitants older than 65 years in the estimation set and 15.2 per 100,000 in the validation set. The model correctly classified five out of the six epidemics of the validation set, while one 'high' epidemic was misclassified as 'moderate', although the absolute difference was small. In the validation set, Spearman's rank correlation coefficient between observed and predicted mortality burdens was 0.83 (P=0.002). Conclusion: The method described here can be used to estimate the influenza mortality burden of epidemics in countries where specific P&I mortality surveillance data are not available.

Abstract P116

Mortality Due to Pneumonia and Influenza in the Population of Elderly of 60 Years and Older and Circulation of Influenza Virus in the City Of São Paulo, Brazil, From 1991 to 2004

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Research on influenza and its complications have been encouraged by the World Health Organization (WHO).As a result, many countries, including Brazil, have increased surveillance activities for influenza-associated morbidity and mortality. Influenza virologic surveillance is essential for determining the composition of the annual vaccine. Indicators of the severity of the influenza season are also important for understanding the epidemiology of influenza. This study investigated the association between mortality due to pneumonia and influenza (P&I) and the circulating strains of influenza viruses in the city of São Paulo. Population under study: The metropolitan region of São Paulo is the fourth largest urban conglomerate in the world (IBGE, 2000). We analyzed mortality data from 1991 through 2004 for individuals aged $\geq$ 60 years living in the city of São Paulo, which is the primary age group targeted for annual influenza vaccination in Brazil. Data Source: Death certificates data were obtained from the Program for Perfecting Mortality Information in the City of São Paulo – PRO-AIM from the Municipal Secretary of Health (SMS) of São Paulo. Deathes registered for both residents and non-residents were included in the study. Mortality Causes: Cause of death was codified by PRO-AIM based on the International Disease Classification (ICD) IX and X – ICD-9 and ICD-10, respectively, for the period from 1991 to 1995 and 1996 to 2004. Virological Surveillance data: Influenza viruses identified from 1991 to 2004 were provided by the Instituto Adolfo Lutz World Health Organization Influenza Collaborating Center. Results: From 2001 through 2004, mortality coefficients for P&I deaths increased. During this time period, vaccine coverage increased among individuals aged $\geq$ 60 years of age from 68,7% to 75,3%. In addition, during influenza virus

season 2003 the circulation of H1N2 strain in the city of São Paulo was also detected. Conclusions: Similar to several recent studies of influenza-associated mortality, we did not find a significant decrease in influenza-associated mortality during a time period of increasing vaccine coverage among the elderly. However, many factors affected P&I mortality estimates, including demographic factors, the severity and length of influenza seasons, and other individual factors not available in ecologic analyses.

Abstract P117

Viral Circulation and Latitude in Argentina

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Argentina is a Southern Hemisphere country extended from latitudes –21º 46' to –55º03' with temperate climate, so influenza season occurs usually from May to September. Occasionally, influenza outbreaks have been observed out of the expected seasonality, mainly in cities located in extreme latitudes. Therefore, data of viral circulation obtained during 7 consecutive years in 4 Argentine cities located in different latitudes will be analysed in order to establish if there is any relationship between geographical location and viral circulation. Materials and methods: Retrospective influenza data obtained at collaborating laboratories from the National Network located in Salta (latitude-24º48'); Buenos Aires (-34º38'); Neuquen (-38º55') and Ushuaia (-54º48') cities from years 2000 to 2006 will be used. Normal seasonality (NS) is considered when the peak occurs between May and September; early peak (EP) from January to April, and late peak (LP) from October to December. Results: Salta city presented EP in 2003 and 2004 (single); NS in 2003 (second peak), 2005 (single) and 2006; and LP in 2001 (single), 2002 (single) and 2006 (second). Buenos Aires city never presented EP but NS was observed during all the period usually peaking in June-July except for years 2003 and 2004 peaking in May. Mild LPs were observed in 2000, 2004 and 2006 as a second peak in September-October. Neuquen city presented a similar profile as Buenos Aires city for EP and NS peaking always in June. LPs occurred in 2002, 2003 and 2004 as second peaks. Ushuaia city presented EP in 5 out of 7 years usually followed by a second peak either as NS or LP. In 2005, there was a single EP in March. Viral antigenic characterization from EPs in Salta and Ushuaia were not always available but for year 2003 the viruses circulating in January in Salta and in April in Ushuaia were not related with the new variant that caused a severe NS season in the rest of the country. Conclusions: Influenza circulation in Argentina is usually observed as a winter important peak. In the cities located at extreme latitudes influenza also peaks during summer or early autumn. Salta presents a viral circulation similar to that observed in tropical areas; LP are usually produced by influenza B viruses. However, a co-circulation A and B is also observed; EP in Salta and Ushuaia do not predict which virus will cause NS. An extended surveillance in Salta and Ushuaia will be necessary to better understand the behaviour of influenza circulation in these areas.

Abstract P118

Area of Jurisdiction and US Population Coverage of the 122-Cities Mortality Reporting System

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Background: The 122 City Mortality Reporting System (122 CMRS) provides the Centers for Disease Control an early indication of the impact of influenza on mortality in the US. A telephone survey was launched in 2006 to evaluate key attributes of the system, including US population coverage. Methods: The 122 CMRS receives weekly reports from 122 cities summarizing the total number of death certificates received and the number of any-listed pneumonia and influenza (P&I) deaths, by age category. In October 2006, a telephone survey of the 122 CMRS reporters was initiated to determine each participant's area of jurisdiction, methods of mortality data collection, and adherence to the 122 CMRS P&I case definition. The proportion of the US population covered by the 122 CMRS was estimated from survey responses and US Census data. Results: Complete responses were received from 121 CMRS reporters. Sixty-nine (57%) participants defined their area of jurisdiction for mortality data collection as within their city limits, 50 (41%) defined their area as a county, and 3 (2%) defined their area as metropolitan or other specified geographic area. The majority of sites (88%) reported data on all deaths that occurred within their jurisdiction, regardless of the residence of the decedent. Ninety percent of reporters adhered to the 122 CMRS P&I case definition. Three cities (2%) reported only underlying P&I deaths. When comparing the reported area of jurisdiction with US Census data estimated for 2005, the population covered by the 122 CMRS is approximately 69,113,320 persons or 23.2%. Conclusions:

The survey responses indicated that there is a high degree of adherence to the 122 CMRS P&I case definition. The results indicate that population-based denominators can be approximated for the 122 CMRS based on the reported jurisdictions of the participating cities. The 122 CMRS covers roughly one-quarter of the US population. Future analyses will include comparing city level 122 CMRS data to city level mortality data collected by the National Center for Health Statistics.

Abstract P119

Influenza Virus Surveillance by Adolfo Lutz Institute, São Paulo, Brazil, During 2006: Influenza the Biggest Challenge for the Global Public Health

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Worldwide Public Health Authorities face an imminent risk of influenza virus pandemic. An unprecedented epizootic avian influenza A (H5N1) virus that is highly pathogenic has crossed the species barrier in Asia to cause many human fatalities and poses an increasing pandemic threat. The key to influenza preparedness is effective global surveillance. The Ministry of Health of Brazil has implemented this surveillance activity by creating the National Influenza Surveillance Network. Population under study: From January to September 2006, 958 respiratory secretions were collected from patients 0 to 75 years of age, with acute respiratory illness living in the Southeast, Center East, North and Northeast regions of Brazil. Virus isolation:Virus isolation attempts were performed in cell culture of MDCK, Vero, and Hep-2 and embryonated hen's eggs. IFI assay using monoclonal antibodies of the respiratory Panel 1 Viral Screening & Identification Kit (Chemicon International Inc., Temecula, CA) was performed for identification of the isolates. Influenza viruses subtype by the hemagglutination inhibition test using antiserum provided by the World Health Organization or PCR by using primers kindly provided by CDC were sent to the Influenza Branch at the Centers for Disease Control and Prevention - Atlanta, GA, for further antigenic and genetic analysis towards strains vaccine selections for South Hemisphere Vaccine Composition. Results: This virological surveillance activity shows the circulation of the following influenza strains: Influenza virus A/New Caledonia/20/99 – Like (H1N1); Influenza A/Wisconsin/67/2005 – Like (H3N2); Influenza B/Florida/07/2004 – Like; B/Ohio/1/2005, B/Hong Kong/330/2001 – Like. Discussion and Conclusion: According to the Southern Hemisphere surveillance influenza virus strain H1N1 detection - during influenza virus season 2006 - has been increased when compared with the previous influenza virus seasons and was responsible for epidemics in Brazil. Whilst outbreaks due to influenza A (H3N2) occurred in New Zealand and South Africa, activity elsewhere in Africa and Oceania was low. Regarding influenza virus of type B three different strains has been circulating during influenza virus season 2006, in Brazil, the strain B/Florida/07/2004 – Like, B/Hong Kong/330/2001 – Like and B/Ohio/1/2005 a recent B/Malaysia/2506/2004 – Like reference strain.

Abstract P120

The Respiratory Virus Profile of Medically-attended Influenza-like Illness Among Patients Presenting to a Sentinel Physician Network in British Columbia, Canada

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Introduction: Influenza causes only a proportion of influenza-like illness (ILI). The Tm Biosciences ID-Tag™ Respiratory Viral Panel (RVP) multiplex RT-PCR, used on the Luminex® xMAP® system, offers an array of virus diagnostic capability. We assessed respiratory virus detection by RVP among community-dwelling patients presenting with ILI to sentinel physicians during the 2005-06 influenza season in British Columbia (BC). Methods: Respiratory specimens are routinely collected by sentinel physicians in BC from patients presenting with ILI. All are accompanied by epidemiologic detail and tested at the BC Centre for Disease Control (BCCDC) by RT-PCR and virus isolation. Stored specimens were subsequently tested by RVP. Results: Between November and April, 524 specimens were submitted to BCCDC by sentinel physicians; 477 were available for RVP testing. These included patients 10 months-95yrs (median=30yrs) of whom 26% were vaccinated against influenza. RVP established viral diagnosis in 56%(265/476). Influenza A and B were detected in 95(20%) and 88(18%), respectively. Among the 294 influenza-negative specimens, RVP detected an alternate viral diagnosis in 82(28%). This included: enterovirus/rhinovirus (n=34), metapneumovirus (n=22), HCoV-HKU1 (n=4), HCoV-

NL63 (n=4), parainfluenza-1 (n=8), parainfluenza-3 (n=1), parainfluenza-4 (n=3), RSV-B (n=3), and adenovirus (n=3). No specimens were positive by RVP for parainfluenza-2, RSV-A, HCoV 229E, OC43, SARS or influenza A(H5). One influenza A-positive patient was co-infected with enterovirus/rhinovirus and one influenza B-positive patient with parainfluenza-1 and enterovirus/rhinovirus. RVP identified two influenza-positive specimens that were not detected by conventional methods, conventional PCR identified 22 influenza-positive specimens (12 culture-positive) that were not detected by RVP and two specimens results were mismatched (influenza A-positive by RVP and influenza B-positive by conventional PCR and culture). RVP detected influenza from 25%, 59%, 30%, 27% and 32% of specimens submitted from patients aged ≤5, 5-19, 20-49, 50-64 and ≥65 years, respectively, with other viruses identified among 44%, 12%, 19%, 18% and 17%. Discussion: During the winter season, respiratory viruses other than influenza play a significant role in medically-attended ILI. RVP appears less sensitive than conventional PCR for influenza detection but is able to provide alternate viral diagnosis for ILI in a substantial proportion of influenza-negative specimens. This may be important in certain clinical and public health contexts.

Abstract P121

Age-related Trends in the Timeliness and Prediction of Medical Visits, Hospitalizations, and Deaths Due to Pneumonia and Influenza, British Columbia, Canada, 1998-2004

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Introduction: In a recent US study, medical visits for respiratory illness among children <5yrs of age were the first and strongest predictors of overall peaks in mortality due to pneumonia and influenza (P&I). Another US study instead emphasized the role of working-age adults. We explored similar age-related trends in British Columbia (BC), Canada, in order to inform influenza immunization policies. Methods: Influenza surveillance reports were reviewed to characterize each influenza season from 1998-99 to 2003-04. Age-related associations were examined from medical service plan (MSP) billings, hospital discharge and vital statistics data for each season. Timeliness was the difference between the first peak in influenza medical visits and first peak in hospitalization or death directly due to P&I for each age group and season. Poisson regression was run per age group and season: dependent variable was hospitalization or mortality counts with prediction based on medical visits for the period derived from the timeliness analysis. Prediction was expressed as the average proportion of the deviance explained by each age group. Results: Seasonal variation in the dominant influenza type/subtype, magnitude and timing of peaks was identified. Highest medical visit rates were in children 6-23m, 2-4yrs and 5-9yrs (2.1, 1.8 and 1.3/1000). Highest P&I hospitalization rates were in children <6m, 6-23m and adults ≥65yrs (0.6, 0.5 and 0.5/1000), and highest death rates were in children <6m, 6-23m and adults ≥65yrs (0.05, 0.01 and 0.1/1000). After adjustment for season, medical visits in children aged 6m-4yrs and adults 20-49yrs were the strongest predictors of P&I hospitalizations (73% of the deviance each) with children aged 2-4yrs demonstrating earliest medical visits. Conclusion: Young children and the elderly suffer the greatest direct effects from influenza and have the highest rates of related healthcare use. Infection in young children, especially pre-school aged (2-4 years) and working-age adults may amplify the indirect risk to other groups, including vulnerable populations. Administrative data are useful for population-wide trend analysis but should be used cautiously in guiding age-targeted recommendations. The indirect benefits of immunizing healthy children and adults as a means of protecting those most significantly impacted by influenza should be further evaluated.

Abstract P122

Influenza Surveillance in the Eastern Mediterranean Region, Africa, Eastern Europe, and the Central Asian Republics

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NAMRU-3 continues to expand influenza surveillance within the Eastern Mediterranean Region and other regions as an integral part of the World Health Organization (WHO) global influenza surveillance program. Trilateral agreements between NAMRU-3, WHO-EMRO, and host countries have been established to support regional surveillance. Additional countries from Africa, Eastern Europe, and Central Asia have entered into bilateral agreements with NAMRU-3. Isolation and characterization of circulating virus strains from these regions contributes to the overall decision for the annual

vaccine formulation. Participating laboratories were assessed and provided necessary equipment, reagents, and training. Throat swabs collected from patients with influenza-like illness were processed for virus isolation and subtyping conducted using hemagglutination inhibition kits provided by a WHO Collaborating Center for Reference and Research on Influenza (Centers for Disease Control (CDC), Atlanta, Georgia). Positive specimens or putative isolates from collaborating sites were forwarded to NAMRU-3 for additional testing and/or confirmation with representative isolates being forwarded to the CDC. From July 2005 to June 2006, a total of 4,524 specimens were collected from 12 countries. A total of 397 (10%) influenza viruses were isolated from 3,983 samples collected in Egypt. Seventy-three isolates (18.4%) were confirmed as influenza type A and 324 (81.6%) as type B. Seventy-one of the 73 influenza A isolates were H1N1 (A/NEW CALEDONIA/20/99-LIKE) and two were H3N2. Influenza B isolates were B/OHIO/01/2005-LIKE.From other countries, influenza viruses were isolated from 65 (12%) of 541 specimens from Kazakhstan, Uzbekistan, Kyrgyzstan and Ukraine. Of these, 57 were influenza B, five were influenza A H1N1, and the remaining three were influenza A H3N2. Between July 2006 and January 2007, an additional 1,984 specimens were processed resulting in 42 (2.4%) influenza isolates from Jordan, Kyrgyzstan, Oman, and Syria. Of these isolates, 35 were influenza B, two were influenza A H1N1, and five are pending subtyping. Development of influenza surveillance networks is crucial for identifying circulating influenza strains for incorporation into the seasonal vaccine. Capacity building in countries with the development of National Influenza Centers to expand diagnostic capabilities strengthens these efforts. Training to enhance the quality of sample collection and processing is required during this process.

Abstract P123

The Impact of Influenza on Families of Children With Influenza

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Background: Influenza contributes to significant economic and societal costs. The indirect costs of influenza infection on individual households are difficult to estimate. Parents of children with confirmed influenza presenting to secondary care were invited to complete a questionnaire to evaluate indirect costs to the family. Methods: During 2006-07, we identified influenza infection among febrile children presenting to emergency secondary care by rapid antigen testing. Parents were requested to complete a short questionnaire around 7-10 days following diagnosis. We collected information on number of adult and children household contacts, duration of illness in any household member, number of medical consultations and any treatments received, absenteeism from work or school and additional childcare provision. Results: At the time of writing, 35 influenza-positive children were identified; questionnaires are currently being returned and evaluated. Of adult household contacts, 35% experienced an influenza-like illness of 2 day mean duration. Of ill adults, 83% received antipyretic medications, 25% consulted primary care, 20% received antibiotic prescriptions and a mean of 1.1 days work absenteeism was reported due to ill health. Of children in affected households, 63% experienced an influenza-like illness of 6.5 day mean duration. Of ill children, 88% received antipyretic medications, 56% consulted primary care, 36% received antibiotic prescriptions and a mean of 3 days school absenteeism was reported to ill health. In 35% of affected households, at least one parent took time off work to care for children (mean 1.7 days per household). The number of adult workdays lost due to adult illness was approximately half the number of those lost to childcare. 17% households needed to organise additional child care. Discussion: Influenza and influenza-like illness has appreciable impact on working families and medical providers of healthcare.

Abstract P124

Do Children Drive the Spread of Influenza-like Illness in the Community?

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Introduction: There has been considerable debate surrounding, and conflicting evidence supporting the notion that young children are the main transmitters of respiratory infections in the community. Recently, the administration of influenza vaccine in young children has become routine in some countries; part of the rationale for these policies is that controlling the spread of infection in younger age groups limits the transmission of the virus to older age groups thus reducing the morbidity, mortality and economic burden associated with influenza infections in the elderly. For this rationale to be accepted, theory dictates that children need to be the first age group in the community to encounter annual epidemics of respiratory disease. Aims: We analysed 40 years of clinical incidence data collected from the Royal College of General Practitioners Weekly Returns Service (WRS) sentinel surveillance network. We aimed to estimate the spread of

influenza-like illness (ILI) and acute bronchitis from children to older age groups. Methods: New episode incidence rates for ILI and acute bronchitis per 100,000 population were collected from a network of general practices located across England and Wales. Clinical incidence data recorded by doctors were analysed for winters (weeks 40-20) over the period 1966/67 to 2005/06. Data were stratified by age band (0-4, 5-14, 15-44, 45-64 and 65+). Weeks containing the peak incidence rates of ILI and acute bronchitis were compared across each age band and cross-correlations tabulated to calculate lag periods between the different series. Results: The occurrence of ILI showed considerable variation across the winters studied; the timing of annual influenza epidemics ranged in peak incidence from week 45 (early November, 1993/94) to week 15 (mid-April, 1983/84). The incidence of acute bronchitis showed significantly less variation and over the study period, epidemics of acute bronchitis were generally confined to weeks 48-05. The clinical incidence rates of ILI was spread across all age groups although, the precise burden differed from winter to winter; for acute bronchitis the burden of disease was limited to the young (0-4 years) and elderly (65+ years) populations. Lag periods were calculated for ILI and acute bronchitis across all age groups for individual winters, and for the combined 40-year study period. There were no consistently discernable lag periods detected between any age groups for ILI. Lag periods were cross correlated for acute bronchitis; there were no lags between 0-4 years and 5-14 years groups; 1 week between 0-4 and 15-44; 2-3 weeks between 0-4 and 45-64 and 2-3 weeks between 0-4 and 65+. There were also lags detected between 5-14 and 45-64 years (2 weeks) and 5-14 and 65+ years (2-3 weeks). Incidence of acute bronchitis always peaked in children before the elderly. Discussion: We found no evidence of delayed spread of ILI between children and the elderly. Our data do not support the routine vaccination of young children as a means of reducing the spread of influenza into older age groups. We conclude that vaccination of healthy children should be administered purely for the benefit and cost-saving in these young age groups. In respect to acute bronchitis, we found a 2-3 week lag between the 0-4 and 65+ age groups. It is widely accepted that respiratory syncytial virus (RSV) is one of the leading causative agents in acute bronchitis suggesting that future paediatric vaccines developed against RSV could have protective effects in reducing morbidity and mortality rates in the elderly associated with acute bronchitis.

Abstract P125

Is Routine Seasonal Influenza Vaccination of Elderly People an Effective Community Policy?

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Introduction: In the UK, a policy of routine influenza vaccination has been encouraged for persons with co-morbidities since 1990, for those over 75 years since 1998 and over 65 years since 2000. Whilst most influenza vaccine trials have shown effectiveness against laboratory confirmed influenza in reducing deaths, hospital admissions and minimizing clinical incidence, excess deaths and high pressures on health care resources in mid-winter remain and challenge the economic value of this policy. Aims: We investigated four influenza related outcomes (the clinical incidence of influenza-like illness [ILI] and acute bronchitis reported in a sentinel practice network; national hospital admissions for respiratory illness; and all-cause mortality) over 10 winters (1994/95 – 2003/04) to discern the impact of the routine influenza vaccination policy on persons over 65 years. Methods: We examined weekly data for the four outcomes in the age groups 45-64, 65-74 and 75+ years. Virus active periods of influenza and respiratory syncytial virus (RSV) were defined from national laboratory data. Active periods included weeks embracing 70% of the isolates reported that winter. Adjustments for age were necessary because almost all RSV reports are in children less than 5 years. Rates of the four outcomes were analysed in relation to the adjusted periods of circulating virus. Results: In 4 of the 10 winters, influenza and RSV were active at similar times; in 5 RSV preceded influenza and in 1 influenza preceded RSV. Peaks of ILI and acute bronchitis were evident in all winters and in all age groups; peaks of respiratory admissions and all-cause deaths were evident in age groups 65-74 and 75+. Peaks were maximal in those winters where both viruses co-circulated. In those where virus active periods were separable, all 4 outcome measures peaked in RSV active periods. Discussion: Over 10 winters of increasing influenza vaccine uptake, increases in respiratory illness, respiratory admissions and all-cause mortality predominantly occurred during periods of RSV activity. A policy of routine vaccination has reduced the impact of influenza even in persons over 75 years. Complementary virological information on RSV and influenza is needed when assessing the impact of influenza vaccination.

Abstract P126

Surveillance of Influenza and Respiratory Illness Over 40 Years Using the Weekly Returns Service

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Aim: To provide information on a general practice-based sentinel surveillance scheme that has monitored the incidence of influenza-like illness (ILI) in England and Wales for 40 years. Introduction: The Weekly Returns Service (WRS) of the Royal College of General Practitioners (RCGP) is a clinical information system based on a sentinel network of general practitioners who record diagnostic data from every consultation. Since 1966, the WRS has collected data on a selected number of diseases; since 1998 all data have been entered into electronic patient records and tabular extracts are taken on a twice weekly basis allowing analysis of every disease/condition reported. The RCGP Respiratory and Communicable Disease Report presents surveillance data for a range of "alert" conditions including respiratory illnesses, common infectious diseases and symptom diagnoses which might be associated with bioterrorist attack. Data are analysed by region, age group and gender and presented in the context of historical baseline levels. Results: The WRS is best known for its reporting on ILI and has monitored events such as the 1968/69 pandemic, the 1977 pandemic and the last major epidemic in the UK (1989/90). The main clinical impact of the 1968/69 pandemic was felt during the winter of 1969/70, when the WRS recorded the highest rates of ILI. Incidence was highest in the age group 15-44 years. In the subsequent ten winters, incidence remained elevated but then has gradually decreased and is now less than a fifth of the 1969 rate. In the interpretation of any current epidemic situation, the age specific rates of a wide range of respiratory diagnoses are considered. In collaboration with the UK Health Protection Agency, the RCGP co-ordinates an influenza swabbing scheme. Patients presenting with ILI are swabbed using a nose/throat swab, which is tested for influenza A (H3 and H1), B and RSV. Clinical and virological data from the same population are integrated. Conclusions: The WRS provides a reliable surveillance service that is based upon a sentinel network of doctors who record clinical data to very high standards of quality. Integration of microbiological testing increases the potential of the system to interpret clinical data.

Abstract P127

Variability of Estimators for Excess Mortality

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Background: Excess mortality is often used as a population-level measure of epidemic or pandemic influenza severity. Following Serfling, regression-based methods use a two-step procedure to estimate excess mortality: (1) establish seasonal baseline with a periodic regression model; and (2) calculate aggregated positive deviation above predicted baseline. Although excess mortality serves as the primary outcome in many epidemiological studies of influenza data, the variability of its estimator has rarely been studied closely. Ignoring sources of variability can lead to invalid statistical inferences or (potentially) incorrect conclusions. Methods: Time series with dynamic behavior typical of influenza surveillance data were repeatedly simulated with known parameters, and an estimator based on Serfling's model was used to quantify bias and variability. Factors that could affect variability were investigated, including number of observations used for model-fitting; temporal aggregation of data; or timing and duration of epidemic. Expressions for standard error in a general setting were derived and applied to historical data. Results: Relative variability is expressed as a Standard Error Inflation Factor (SEIF). Altering the epidemic timing or duration did not introduce bias or affect the variability of Serfling's estimator. As expected, exclusion of observations during model fitting resulted in larger standard errors, with SEIF ranging from 9% to 66%. Temporal aggregation from weekly to monthly data also resulted in a substantial increase in variability (SEIF 63%) and underestimation (relative bias 3%). Conclusion: Differences in implementation (e.g. temporal scale) can adversely affect the accuracy and precision of Serfling's estimator. Future work is needed to improve understanding of the statistical properties of these and other commonly used measures relevant to the epidemiology of influenza.

Abstract P128

A Generic Guideline for Influenza Surveillance and Early Pandemic Detection

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In 2005, the World Health Assembly adopted a resolution calling for the fortification and coordination of national strategies to prepare for an influenza pandemic. The WHO Global Influenza Surveillance Network provides virus strain information for the selection of seasonal vaccine strains, but it has limited capacity to provide the corresponding epidemiological information needed to characterize an emerging pandemic. Developing countries are under-represented in the system, and most of the assumptions underlying pandemic models are derived from epidemiological data collected in Western Europe and North America. We describe a generic guideline for use by member states of the Pan American Health Organization to collect data on severe acute respiratory infections (SARI), influenza-like illness (ILI), and laboratory-confirmed influenza that can be used to estimate disease burden, describe the epidemiology of seasonal influenza, and trigger an early response to an emerging pandemic strain. The system consists of sentinel hospital-based surveillance for SARI and sentinel clinic-based surveillance for ILI, combined with triggers for immediate reporting by all health care workers and the general public using the sentinel surveillance reporting infrastructure. SARI is defined as a fever >38ºC, with cough or sore throat, and shortness of breath or difficulty breathing among hospitalized persons. ILI is a fever >38 ºC with cough or sore throat. Specimens from a sample of cases are tested for influenza by Immunofluorescence Antibody testing, and detailed demographic and risk factor data are gathered on a standardized questionnaire. In addition, health care practitioners nationwide are instructed to immediately report SARI cases with characteristics including clustering, a history of exposure to sick birds or other animals, or recent travel to areas of known transmission of influenza A (H5N1). Reports from the news media, schools, and agricultural workers are also investigated. Establishment of a sentinel system provides the reporting and response mechanisms, clinical sample transport and processing systems, and baseline data for investigating reports. In addition to providing the necessary infrastructure for a pandemic early warning system, the data can be used for country specific pandemic modeling and provide a greater understanding of influenza in developing nations where the disease is currently poorly understood.

Abstract P129

Influenza Vaccine Uptake Amongst Adult Canadians; A Progress Report

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Introduction: Immunization against influenza is a key public health intervention in reducing the burden of influenza each year. In 2006 the Public Health Agency of Canada conducted a National Immunization Coverage Survey to assess coverage rates for a select number of adult immunizations including the annual influenza vaccine and to measure progress against national targets. National targets for influenza immunization coverage are 80% in seniors (≥ 65 years), adults with high risk medical conditions and health care workers (HCW). Methods: A random digit dialling telephone survey was conducted between April 29th - July 4th, 2006 on a sample of non-institutionalised adult Canadians 18 years and older. Coverage rates were reported by antigen for the general population as well as NACI (National Advisory Committee on Immunization) recommended high risk groups. Results: The overall response rate for this survey was 12% (n=2237). The crude national influenza immunization coverage estimate for the general population was 37% (±2.1). When analyzed by province and age group, rates were higher in Ontario (46.2%±3.2) and for those aged 75+ (76%±8.6). Influenza coverage estimates by target group were: 69.9% (±5.8) for seniors, 38.2% (±4.9) for adults 18-64 years with chronic medical conditions and 64% ±2.9 for health care workers. Rates for these groups in 2001 were 64.1%±4.1 (seniors), 38.4%±3.3 (adults with chronic medical conditions) and 54.8%±5.8 for HCW. The majority of those who received a recommendation from a health care professional since Oct 2005, say they were immunized (38%±2.1 received a recommendation and 78.8%±4.1 of these were immunized). The most commonly cited reason for not receiving an influenza immunization included: "I'm healthy, don't think I need it" (36%). Conclusions: Influenza immunization estimates have increased since 2001 for HCW and seniors, but have remained similar over time for adults with chronic medical conditions. Provincial rates vary, with the highest coverage rates in Ontario where there exists a universal influenza program. Coverage rates for high risk groups remain below national targets. There is opportunity to increase influenza immunization uptake by encouraging HCW to recommend vaccination to their clients and through public education.

Abstract P130

Emerging Infections Program: Pediatric Laboratory-confirmed Hospitalization Surveillance, 2003-2005

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Background: Pediatric influenza hospitalization surveillance was implemented by CDC and the EIP—a network of state health departments and academic institutions—during 2003-04, a season marked by media reports of increased pediatric influenza-associated deaths. We compared the characteristics of hospitalized children during the 2003-04 influenza seasons with subsequent seasons. Methods: We implemented population-based active surveillance for children hospitalized with community-acquired, laboratory-confirmed influenza in10 sites for three influenza seasons: 2003-04, 2004-05 and 2005-06. Case children were aged <18 years and admitted to a surveillance hospital within 14 days of a positive influenza test result. We collected demographic and clinical data by medical record abstraction. Cases were evaluated using the 2003 Advisory Committee on Immunization Practices (ACIP) list of medical conditions for which influenza vaccination was recommended. Results: The number of children with influenza hospitalized in seasons 2003-04, 2004-05, and 2005-06 was 1306, 615, and 717, respectively. Hospitalization rates during 2003-04 for children aged <6 months, 6–23 months, 24–59 months, and 5–17 years were 29.9, 11.5, 3.9, and 0.8 per 10,000 children, respectively. Except for children aged 5–17 years, each 2003-04 hospitalization rate was ≥2.2 times greater than any subsequent season. Sex and race/ethnicity distributions were similar throughout each season. Median time from illness onset to hospital admission and length of hospitalization (LOH) was 2 days in all seasons. The LOH interquartile range in 2003-2004 compared with 2005-06 was 1–3 days and 1–4 days, respectively. During 2003-04, significantly fewer hospitalized children required mechanical ventilation than in 2005–06 (3.5% vs. 6.8%). Children with ≥1 ACIP medical conditions increased from 25.4% (95%CI [confidence interval]: 23.1–27.8%) in 2003-04 to 32.1% (95%CI: 28.7–35.5%) in 2005-06. Discussion: The 2003-2004 season had the highest rates of hospitalization, the lowest proportion of children with mechanical ventilation and medical indication for vaccination, and tended toward shorter LOH. Despite high hospitalization rates in 2003-2004, hospitalized children may have been healthier at baseline. The increased hospitalization rate may reflect greater testing and identification of less severely ill children with influenza or possibly a lowering of the threshold for hospitalization during a time of much media attention.

Abstract P131

Monitoring Influenza Laboratory Detections in Canada, Evaluation to Inform Future Directions

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Introduction: Influenza is a significant public health issue in Canada, resulting in yearly epidemics and contributing to increased morbidity and mortality. Laboratory detection is important for confirming the arrival and spread of influenza across Canada, establishing predominant circulating strains and monitoring virus characteristics to inform vaccine and antiviral use. Global laboratory monitoring began in the early 1950s and by 1975 Canada was conducting weekly systematic monitoring of influenza viruses. Since then, laboratory and epidemiologic surveillance activities have developed into the present Respiratory Virus Detection Surveillance System (RVDSS, est. 1993) within a comprehensive influenza surveillance program (FluWatch, est. 1996). This descriptive study looks at the history, current status and future development of laboratory surveillance for influenza in Canada. Methods: Archival records search on the history and development of influenza virus surveillance in Canada. Review and descriptive analysis based on RVDSS historical data (1993-2006). Evaluation plan aimed at development of recommendations for continued improvement of RVDSS to meet current routine and anticipated pandemic influenza surveillance demands. Results: Since 1993, the RVDSS has grown from an initial 22 labs to a network of 33 sentinel public health and hospital laboratories across Canada. Laboratory detections data have contributed to weekly reports for dissemination to public health professionals in Canada and internationally (WHO FluNet); annual and long-term epidemiologic trend analysis, and global vaccine decision-making. Over 13 years, the number of labs and the amount of testing has increased from 16,000 tests reported by 22 labs to 90-100,000 tests reported by 33 labs, annually. Conclusion: RVDSS data continue to signal the arrival and progression

of annual epidemics in different regions in Canada and also contribute representative strains for antigenic characterisation and antiviral resistance monitoring. The capacity for influenza laboratory identification has increased considerably over the years and provides a platform for the rapid detection of a novel influenza strain. A critical review of the development and current status of RVDSS in Canada is timely given the evolving use of laboratory surveillance information and the need to develop surveillance protocols to cope with the resource challenges in a pandemic.

Abstract P132

Evaluation of Influenza-like Illness Reporting by U.S. Sentinel Physicians

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Objective: To determine the comparability of data from the U.S. Influenza Sentinel Provider Surveillance Network (SPN) with those obtained from two other surveillance systems. Background: Approximately 2,500 participating physicians report weekly counts of patients with influenza-like illness (ILI; fever [temperature ≥100ºF], cough and/or sore throat in the absence of a known other cause) versus those seen for any reason. Data are weighted by state population, and then national and region-specific percentages of visits for ILI are calculated. Methods: Comparison of SPN ILI reports from three influenza seasons (2003-06) with two other surveillance data sources: the total influenza isolates reported through the World Health Organization (WHO) laboratories (the gold standard) and BioSense. BioSense receives diagnosis codes from the Veterans Affairs and Department of Defense outpatient clinics, including ICD-9-CM code 487 for influenza. Results: Correlations were high between the weekly SPN percentage of ILI patients and total number of WHO laboratory isolates (r=0.95) and the percentage of BioSense patient visits coded for 487 (r=0.90). All three systems showed peak influenza season activity during the same week (week 7) in 2004-05. SPN and BioSense systems both showed peak activity during week 52 in the 2003-04 and 2005-06 seasons; however, number of WHO isolates peaked during weeks 50 and 10, respectively. Significant variability was observed in the timing of the season activity peak when stratified by US Census region. For example, early activity occurred in three western regions in the 2005-06 season creating an early peak (week 52) in SPN and BioSense national data, while other regions peaked later creating a second slightly lower peak (week 10). WHO laboratory isolates showed a predominant peak at week 10. Conclusions: Trends in changes of influenza activity detected by WHO and BioSense aligned well with the SPN system over three different seasons. However, significant differences were seen between regions, making national summary of these data difficult. The SPN system detected increases in ILI earlier than the WHO laboratory system in 2004-05 and 2005-06. The 2003-04 season was associated with severe, well-publicized outcomes in children, which may have influenced both testing practices and patterns of health care.

Abstract P133

Among All Ages School Age Children Have the Highest and Elderly the Lowest Relative Affinity to Influenza B

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Introduction: Among all age groups school age children are considered to be most prone for influenza B infections. However, little evidence can be found in the international literature to support this assertion, because age-group and type-specific incidences are difficult to measure and to interpret, for example because younger persons are at higher risk for any kind of influenza anyway. Methods: In four of the past five influenza seasons (2001/02, 2002/03, 2004/05 and 2005/06) influenza B circulated in Germany. The proportion of B viruses identified among influenza patients ranged from as little as 14% (2002/03) to 70% (2005/06). Our goal was to analyse the relative disposition of different age groups (0-4, 5-15 ("school age children"), 16-34, 35-60, >60 years old) to influenza B relative to influenza A. We used the database of all laboratory confirmed influenza cases as diagnosed by the German national reference centre for influenza in the respective seasons. We investigated the odds of being diagnosed with influenza B vs. A using logistic regression. Results: Analysis was based on results from 4918 influenza patients, 3545 (72%) were influenza A and 1373 (28%) were influenza B-patients. Controlling for region and using the elderly as the reference (odds ratio (OR)=1) we found in each of the four seasons a very stable pattern with the lowest OR in the elderly. In all four seasons school age children had the highest OR. ORs of the remaining three age groups (0-4, 16-34 and 35-59 year old) were approximately between school age children and the elderly. In a pooled analysis of all seasons, the OR of school age children was 6.6 (95% confidence interval=3.8-11.5), significantly higher than the OR in those aged 0-4 and 35-59, but not significantly higher than in 16-

34 year old patients. Conclusion: Our findings confirm the notion that school age children are the age group that has the highest affinity to influenza B. The relative disposition of influenza B-infection in children younger than 5 years of age was significantly lower and did not differ from that of young adults.

Abstract P134

Monitoring Influenza Infection in Children Using a Hospital-based Surveillance System, Canadian Immunization Monitoring Program, Active (IMPACT)

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Background: Influenza is common among children and associated with significant morbidity and mortality. The Canadian Immunization Monitoring Program, Active (IMPACT) sponsored by the Public Health Agency of Canada and coordinated by the Canadian Paediatric Society has carried out nationwide surveillance of children hospitalized for influenza since the winter of 2003-4. Methods: The IMPACT surveillance network consists of 12 sites representing over 90% of pediatric tertiary care beds in Canada. Teams of trained nurse monitors and pediatric infectious disease specialists identify children age 0-16 years admitted with laboratory-confirmed influenza using admission logs, laboratory reports, and discharge diagnoses. Case totals are submitted weekly and detailed case report forms monthly to the IMPACT data center (Vancouver, BC) for analysis. Results: During 3 influenza seasons, 1270 children were admitted because of influenza infection to IMPACT centers in Canada. Numbers of patients varied by season: 505 in 2003-4, 391 in 2004-5 and 374 in 2005-6. 646 (50.9%) were age < 24 months. The proportion of children age 6-23 months decreased from 34% in 2003-4 to 24% in 2005-6. Fifty-five percent of children were previously healthy. 1002 (78.9%) had influenza A, 267 (21.0%) B and 1 (0.08%) A and B. Serious complications included encephalopathy (19 reports), myocarditis (3) and aseptic meningitis (1). 84 (6.6%) received antiviral therapy [70 (5.5%) oseltamivir]. Of 10 deaths, the mean age was 5.8 yrs (range 0.5-14 yrs) and 4 (40%) were previously healthy. Among the 588 children who were vaccine candidates, 88(15.0%) were documented to have been vaccinated appropriately [7(4.5%) of 154 in 2003-4, 41(18.5%) of 222 in 2004-5, 40 (18.9%) of 212 in 2005-6]. For the 2006-7 season, 140 children (130 with type A, 10 type B) have been reported via weekly updates up to February 24. Conclusions: Influenza hospitalizations represent a significant health burden among children. IMPACT is able to capture the variability of influenza seasons including changes in age profile and severity as well as circulating influenza type (A vs B). By providing real-time case totals supplemented by detailed clinical information, IMPACT offers an integrated tool for monitoring disease patterns and vaccine programs in Canada.

Abstract P135

A Sentinel Surveillance System for Influenza in Kenya

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Background: In sub-Saharan Africa, little data on the epidemiology and disease burden of influenza exist, and poor disease surveillance makes the region ill-prepared to detect a new influenza strain or clusters of human cases that could be associated with a pandemic. To address these gaps, the Kenyan Ministry of Health (KMoH) and CDC–Kenya developed a national sentinel surveillance system for influenza-like illness (ILI), Severe Acute Respiratory Illness (SARI), and suspected Avian Influenza (AI). Methods: We targeted 11 sentinel sites in Kenya: 8 provincial hospitals, 2 refugee camp health facilities, and one private hospital. Health workers at each site were trained on case definitions for ILI, SARI, and suspected AI. For every patient who met the case definition for SARI and suspected AI, and for the first three patients of the day who met the case definition for ILI, a questionnaire was administered, and nasopharyngeal and oropharyngeal samples were taken. Specimens were sent to National Influenza Center in Nairobi, and tested at the CDC-International Emerging Infections Program laboratory using RT-PCR. Results: From October 5, 2006 through February 19, 2007, the KMoH and CDC-K initiated influenza surveillance at 4 of the 11 sites. Of the 203 patients who were identified through the surveillance system, 10 (4.9%) had samples that tested positive for influenza B. No samples tested positive for influenza A. The surveillance system identified 104 patients with SARI (51.2%), 83 patients with ILI (40.8%), and no patients with suspected AI. No distinction was made between ILI and SARI for 16 patients (8%). All positive influenza samples came from patients who had presented with SARI. Conclusions: A sentinel surveillance system for influenza

in Kenya was effective in identifying patients with SARI and ILI. In Sub-Saharan Africa, establishment of sentinel surveillance systems for influenza in countries may be an effective way to evaluate the burden of disease of influenza, identify circulating influenza strains, and detect clusters of human cases of SARI that could signal the start of a pandemic.

Abstract P136

Predictions by Early Indicators of the Time and Height of the Yearly Influenza Outbreaks in Sweden

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Summary: Methods for prediction of the spread of the influenza from early observations are urgently needed for societal planning during a large influenza epidemic. In this study, new methods are described and applied for Swedish data on influenza-like illness (ILI) reported weekly by sentinel physicians and for weekly laboratory diagnoses of influenza (LDI) from the routine diagnostics of all microbiological laboratories. It was found that the ILI variable is not a good leading indicator for LDI, since their relation has a complicated time dependency. Both simple and advanced rules for how to predict the time and height of the peak of LDI by means of very early observations of laboratory data are suggested. The simple rules are based on observed incidences while the advanced ones are based on smoothing by unimodal regression. The suggested predictors were evaluated by cross-validation and by application for the observed seasons. The advanced rules have a median error of 0.9 weeks for the times of the peak and a median deviation of 28% for heights of the peaks of the 8 seasons. This indicates their practical usefulness.

Abstract P137

Molecular Epidemiology of Influenza Virus A H1N1 and H3N2 in the South and Southeast Brazil During the 2004-2006 Epidemic Year

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In this study, influenza A samples detected in five Brazilian States (South, South-East and North-East regions) were compared to A/H1N1 and A/H3N2 vaccine prototypes used in 2004, 2005 and 2006 epidemic seasons respectively. The detection of influenza virus, subtyping of haemagglutinin (HA) in samples and further sequence analysis of HA1 domain, were accomplished by RT-PCR based protocols, directly from clinical specimen. The homology to vaccine strains was assessed by comparison to sequenced HA1 domain of forty-three A/H3 and eight A/H1 influenza samples to their vaccine prototype/year. Phylogenetic analyses demonstrated that first 2004 Brazilian influenza A/H3 isolates matched the vaccine prototype A/Fujian/411/02, however, presenting a progressive lost in this homology. Most of 2004 and 2005 were sprayed in different clusters in the tree, conversely the noticed with 2006 isolates, that were grouped in the same sub-tree, closer to chosen vaccine prototype for 2006-2007 influenza season in the North hemisphere, A/Wisconsin/67/05. In the same period, A/H1 detected samples showed less variation, presenting a great identity to the vaccine prototype. Our study suggests for 2007 epidemic in Brazil a vaccine composition similar that used in 2006-2007 epidemic in the North hemisphere, and indicates the molecular analysis of HA1 gene as complement of standard protocols, for more accurate analysis of virus evolution. Financial support: CGLAB/MoH and FIOCRUZ

Abstract P138

Options, Feasibility and Use of Real-time Mortality Data for Influenza Severity Monitoring and Signal Detection in Canada

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Background: Timely access to mortality data is useful for real-time monitoring of influenza severity and early detection of unexpected demographic patterns. However, the current 5-year delay for vital statistics data in Canada precludes use for timely public health action during routine influenza seasons or emergencies such as pandemic influenza. A feasibility study was conducted to explore alternatives for real-time mortality surveillance in Canada. Methods: An environmental scan was conducted to identify and explore existing mortality surveillance systems including international programs and pilot projects as well as novel strategies for collecting real-time mortality data. Key system attributes for real-time mortality surveillance were reviewed to develop an evaluation framework. Results: The initial environmental scan identified a wide range of options including traditional health care/administrative settings (ERs, hospitals, vital statistics offices etc.) as well as non-traditional settings (funeral homes, on-line obituaries). The approaches varied based on data quality and reporting complexity. Key attributes for a routine real-time surveillance system were identified as simplicity, acceptability and representativeness. However, flexibility is expected to be important for sustainability during a pandemic. While longer term options such as accessing data from vital statistics or other administrative databases proved promising in terms of data quality, these are limited by lack of rapid reporting mechanisms and limited geographic participation. Medium to short term options tended to have more crude data quality but were acceptable when weighed against the benefits of real-time, representative data. One short term option, weekly reporting of all cause mortality through a network of funeral homes, showed promise. Conclusion: From the initial evaluation of surveillance options, accessing real-time mortality data from funeral homes is considered the most feasible short-term option, based on: preliminary interest/acceptability among stakeholders, existing collection of all-cause mortality, simplicity of reporting and potential to meet desired attributes. Next steps include working with stakeholders in a detailed evaluation of attributes and testing potential reporting mechanisms among a network of funeral homes. Pending favourable results from detailed feasibility and pilot testing, assessment of sustainability for year-round reporting and correspondence with other influenza surveillance data (e.g. laboratory detections, influenza-like illness consultations) will be needed.

Abstract P139

A Model for Real-time, Web-based Electronic Reporting of Influenza Activity

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Background: National influenza surveillance in Canada is co-ordinated through the FluWatch program which collects information from a variety of sources. A secure web-based application was proposed to 1) reduce the inefficiencies in data flow, 2) allow for reporting sources to enter data directly for others to edit or view and 3) replace manual data validation with automated validation routines. The process of developing the application and features of the application will be presented. Methods: The FluWatch application was developed in September 2004 and subsequently piloted among several Atlantic provinces in Canada. Results from the pilot revealed some inconsistencies in algorithms used. A second version was developed in late 2006 to resolve these and new features were added to enhance user friendliness and applicability. Results: The FluWatch application provides a secure, web-based platform for users to enter weekly influenza surveillance data, including the number of laboratory detections and outbreak information, after which the appropriate activity level for the surveillance region is automatically determined. Weekly summary reports are generated for each sentinel site (i.e. long-term care facility, hospital, school, workplace), sub-region, region and province/territory (P/T). Additional features include: a chart/summary statistics generator, notification options (e.g. e-mail reminders and alerts); edit trail monitoring; reports archive; and fields that auto-populate where necessary to minimize errors. Discussion: The FluWatch application allows for a secure, web-based environment, with real-time monitoring and dissemination of information and data input at various levels. The original application is currently well accepted and being used by several Atlantic provinces to report influenza activity levels. Part of this success has been the incorporation of feedback from users and regular communication between users and administrators of the application. The second version will be piloted among interested P/Ts in the summer of 2007 and is anticipated to be fully operational by fall 2007. Future plans include continuous evaluation and improvement, increasing the number of users and indicators, and increasing the representativeness and applicability of the data.

Abstract P140

FluWatch: 11 Years of Influenza Surveillance in Canada

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Background: Since 1996 Canada has had a co-ordinated national influenza surveillance program, FluWatch, which monitors the occurrence and spread of influenza activity. FluWatch consists of a network of sentinel laboratories and primary-care practices, provincial and territorial health ministries, paediatric hospitals and the National Microbiology Laboratory. A description of the program with results from the past eleven seasons, a discussion of its strengths and limitations, and how it compares to other international influenza surveillance systems will be presented. Methods: A descriptive analysis of FluWatch surveillance data from the 1996/1997 to 2006/2007* seasons was conducted. Indicators and key attributes of FluWatch were compared to international influenza surveillance systems. (*Preliminary results) Results: In seven seasons since 1996/1997, influenza A (mostly H3N2 subtype) constituted ≥85% of isolates in which those ≥65 years or children <5 years were largely affected. In contrast, in four seasons where influenza B detections were >35%, the majority of cases involved children. On average, between 5–14% of all influenza tests per season were positive (weekly peak ranging from 13–33%). The weekly peak in influenza-like illness (ILI) consultation rates ranged from 31–149 per 1,000 patient visits during the influenza season, compared to an average rate of 8 per 1,000 in the off season. Since 2001/2002, between 46–762 outbreaks in long-term care facilities were reported per season whereas influenza-associated pediatric hospitalizations ranged from 374–505 cases (since 2003/2004). Surveillance data indicate that the majority of the seasons were relatively mild, except for the 1998/1999 (A/Sydney/3/97(H3N2)) and 2003/2004–2004/2005 (A/Fujian/411/02(H3N2)) seasons which were moderate in severity. Key features of the FluWatch program include year-round surveillance and high per capita influenza testing and strain characterization rates compared to other countries. Discussion: Overall, Canada has a comprehensive system for timely surveillance of seasonal influenza activity, in line with other international influenza surveillance systems. Since its inception, FluWatch has expanded and improved over the years through the strengthening of its components, addition of indicators, and implementation of mechanisms for more timely data collection and information dissemination. However, it lacks real-time severity indicators of adult hospitalizations and mortality.

Abstract P141

The Centers for Disease Control and Prevention's Global Influenza Program and Strategy: An Overview

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Background: This project summarizes the Influenza Division's global influenza program and strategy to improve global ability to rapidly identify avian influenza viruses with the potential to cause the next influenza pandemic and simultaneously build sustainable capacity for influenza surveillance. The Influenza Division, Centers for Disease Control and Prevention, officially began its global influenza program in March 2004 as a result of the significant and on-going threat of highly pathogenic avian influenza A (H5N1) that caused outbreaks in poultry and humans in Southeast Asia. The overarching goal of the Influenza Division's comprehensive strategy is two-fold: 1) to enhance the existing World Health Organization (WHO) Global Influenza Surveillance Network (GISN); and 2) to provide support to countries to build sustainable influenza surveillance capacity. Efforts to support these goals include the provision of training, direct bilateral funding, supplies, reagents, and technical assistance to Ministries of Health, other United States Government (USG) Agencies and non-governmental organizations (NGOs) and enhanced collaborations with WHO globally. Since 2004, the Influenza Division's support of global surveillance activities has expanded dramatically in geographical coverage and scope of activities as the H5N1 virus has been identified in human and poultry outbreaks in over fifty countries around the world. Methods: An analysis will be conducted of the following program elements: 1) specific strategies/mechanisms employed; 2) scope of activities; 3) funding; 4) geographical coverage; 5) capacity building; and 6) progress in all of these areas. Conclusion: A multi-faceted, systematic approach is necessary to build sustainable influenza detection, surveillance, outbreak investigation, and rapid response capacity in developing countries in order to increase the United States' ability to detect novel influenza viruses early and reduce the threat to human and animal health posed by viruses with pandemic potential.

Abstract P142

Rapid Estimates of Influenza-associated Deaths in the United States

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Background: Rapid assessments of influenza-associated deaths in the US have been limited to qualitative annual summaries using the 122 Cities Mortality Reporting System (122CMRS). We compared four methods for estimating influenza-associated deaths using the 122CMRS and propose a method for rapidly estimating weekly national numbers of influenza-associated deaths. Methods: The 122CMRS collects pneumonia and influenza (P&I) deaths and all-cause deaths on a weekly basis from 122 US cities. We fit four regression models to weekly death data from the 1972/73 through 2005/06 seasons: three robust regression models (Andrews, Huber, and Bi-square models) and one previously published ordinary least squares regression (OLS) model. For the robust regression models, we modeled the ratio of P&I deaths to all-cause deaths and compared the model fits. The OLS model was fit to non-epidemic data and modeled absolute numbers of P&I deaths. We could not compare the fit of the OLS model to the other three robust regression models. All four regression models were compared in terms of annual estimates. We then compared the estimates from the 122 CMRS models to national estimates using National Centers for Health Statistics multiple-cause of death data. Results: The estimated annual numbers of influenza-associated P&I deaths for the 122 CMRS from the 1972/1973 through 2005/2006 seasons were 1393 (range 0 – 4,590), 1061 (range 0 – 3,732), 951 (range 0 – 2,823), and 1651 (range 0 – 6,251) for the Andrews, Bi-square, Huber, and OLS models, respectively. Among the robust regression methods, the Andrews model was the best fitting model based on the percentage of the standard error over the estimate. The annual estimates from all four methods were all highly correlated (r>0.93). The correlation between the 122CMRS estimates using the Andrews method and national estimates using the Andrews method was statistically significant (r=0.88), allowing us to estimate rapidly the national weekly numbers of influenza-associated deaths. Conclusions: Similar estimates of influenza-associated deaths were made using four different regression models. The Andrews model was the best-fitting robust regression model and can be used to make rapid estimates of weekly numbers of influenza-associated deaths in the US.

Abstract P143

Comparison of Methods for Defining Influenza Epidemic Periods on Estimates of Influenza-associated Deaths From the 1983/1984 Through 2001/2002 Seasons

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Background: Several studies of influenza-associated deaths have used ICD-coded influenza deaths to define influenza epidemic periods. We examined similarities and differences in methods using ICD-coded influenza deaths to define epidemic periods and compared them with viral circulation data and other methods for estimating influenza-associated deaths. Methods: We used multiple cause-of-death data collected by the National Centers for Health Statistics from 1983 through 2002. Influenza deaths were coded using the International Classification of Diseases (ICD) -9th and 10th revision (ICD-9 487; ICD-10 J10-J11). Influenza epidemic periods were defined using several methods. The first method, the Andrews method, involved fitting a robust regression model to ICD-coded influenza deaths and using the 95% confidence limits to define epidemic periods. The second method replicated the methods used by Simonsen et al (2005) by removing epidemic periods and fitting a regression model through the non-epidemic data. The 95% confidence limit was used to define epidemic periods. The third method used a fixed-rate threshold of 2 deaths per 10,000,000 person-weeks to define epidemic periods. All three methods were then used to estimate excess underlying pneumonia deaths (ICD-9 480-486; ICD-10 J12-J18). To estimate all influenza-associated P&I deaths, all influenza deaths and excess underlying pneumonia deaths were summed. Results: On average, the peak week rate of influenza coded deaths was 1 week later than the peak week percentage for the viral surveillance data. The average annual numbers of epidemic weeks estimated using the Andrews, Simonsen, and the fixed-rate threshold model were 7.5 (range 0 – 13), 8.8 (range 0-14), and 8.6 (0-14), respectively. The average annual numbers of influenza-associated P&I deaths estimated using the Andrews, Simonsen, and fixed-rate threshold model were 5,300 (range 400-12,000), 6,400 (range 400-13,500) and 5,513 (range 400-12,200), respectively. All three estimates were highly correlated (r > 0.99) and were significantly correlated with national estimates using methods modeling underlying influenza and pneumonia deaths together (r=.80). Conclusions: The three methods used for defining influenza epidemic periods using ICD-coded influenza deaths resulted in similar estimates of influenza-associated deaths. The estimates were also correlated significantly with other methods for estimating influenza-associated deaths.

Abstract P144

Evaluation of the State and Territorial Epidemiologists' Report of Influenza Activity

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Objective: To compare the State and Territorial Epidemiologists' Report of Influenza Activity ("the Report") with U.S. virologic surveillance and sentinel provider network's (SPN) influenza-like illness (ILI) surveillance. Background: During influenza season, each state reports weekly a level of influenza activity: none, sporadic, local, regional, or widespread. Multiple sources of data, including SPN physicians, nursing homes, and laboratories, are used. Data are displayed on a color-coded U.S. map that reflects the geographic spread of influenza by state. Methods: Each activity level was assigned an activity score (0—no activity, 1—sporadic activity, etc.). A national weekly cumulative activity score was compared (using Pearson's correlation) with the national weekly percent of physician visits for ILI and the national weekly percent of respiratory samples testing positive for influenza. For state level comparisons between surveillance systems, all states were assigned a composite rank reflecting the combined strength of each state's virologic surveillance (samples tested per 100,000 population) and ILI surveillance (sentinel providers per 250,000 population). Analyses were performed for states with the five lowest and highest composite ranks. Results: Throughout the 2004-2005 US influenza season, national activity scores were highly correlated with both weekly percent visits for ILI (r = 0.95) and percent samples testing positive for influenza (r = 0.96). In the 5 states with strong overall influenza surveillance, weekly influenza activity scores correlated well with either one or both of a state's SPN and virologic surveillance systems. For example, in state A, weekly state activity score and weekly virologic surveillance were highly correlated (r=0.95); however, weekly state activity score and weekly percent visits for ILI were only modestly correlated (r=.68). In the 5 states with less robust influenza surveillance, weekly influenza activity scores overall correlated less well with ILI or virologic surveillance. Conclusion: At a national level, the Report correlates well with ILI and virologic surveillance in reflecting the timing and intensity of the U.S. influenza season. However, at a state level, even in states with strong overall influenza surveillance, the Report appears to provide additional information on state-level weekly influenza activity that other components of national influenza surveillance may not capture.

Abstract P145

Enhanced Support to Countries Impact on Participation in the WHO Global Influenza Surveillance Network

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Background: To support countries and regions experiencing avian influenza outbreaks and to enhance the WHO Global Influenza Surveillance Network (GISN), the U.S. Centers for Disease Control and Prevention (CDC) began providing funds to countries through direct bilateral agreements in 2004. Nine countries were funded in 2004; three additional countries were funded in 2005. The main focus of the bilateral agreements was to enhance countries' participation in the WHO GISN by developing or expanding their epidemiologic and virologic influenza surveillance network and improving the reporting of data and sharing of isolates from surveillance sites, laboratories, and National Influenza Centers (NIC). CDC funded China, India, Indonesia, Malaysia, Mongolia, Pakistan, Philippines, Republic of Korea, and Thailand in 2004. Kazakhstan, a consortium of islands that contribute to the NIC in New Caledonia, and Viet Nam received funding in 2005. Methods and Results: To examine the trends in participation in the WHO GISN, data will be collected from multiple sources. Data will be collected from the grantees and their progress reports, WHO CCs, FluNet, and information from WHO Geneva. Data summarizing the total number of seasonal influenza isolates submitted to WHO GISN and frequency of reporting through FluNet in 2003 will serve as the baseline for the funded countries, and these data will be compared to data, specimens, and isolates submitted during 2004-2007. The 2003-2007 data will be analyzed to determine trends, as it relates to the WHO GISN.

Abstract P146

A Surveillance Network for Influenza Virus and Other Respiratory Viruses in Abidjan, Ivory Coast, From 2002 to 2006

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Background: Influenza is an acute respiratory infection due to influenza virus. In countries with moderate and cold climates, it is known to cause great morbidity and considerable mortality. Influenza activity remains underestimated in tropical countries, and its impact in most parts of Africa is poorly understood. The Institut Pasteur of Ivory Coast together with Sanofi-Pasteur, Ivory Coast, established a surveillance network for respiratory illnesses in Abidjan from 2002-2006 in order better understand influenza activity in the Ivory Coast. Methods: 24 health in Abidjan were selected as surveillance sites. Doctors at these sites were charged to take naso-pharyngeal swabs from patients presenting with a flu-like syndrome or symptoms compatible with a febrile acute respiratory illness. Samples were transported to the laboratory at the Institut Pasteur-Ivory Coast, in Abidjan, for analysis. Samples were tested for influenza, RSV and hMPV. Analysis of samples was made by: systematic inoculation in MDCK cells followed by identification by ELISA immunocapture; ELISA immunocapture on origin samples; and multiplex PCR for identification of the RSV and hMPV. The samples that tested positive for influenza were sent to the Pasteur Institute of Paris or the National Influenza Centre of South Africa for confirmation and antigenic characterization. Results: 13 health centers participated in the surveillance network in 2002 – a participation rate of 65%. Over the period of 2002 to 2006, we observed a 79% fall in the participation of sentinel sites. During the four years, 572 samples were analyzed with a rate of positivity for the influenza of 10.3%. 88.1% of the positive cases occurred during June, May, July, and October, which correspond to the rainy season, and during the month of December, during which dry winds and cold weather come in from the Sahel. The PCR multiplexing enabled us to highlight the concomitant circulation of the RSV and the hMPV. Conclusion: The establishment of a surveillance network for acute respiratory illness in Abidjan from 2002-2006. made it possible to monitor the circulation of influenza viruses and other viral agents responsible for influenza-like illness in Abidjan, Ivory Coast.

Abstract P147

Impact of Influenza Epidemics Over 7 Winter Seasons

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Introduction and objectives: The probability of emergence on an influenza pandemic has been one of the major public health concerns worldwide. Still, one can only speculate when this pandemic will occur and how severe it will be. Seasonal influenza, however, is a reality almost every winter, with an important social and economical impact. The objective of this study is to contribute to the evaluation of the impact that seasonal influenza represents each winter, presenting the morbidity and virological data analysed by the National Influenza Centre over the last seven influenza winter seasons (1999/2000 to 2005/2006). Methods: Clinical and epidemiological data from Influenza-like illness (ILI) cases were reported to the National Influenza Centre and the National Health Observatory and the distribution by age group, gender and region, and the signs and/or symptoms present were analysed. The intensity and duration of the epidemic periods were described based on the weekly incidence rates for ILI. Also, the excess of population with ILI was found as a function of the baseline for the incidence of ILI. Nasopharyngeal swabs were collected for virological characterisation of influenza viruses in circulation, by typing and genetic and antigenic analysis. Results: The majority of notifications originated from individuals with 15-44 years, of the female gender, and mainly from the north of Portugal. A statistically high association with a confirmed influenza case was found for high fever, cough and contact with another ILI case. Influenza A(H3) viruses were dominant during the 1999/2000, 2001/2002, 2003/2004 and 2004/2005 winter seasons. Influenza B viruses were mainly detected during 2000/2001 and 2002/2003. During the 2005/2006 season, both influenza B and A(H1) viruses were detected. With the exception for the 2003/2004 winter, the majority of influenza strains analysed were similar to those included in the influenza vaccine for the respective season. On average, the epidemic periods associated with the circulation of influenza type A(H3) viruses were longer (8-9 weeks) and the incidence rates recorded were higher (peak incidence reached within 3 weeks). The highest increase in the excess of population with ILI, 0.8%, was recorded for the 2001/2002 winter, associated with the circulation of influenza A(H3) viruses. Discussion: This study aims for a better knowledge of the impact of influenza epidemics in Portugal, contributing to the improvement of clinical and virological monitoring systems for influenza and for a better planning and, if necessary, the implementation of preventive and/or therapeutic measures in future influenza epidemics.

Abstract P148

Length of Influenza Seasons in the United States, 1976-77 to 2001-02

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Background: Influenza epidemics of variable duration and severity occur each winter in the US. We examined virologic and mortality data from 1976-77 through 2001-02 for changes in season length over time and differences in season length by influenza type/subtype. Methods: Influenza season length (number of weeks above a specified threshold) was calculated using several different models and data sources. Three mortality-based models (Andrews, fixed rate, and Simonsen 2005) estimated season length by modeling influenza deaths coded as ICD-9 487 and ICD-10 J10-J11. The fourth model estimated season length by modeling underlying respiratory and circulatory deaths by age group (<65 years and ≥ 65 years) and the fifth model used US virologic surveillance data with thresholds of 5%, 10% and 15% of specimens testing positive for influenza. Linear trends from 1976-77 to 2001-02 of the number of weeks above threshold for each model were determined. The virologic surveillance data were also used to calculate season length for each influenza type/subtype. Results: The average season length during this time period ranged from 3.7 to 10.0 weeks for the mortality-based models and 7.6 to 18.4 weeks for the virologic-based models. Mortality-based models using influenza coded deaths showed a decrease in season length over time (range: 0.17 to 0.28 week decrease per year). The models of underlying respiratory and circulatory deaths showed an increase in season length over time for both age groups (0.42 and 0.24 weeks per year increase respectively for those ≥ 65 years and those <65 years). The virologic surveillance based method also showed an increase in season length during this time period (range: 0.20 to 0.39 week increase per season) and showed that H3 viruses circulate at elevated levels for longer periods of time than H1 or B viruses. Conclusion: Analyses of virologic surveillance data and models of underlying respiratory and circulatory deaths indicate that influenza seasons increased in length from 1976-77 to 2001-02. However, analyses based on influenza coded deaths show season length to be decreasing. Further investigation is needed to determine the impact of changing testing practices and to explore potentially changing mortality coding practices.

Abstract P149

Use Of Early Aberration Reporting System (EARS) Outbreak Detection Algorithms in Influenza Sentinel Provider Surveillance

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Background: CDC monitors the weekly proportion of outpatient visits to health care providers for influenza-like illness (ILI) and compares national and regional proportions to baseline values calculated as the mean percent ILI during non-influenza weeks plus 2 standard deviations. State and local level analyses are desirable, but data variability makes local baseline development problematic and comparison to regional or national baselines invalid. An outbreak detection algorithm using site-specific rather than aggregate baselines may be useful in identifying and monitoring influenza activity. We applied a CDC outbreak detection method, EARS, to weekly ILI data by reporting site. Methods: Two EARS methods that differ in terms of sensitivity (C2-MEDIUM with moderate sensitivity and C3-ULTRA, the most sensitive) were applied to ILI data. A moving average of the number of ILI cases for each site was also calculated. A baseline number of ILI cases for each sentinel provider site was established when both C2 and C3 aberrations occurred for two consecutive weeks. Sites were monitored to determine whether they exceeded their site-specific baseline and/or ILI moving average. The number of regularly reporting sites for which baselines were established, the number of weeks above both baseline and the ILI moving average, and the weeks above either baseline or moving average were examined for the 2005-06 influenza season. The weekly number of sites above their respective baselines was compared to national virologic data. Results: Site-specific baselines were established for 786 of the 1,293 sentinel provider sites. Of sites reporting increased ILI, the mean number of weeks above both baseline and the moving average was 3.4 weeks (median = 2). The mean number of weeks above either the baseline or the moving average was 5.4 weeks (median = 4). The weekly number of sites above both measures or above at least one measure was highly correlated with virologic data (R=0.82, R=0.91 respectively). Conclusions: Use of site-specific baselines detected ILI increases slightly shorter than the typical length of community influenza outbreaks but correlated to virologic activity. This method allows for better detection of ILI increases in sites with low patient numbers and mapping of local-level ILI data.

Abstract P150

Age as an Independent Risk Factor for Deaths From Pandemic Influenza

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The great 'Spanish Influenza' pandemic of 1918 was unique in the 20th century both in its virulence, causing greater than 40 million deaths worldwide and in the age groups that fell victim to the disease and died. Unlike seasonal influenza, both then and now, where it is the old, young and infirm who are its usual victims, in 1918, there was an unprecedented number of deaths in the age range 20 to 44 years. Many of the individuals in this age range who died, did so within 24 hours of falling ill, with well described symptoms and signs of primary viral pneumonia. Many theories have been put forward for both the scale and age distribution seen for deaths in 1918, ranging fron social unrest, malnutrition and lack of social distancing in the army camps along the Western Front in Europe. This study will explore the role of age as an independent risk factor for deaths due to new emergent pandemic influenza virus. Results will be presented on the subsequent pandemics of Asian Influenza in 1957 and Hong Kong in 1968 and the impact of these across the age spectrum. These finding will be discussed in the context of pandemic planning and the defining of 'at risk' groups for antiviral drugs and vaccine.

Abstract P151

Early Analysis of the 1889 Russian Pandemic

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The past two years have seen a dramatic interest in the emergent threat of Pandemic Influenza, fuelled by fear that highly pathogenic H5N1 Avian Influenza will jump the species barrier and transmit in man. Fears that such a zoonotic episode could have the same devastating consequences as the 'Spanish Influenza' pandemic of 1918, has renewed interest in the lessons that may be learnt from the past. This present study concentrates on the much forgotten pandemic of the 19th century, when 'Russian 'Flu' potentially killed over 25 million people worldwide. After many years of quiescent activity, from the mid nineteenth century, Influenza made a dramatic return during the winter of 1889/90 and subsequent years. The pandemic emerged without warning to a world ill equipped to cope with the disease and a medical profession lacking the experience or expertise to deal with its consequences. From its origins in Asia it spread within weeks to Western Europe and North America, devastating communities and causing the deaths of millions worldwide. The presentation will explore the epidemiology of influenza in the years prior to the emergence of the pandemic in 1889, it will analyse the impact on nations and individuals of the disease exploring its clinical presentation as well as its epidemic impact. Finally the paper will draw together evidence as to the causative agent for this pandemic, its relationship to current viruses and the implications for pandemic planning in the future.

Abstract P152

The Annual Impact of Seasonal Influenza in the US: Measuring Disease Burden and Costs

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Background: Despite preventive efforts, influenza epidemics are responsible for substantial morbidity and mortality every year in the United States (US). Various strategies to reduce disease burden have been implemented. However, no previous studies have systematically estimated of the annual economic burden of influenza epidemics, an estimate that may guide policy makers. Objective: To estimate age- and risk-specific disease burden, medical and indirect costs attributable to annual influenza epidemics in the United States. Methods: Using a probabilistic model and publicly available epidemiological data we estimated the number of influenza-attributable cases leading to outpatient visits, hospitalization, and mortality, as well as time lost from work absenteeism or premature death. With data from health insurance claims and projections of either earnings or statistical life values, we then estimated healthcare resource utilization associated with influenza cases as well as their medical and productivity (indirect) costs. Results: Based on 2003 US population, annual influenza epidemics were responsible for an average of 610,660 life-years lost (95% confidence interval, [CI] 360,000, 953,000), 3.1 million hospitalized days (CI 2.1, 4.5), and 31.4 million outpatient visits (CI, 22.6, 43.5). Direct medical costs

averaged annually $10.4 billion (CI, $4.1, $22.2). Projected lost earnings due to illness and loss of life amounted annually to $16.3 billion (CI, $8.7, $31.0). The total economic burden of annual influenza epidemics using projected statistical life values amounted to $87.1 billion (CI, $47.2, $149.5). Conclusions: These results highlight the enormous annual burden of influenza in the US. While hospitalization costs are important contributors, lost productivity from missed work days and lost lives comprise the bulk of the economic burden of influenza.

Abstract P153

SCHOLARSHIP International Scholarship

Laboratory Surveillance for Influenza in Cuba

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World Health Organization created more than 50 years ago the World Influenza Surveillance System, which has contributed to the knowledge and the understanding of the epidemiology of influenza viruses. However, it still is necessary to improve and strengthen the surveillance and control of this disease all over the world. There is an urgent need to get ourselves ready to face the next pandemic. In Cuba, infection by Influenza virus and its complications represents the first cause of mortality among the infectious diseases. In our National Centre of Influenza (NCI), the introductions of the powerful tools of molecular biology are contributing to improve the national surveillance system of Acute Respiratory Tract Infection. During the 2005-2006 influenza season, the totality of respiratory samples were analyzed using two multiplex RT nested-RCP multiplex for the detection of 14 respiratory virus. Recently, we introduce two RT nested-RCP for the typing and subtyping of hemagglutinin and neuraminidase of influenza A virus, including the subtype A (H5N1). During this season, virological surveillance highlighted the predominant circulation of B viruses (71% of isolates) in Cuba, in contrast to many other countries in Europe and North America where AH3N2 viruses were isolated most frequently, and in contrast to the infrequent isolation of B viruses in Cuba during the previous years.Influenza A (H1N1) viruses were not identified. However, the surveillance through serum pair studies showed a predominant positively to influenza A (H3N2). Three viruses influenza B were characterized by direct sequencing of haemagglutinin (HA1). Preliminary phylogenetic analysis indicated that the totality of the B viruses belonged to the B/Victoria/2/87-lineage. With the implementation of new advanced techniques in the NCI of Cuba, we will be able to undertake an effective monitoring of the circulation of influenza virus in our country and will keep the national health authorities alert and the WHO about the strains circulating in the country and will allow to put into practice, if needed, a Control and Prevention Measures Plan to face the pandemic.

Abstract P154

Influenza Surveillance in China

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China started influenza surveillance since 1950's, and joined the WHO global influenza surveillance network since 1983. Currently a national-wide surveillance system has been developed which including 197 sentinel hospitals and 63 influenza laboratories, the sentinel hospitals are responsible for epidemiological data report and for sample collection, the influenza laboratories are responsible for virus isolation and characterization. All epidemiological and virological data including all virus isolates will be submitted to Chinese national influenza center (CNIC) for further confirmation and genetic and antigenic analysis, and CNIC is also responsible for information and virus exchange with WHO GISN. An internet based information system also has been developed for our surveillance system in order to promote the information exchange. Here we summarized and reported the surveillance results since 2003, and compared with the results from WHO vaccine components recommendation data, our results definitely help us for influenza control and prevention in china and also contribute to the WHO GISN.

Abstract P155

Clinical Features of Influenza C Virus Infection and Impact of Antigenicity of Hemagglutinin Esterase Protein

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Background: Among influenza C viruses isolated in Japan, five distinct antigenic groups can be recognized. Outbreaks of influenza C virus infection occurred in Yamagata and Miyagi at intervals of almost 2 years between 1996 and 2004, and Yamagata/81 antigenic group (YA81-group) circulated from 1996 to 2001 and Kanagawa/76 antigenic group (KA76-group) predominated between 2002 and 2004. We revealed the clinical features of influenza C virus infection and evaluated the clinical impact caused by these different antigenic groups. Subjects and Methods: We examined the clinical data on 142 children infected with influenza C virus between 1996 and 2004 in Yamagata and Miyagi, Japan, and compared them between 60 children infected with YA81-group and 71 children infected with KA76-group. Results: Between 1996 and 2004, 51,366 respiratory specimens were obtained from patients <15 years old, and 161 specimens (0.31%) were positive for influenza C virus by cell culture system. Of 142 children, 130 (91.5%) were <6 years old. Fever (90.1%), cough (75.3%) and rhinorrhea (62.0%) were the most frequent symptoms. Overall, 93 (65.5%) were diagnosed with either a URT illness or "influenza", and 30 (21.1%) were diagnosed as having a lower-respiratory-tract (LRT) illness such as pneumonia, bronchitis or bronchiolitis. The median duration of fever was 2 days. The rate of hospital admission was 18.3% (26/142). Of the 26 hospitalized children, 14 (53.8%) were <2 years old and 18 (69.2%) had LRT illness. The clinical features (age distribution, symptom, rate of hospital admission) caused by YA81-group were similar to those caused by KA76-group, but diagnosis of "influenza" was more common for patients with KA76-group infection. Conclusion: Typical clinical symptom of influenza C virus infection is a common cold-like illness with a fever that persists for 2 days and the risk of complications with LRT illness is high in children <2 years old. The clinical impact caused by different antigenic groups was not shown. Diagnosis of "influenza" increased in 2002 and in 2004, when outbreaks of influenza C virus coexisted with those of influenza A and B viruses.

Abstract P156

Synchrony of Influenza Virus Activity and Influenza-like Illness in Hong Kong

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Incidence of influenza-like illness (ILI) has been regarded as a good indicator for activities of influenza viruses in community. While such correlation between ILI and influenza virus activity has been investigated in temperate regions, the correlation in tropical/subtropical regions is less well defined. The non-specific symptoms of influenza infection could possibly obscure the predictability of influenza epidemics based on ILI incidence rates. In this study, we investigated the relationship between seasonality of influenza virus activity (measured by proportions of specimens positive for influenza) and incidence of ILI (reported separately by General Out-patient Clinics (GOPC) and General Practitioners (GP)), by using the weekly surveillance data collected by the Department of Health, Hong Kong Special Administration Region from January, 1998 to May, 2006. During the periods 1998-2000 and 2002-2003, influenza virus activity exhibited two peaks each year, one in the winter and another in late spring or early summer. But during other periods of 1998-2006, only one winter peak could be clearly identified. ILI incidence rates in both GOPC and GP share a similar nonstationary seasonal pattern. We used wavelet analysis which is suitable for nonstationary time series and is able to characterize variation of seasonality as well as coherency between two series over time. Our results showed high coherence between ILI in GOPC and influenza virus activity for the annual periodic mode, but only significant during the periods 1998-1999 and 2002-2006 ($p<0.05$). For the half-year periodic mode high coherence ($p<0.05$) was also found significant during the period 1998-1999 and year 2003 when two peaks of influenza were evident. Compared with ILI in GOPC, ILI in GP had a weaker association with influenza virus activity for the annual periodic mode. On average, ILI in GP and ILI in GOPC preceded influenza virus isolation by 4 weeks and 2 weeks respectively. Our findings suggest that incidence rates of ILI can be used as a predictor for influenza epidemics in the tropics/subtropics, but we can expect influenza virus activity to lag several weeks behind incidence of ILI.

Abstract P157

Ontology-driven Influenza Surveillance From Web Rumours

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Background: The recent H5N1 avian influenza epidemic has highlighted the need for improved surveillance infrastructure in the Asia-Pacific region. An emerging modality in surveillance is the ability to exploit unsubstantiated Web news for detecting early disease outbreaks. However several challenges remain including the massive size of the Web, its multilingual nature and the uncontrolled proliferation of terms. We argue that without knowledge intensive methods search times for Web news will overwhelm scarce expert resources. To support the development of intelligent systems we present a multilingual ontology focused on the needs of infectious disease surveillance with vocabulary in six Asia-Pacific languages (English, Chinese, Japanese, Korean, Thai and Vietnamese) and show its application to influenza surveillance. Methods: Top level ontologies were considered for the upper taxonomy and SUMO (the IEEE Suggested Upper Merged Ontology) was chosen. News articles were surveyed and we identified eighteen target classes of terms in news reports including virus, bacteria, disease, syndrome, symptom and host. Key relations were also identified between target classes. A list of high priority pathogens was formed from a survey of notifiable diseases in the region, including the H5N1 subtype of influenza A virus. We then harvested terms from news articles before validating and defining them. Results: The first release of the ontology contains 479 English synonym terms, 274 Japanese, 296 Korean, 283 Chinese, 361 Thai and 265 Vietnamese. 30 pathogens/diseases, 6 syndromes, 108 symptoms, 10 routes of transmission were described. Links were made to external ontologies and nomenclature such as ICD9/10, LOINC, MedDRA, MeSH and SNOMED CD. Conclusion: The ontology rose out of our need to sustain the development of an intelligent disease surveillance system. We make it feely available for download and also as a searchable Web portal in the hope that it will help bootstrap the development of other intelligent search systems.

Abstract P158

Nationwide Influenza Laboratory Surveillance in Korea During 2003/04-2005/06

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Background: The Korea Influenza Surveillance Scheme (KISS) has been established in 2000, which aims to monitor influenza activity by the clinical and virological surveillance. Physicians throughout the region reported cases of influenza-like illness (ILI), which were then confirmed by virus isolation and typing of the virus in 17 provincial laboratories. Since 2003/04 season, it has been strengthened by introduction of web-based reporting system. Laboratory surveillance and characterization of influenza viruses isolated during 2003/04~2005/06 influenza epidemic were represented. Methods: Throat swabs from the patients with ILI during 2003/04~2005/06 had been collected. Virus was isolated by MDCK cell culture and identified by RT-PCR, hemagglutination inhibition assay (HI) and sequence analysis. Results: A total of 1,312 (A/H3N2, 746; A/H1N1, 1; B, 565), 999 (A/H3N2, 777; A/H1N1, 31; B, 191), and 1,473 (A/H3N2, 305; A/H1N1, 747; B, 421) influenza viruses were isolated in 2003/04, 2004/05, and 2005/06 season, respectively. A/H3N2 and B viruses were dominant throughout whole period. A/H1N1 viruses were remarkably increased in 2005/06 season. The vast majority of A/H3 viruses were similar to the vaccine strains of each season, A/Moscow/10/99-like strain, A/Fujian/411/2005-like strain, and A/California/7/2004-like strain. Most of A/H1 viruses were similar to that of vaccine strain, A/New Caledonia/20/99-like strain, respectively. It was observed that during the study period, 2003/04~2005/06 season, both B/Victoria/2/87 and B/Yamagata/16/88-like strains has co-circulated in Korea. Also it tended that vaccine strains of upcoming season were introduced at least one or two years earlier before vaccine selection. Conclusions: It needs to be improved and strengthened the KISS to detect the emergence of pandemic.

Abstract P159

Epidemiological Analysis of Influenza by Laboratory Surveillance in Jeonbuk Province, Korea, During 2004/2005-2006/2007

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Background: Influenza is a common respiratory disease with a high contagiousness every winter season. Influenza virus has the high possibility of appearing new virus subtypes due to antigen variations. As confirmed cases of human infection from several subtypes of avian influenza have been reported since 1997, WHO has recommend to strengthen the influenza surveillance. In this study, we performed the influenza laboratory surveillance in Jeonbuk province during 2004/05-2006/07. Methods: For the influenza virus isolation, throat swabs were collected from patients with influenza-like illness (ILI) who visited one Health Center and seven hospitals in Jeonbuk during 2004/05 - 2006/07 seasons. The specimens were inoculated to MDCK cells and infected cells were observed for cytopathic effect (CPE). Culture supernatants were confirmed by multiplex RT-PCR which could simultaneously differentiate type and subtype of influenza virus. Results: A total of 1,313 specimens were tested for influenza virus during the 2004/2005 - 2006/2007 season. 687 (52.2%) viruses were isolated, of which 411 (59.9%) were A/H3N2, 163(23.7%) was A/H1N1, and 113 (16.4%) were B. The incidence of influenza was the highest in the childhood aged from six to ten during three seasons. Conclusions: Influenza laboratory surveillance in Jeonbuk has been activated from 2004-05 season, which could be contribute the control and prevention of influenza in Korea.

Abstract P160

Sentinel Doctor's Influenza National Surveillance System, Five Years Experience at Provincial Level, Córdoba Province, Argentine Republic

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Influenza Laboratory from Córdoba Virology Institute is OMS National Influenza Centre (NIC) since 1964. During this period we identify and isolate influenza virus from our Province and others, and we collaborate with influenza surveillance by different ways in our country. In 2002 Argentina begun to use a sentinel doctors surveillance system in parallel with the National Epidemiological Surveillance System (SNVS). This new national system was integrated by provincial units. Our Laboratory coordinate Cordoba provincial unit with the Epidemiology Direction from Provincial Ministry of Health. The system has three components the sentinel physicians, Laboratory and epidemiology, what differentiate to the SNVS that have only epidemiological data without laboratory confirmation. Health-care providers, previously designed, report weekly the total number of patients seen by itself and the number of those patients with influenza-like illness (ILI) by age group. ILI was defined as fever (temperature of ≥38°C) with myalgias or postration and cough and/or a sore throat. Specimens were sending to Virology Institute, tested for human respiratory virus including influenza A and B, and cultured for influenza virus isolation. Data collected were plotted into software in the National Surveillance System web site. In order to organize these systems we perform tree meetings to teach all the components involved in the surveillance the first year, organized by National Ministry of Health, the following years one Workshop was done annually. 15 sentinel physicians distributed in pediatrical and adults institutions were working in Córdoba Province. This System was capable to provide weekly epidemiological and virological data to provincial and national level and the sentinel physicians have access to this information at any time. The reported information answers the questions of where, when, and what influenza viruses are circulating. It can be used to determine if influenza activity is increasing or decreasing, but cannot be used to ascertain how many people have become ill with influenza during the influenza season. This work shows the role of a NIC, collaborating actively in local and national influenza virological and epidemiological surveillance organization.

Abstract P161

Influenza-Associated Pediatric Mortality, United States, 2004-06

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Background: Limited data are available on influenza-related mortality in children. In October 2004, pediatric influenza-associated deaths were added to the list of conditions reportable to the U.S. National Notifiable Diseases Surveillance System. We analyzed reports of fatal pediatric cases during the 2004-05 and 2005-06 influenza seasons. Methods: An influenza associated death was defined as a death in a child aged <18 years resulting from a clinically compatible illness with laboratory confirmation of influenza. Influenza-associated deaths in children are reported to state health departments which report cases to CDC through a web-based form. Data from cases that occurred during the influenza season (October to May) were analyzed by descriptive statistics. Results: The number of deaths (46; 43), number of states reporting ≥1 case (19; 17), median age (5 years, range: 23 days to 17 years; 4 years, range: 28 days to 17 years), and frequency of chronic underlying conditions (60%; 65%) were similar for the 2004-05 and 2005-06 seasons, respectively. Male deaths were similar for the 2004-05 (54%) and 2005-06 (60%) seasons, respectively. Cases were predominantly white in both seasons 73% (n=37) and 54% (n=35), respectively and were similar to <18 US population. Influenza A was identified in 61% (n=44) and 66% (n=38) of cases in the two seasons, respectively. Three cases had invasive bacterial infections reported in 2004-05 compared with 6 in 2005-06 (including methicillin-resistant Staphylococcus aureus, methicillin-sensitive Staphylococcus aureus, Group A streptococcus, Streptococcus viridans, and Gram negative species not identified). Of cases aged 6-23 months, 1 of 4 had received 2 doses of influenza vaccine in 2004-05 compared to 1 of 13 in 2005-06. Of cases aged ≥24 months with chronic high-risk medical conditions, 30% (n=20) and 14% (n=14) had received one dose of influenza vaccine ≥14 days prior to illness onset during 2004-05 and 2005-06, respectively. More cases were hospitalized in 2004-05 (63%) compared with 2005-06 (48%). Conclusions: The majority of pediatric influenza-associated deaths reported during 2004-06 in the United States occurred in children with chronic medical conditions. Influenza vaccine coverage was low among cases recommended for annual influenza vaccination. Efforts are needed to increase influenza vaccination coverage among high-risk children.

Abstract P162

Serological Surveillance of Human Influenza Viruses Among Elementary Schoolchildren in Taiwan During the 2005-2006 Flu Season

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Schoolchildren are important community-based influenza epidemic alerting group because they have longer shedding period and higher transmissibility. However, few studies used serology to evaluate their impact of influenza virus infection. In this community-based seroepidemiological cohort study, the specific aims were: (1) to understand the differences and similarities of the sero-incidence rates, the sero-prevalence rates and the geometric mean titers (GMTs) among schoolchildren in different geographical areas [northern (Taipei), central (Changhwa) and eastern (Yilan) Taiwan plus one isolated islet (Kinmen)] during the 2005-2006 epidemic season, and (2) to investigate the possible risk/protection factors associated with their influenza virus infections. We collected the first time point's blood samples of approximately 1600 enrolled students in Nov.-Dec. of 2005 (before the influenza epidemic season). About 80% participants still joined our second blood taken in Apr.-May 2006. Finally, there were nearly 1300 paired serum samples from five schools. Also, the questionnaire information on risk/protection factors was obtained for analysis. Haemagglutination-Inhibition (HI) test was used to screen antibody against the 2005 influenza virus vaccine-like strains in the Northern Hemisphere recommended by WHO. To measure seroprevalence and seroincidence, seropositive was defined as the HI sero-titer greater than or equal to 40 and greater than or equal to 4-fold rise, respectively. The results showed that sero-incidence rates were between 26.09% and 36.37% of the tested schoolchildren but with geographical variations in the subtypes of influenza A viruses. H1N1 was dominant among the children in Taipei, Kinmen and Yilan, the same dominant subtype during the 2005-06 in Taiwan; whereas both H1N1 and H3N2 subtypes were co-circulated in Changhwa. Moreover, Kinmen schoolchildren had significantly higher seroprevalence rate of H1N1 than other areas even before the 2005-06 influenza season, implying that schoolchildren in Kinmen had experienced the H1 epidemic earlier than others. This is the first community-based study to simultaneously measure prevalence of incidence rates against human influenza H1N1 and H3N2 viruses among children at schools in areas with different risk levels in Taiwan for formulating better future vaccine policy. We found Kinmen schoolchildren can serve as the sentinel populations, consistent with our previous findings that the virus sequences changed earlier than those in Taiwan main island.

Abstract P163

Experiences of Automated Telephone Contacts for Surveillance

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Background: Population-based infectious disease surveillance presupposes a capacity to contact large groups of people in a short period of time. Aim: To measure the influenza immunization coverage in Sweden using automated telephone contacts and to evaluate the telecommunication techniques in comparison with personal telephone interviews. Method: A population-based sample of 7700 persons was randomly divided into three groups: (i) SMS (Short Message Service) to mobile phones, (ii) IVR (Interactive Voice Response) to stationary telephones, and (iii) personal telephone interviews to stationary and mobile phones. Telephone numbers were extracted from a computerized directory. Questions enquired about influenza vaccination and about willingness to be interviewed in the future using the allocated technique. A sample of non-responders to IVR and SMS were also followed up with telephone interviews to inquire about their experience with the contact technique. Results: Mobile phone numbers were obtained for 44.0% of 2400 individuals allotted to SMS, stationary phone numbers for 54.2% of 3150 persons allotted to IVR and stationary or mobile telephone numbers for 76.1% of 2150 allotted to personal interviews. Participation was declined by 17.7% in the SMS group, 18.6% in the IVR group and 11.1% in the personal interview group. The subcontractor that administered the IVR calls had technical problems leading to many failed contacts and a final response rate of only 30.7%. SMS had a lower response rate than personal interviews (17.7% and 68.3%, respectively). The follow-up interview showed that many of the mobile phone numbers were outdated or linked to phones that were not in regular use, and some individuals did not know how to use SMS. Interpretation: The phone directory covered mobile phone services moderately well. The lack of complete and updated phone directories, and lagging adoption of new techniques such as SMS, presently precludes automated contacts with representative samples of the population. At first contact via regular mail, people should be given an opportunity to select a preferred technique and asked to provide a functioning phone number.

Abstract P164

The 1918 Pandemic Experience in Japan: Age and Geographic Mortality Patterns

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Background: The 1918 influenza pandemic was the most devastating epidemic in recorded human history. Aside from the magnitude of the mortality impact, there was a striking concentration of mortality among young adults. Here we describe the Japanese experience of the 1918 pandemic, focusing on age and geographic mortality patterns. Methods: Monthly all-cause and pneumonia and influenza (P&I) mortality data were compiled for each of the 47 administrative prefectures of Japan for 1915-1921. Excess mortality estimates were calculated in each prefecture for the two pandemic seasons of 1918-19 and 1919-20 using a seasonal Serfling regression method. We used annual data to estimate age-specific excess P&I mortality using the pre-pandemic years 1915-17 as baseline (no monthly age–specific data were available). Results: The excess all-cause mortality rate in Japan was 4.7/1,000 in the first pandemic season (1918-19) and 3.4/1,000 in the second season (1919-20). Over the three year pandemic period (1918-1920), the excess P&I mortality rate in young adults (20-40 years of age) was 3.7/1,000, approximately 10-fold higher than the annual baseline level. The excess P&I mortality rate in seniors aged $\geq$60 years was 2.3/1,000, about 1.8-fold higher than the annual baseline level. There was a weak negative correlation in mortality rates between the first and second seasons (rho=-.27, P=0.059) suggesting potential cross-immunity or a difference in virulence between the virus variants responsible for each wave. While excess mortality rates declined in the second season in most prefectures, the reverse pattern was found in and around Tokyo (where ~56% of pandemic deaths occurred in the second season). Conclusions: During the 1918 pandemic, Japanese young adults suffered unusually high mortality rates, similar to patterns also found in the US and UK. It is notable that populations in and around Tokyo experienced higher excess mortality rates during the second wave than they did in the first wave, and, taken separately, the second pandemic season was more severe in Japan than in the US or UK. The potential effect of cross-immunity or decreasing virulence between successive pandemic waves suggested here remains controversial and needs to be confirmed in morbidity and mortality data from other sources.

Abstract P165

Importance of the Inclusion of Paediatric Data to Sentinel Surveillance in Predicting the Onset of Seasonal Influenza Epidemic

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Aim: To analyse the correlation between sentinel Influenza Like Illness (ILI) reporting and influenza virus isolation in paediatric and adult sentinel population for prediction of seasonal influenza epidemic onset. Methods: Surveillance was performed on integrated clinical-viral model [PIDIRAC (Daily Acute Respiratory Infection Information Programme of Catalonia), Department of Health, Generalitat of Catalonia, Spain)]. Study period goes from week 45 to 15 of three epidemic influenza surveillance seasons (2003-04, 2004-05 and 2006-07). Data was compiled through weekly influenza virus identification in nasal-pharynx and throat samples, and incidence of ILI consultation reporting by sentinel physicians (29 general practitioners and 20 paediatricians) throughout Catalonia. Correlation of data from combined pre and initial epidemic period A: 2 weeks before onset and 1st half of epidemic and period B: 2nd half of epidemic and 2 weeks after. Difference between proportions was assessed by z test; correlation between total, paediatric (≤14y) and in adult (>14y) sentinel population influenza virus positivity and total ILI reported consultation incidence rates was assessed by Spearman's correlation test (Rho). Results: During the study period a total of 983 samples were analysed with a total positivity for the influenza epidemic virus was of 394/983 (40%) showing no statistically significant difference between proportions [240/572 (42%) positive in children vs 154/411 (38%) in adults (z = 1,2; 95%CI=0,92-1,58; p=0,1769)]. During total surveillance period, weekly ILI consultation rates showed significant correlation in proportion of influenza virus isolated (Rho=0,759; p<0,001); correlation was also significant in >14y and >14y. (Rho= 0,737 and 0,640 ; p<0,001 respectively). Correlation of data from period A and B was significant for total data (Rho=0,699; p=0,003) as well as for ≤ 14y (Rho = 0,561; p=0,02), but not so for >14y (Rho=0,477;p=0,06). Conclusions: ILI reported incidence by sentinel network physicians has proven to be a suitable indicator to predict onset of influenza epidemic activity only when paediatric population is included in the surveillance system; hence the system fails to detect the beginning of the epidemic when only adult population is considered. This fact highlights the relevance of including paediatricians into influenza sentinel surveillance networks.

Abstract P166

Economic Impact of the 2005/2006 Influenza Epidemic in France

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Context: To quantify the economic impact of influenza is not an easy task: the clinical influenza pattern can be very different from one patient to another and precise influenza diagnostic is rarely done. The French national influenza surveillance network (GROG) is based on the data from community health care professionals who weekly describe their activity and take rhinopharyngeal swabs in patients presenting with influenza like illness. Objective: To evaluate the direct cost of the 2005/2006 influenza epidemic through the GROG surveillance data. Methodology: Retrospective study of all the confirmed influenza cases seen in the GROG network. For each described case, an additional form registering the therapeutic attitude (number of consultations, prescriptions, sick leave…) is filled by the GP and paediatricians taking in account the age of the patient and the intensity of symptoms. Results: The final analysis of the 556 influenza cases fully described by 162 GROG doctors is still in process. As a first approach during the 2005-2006 season, each flu case has led to a mean direct cost from 37 to 51 euros, varying according to the age of the patients (mean drugs prescriptions: 13,70 euros, fees: 25 euros). Moreover, the economic impact should take in account the daily allowances and production loss due to sick leaves: 4,8 days of sick leave in 70% of the working adults consulting for flu; 3 days of sick leave to take care of children in ¼ of children flu cases. On an epidemiological point of view, the 2005-2006 season can be described as a very moderate flu season (influenza B). However our data lead to estimate, for France, the total direct cost of this epidemic wave to 100 million euros. These results will be compared with data of previous seasons obtained using the same methodology.

Abstract P167

Indirect and Out-of-Pocket Costs of Influenza-related Illness in Young US Children

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Background: Indirect and out-of-pocket costs associated with influenza illness were important factors considered in revised vaccination recommendations made by the Advisory Committee on Immunization Practices (ACIP). Prior economic analyses of influenza burden have been based on economic data often not attributable to laboratory-confirmed disease. Objective: To determine indirect and out-of-pocket costs among caregivers of young children with laboratory-confirmed influenza. Methods: Using a population-based surveillance network, we studied a representative group of children aged ≤5 years with community-acquired laboratory-confirmed influenza during three consecutive seasons (2003–2006). Cases hospitalized or seen in outpatient settings were identified and data were collected from follow-up interviews with parents and caregivers of cases. Outcomes measured included work time missed and out-of-pocket expenses. Resource and cost estimates were compared for children with and without a high risk condition (HR) for influenza-related complications as defined by the ACIP. Results: Out-of-pocket and indirect cost data were collected from 69 inpatients and 208 outpatients. Preliminary results show that inpatients required a mean of 2.6 hospitalization days for HR and 3.3 days for non HR ($p<0.001$). Inpatient caregivers missed between 54 (HR) to 69 (non-HR) work hours ($P<0.001$). Outpatients required a mean of 1.3 visits and outpatient caregivers missed approximately 6 or 11 work hours for HR and non-HR, respectively ($P<0.001$). Average indirect costs were $284 (range $109 to $695) for outpatients and $1293 (range $1055 to $1775) for inpatients. Average out-of-pocket expenses were $87 for outpatients and $151 for inpatients. Medical costs from billing and charts data will also be presented. Conclusions: Out-of-pocket and indirect costs of influenza-related illness in young children were sizable and considerably higher than previously reported. The presence of longer hospitalizations and higher costs in non-HR inpatients could be explained by a disease severity selection for hospitalization among non-HR children with influenza. *New Vaccine Surveillance Network (NVSN): A 3-site US active sentinel surveillance network on vaccine-preventable disease outcomes.

Abstract P168

Real Time Surveillance of Influenza Like Illness in the Community Using Automated Telephone Calls: Preliminary Results From the Flu Watch Study

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Introduction: Timely surveillance will be vital in the event of an influenza pandemic. Methods: Randomly selected households throughout England were recruited (October-December 2006). Weekly automated telephone calls were made to each household. A voice actor recorded participants' names and telephone scripts. Calls asked about colds or influenza like illness (ILI) with replies entered via the phone's numeric keypad. The administrator can switch on a message to request that participants submit nasal swabs (triggered on 9/Jan/2007 when national surveillance data suggested that influenza was circulating). We report on data collected via this system until 12/Mar/2007. We used a definition of ILI of self reported fever + either cough or sore throat (modified from the CDC definition which requires confirmed fever). We compared trends and rates of community ILI from our cohort with ILI consultation rates reported by the Royal College of General Practitioners' (RCGP) Weekly Returns Service. Results: To date nearly 3000 automated phone calls have been made to 241 households with 584 participants. The proportion of participants developing ILI varied by age (45% of those aged 0-4, 32% of those aged 5-14, 24% of those aged 15-64 and 13% of those aged 65 or more $p=0.001$). Weekly trends in community ILI rates and RCGP ILI consultation rates were very similar: both began to rise in week 4/2007, peaked in week 7/2007 and fell to low levels in week 10/2007. During this period, however, our community cohort rate of ILI was 100 times higher than the GP consultation rate. 253 nasal swabs have been sent out and 222 returned (88%). 3/11 swabs from patients with ILI were positive for influenza A on PCR. The remaining swabs will be processed after the flu season. Conclusions: Expansion of this novel surveillance mechanism is highly feasible and could allow real-time surveillance of respiratory illness in the community with laboratory confirmation of causative organisms.

Abstract P169

Seasonal Burden of Influenza-Like Illness (ILI) in a Community-Based Health Care Facility in Urban Subtropical Brazil

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Background: Influenzavirus (Flu) epidemics are associated with high morbidity and mortality, mainly in high-risk persons. Children younger than five may experience a particularly high Flu illness burden and thus represent a significant proportion of impact in terms of medical visits and hospitalizations. An overall increase in medical visits has been reported during Flu season. We evaluated the proportion of medical visits related to influenza-like illness (ILI) in the 2006 influenza seasonal outbreak in a community-based primary health care facility in Ribeirao Preto, SP, in southeast Brazil. Methods: All medical visits, ILI-related or not, recorded from May 2006 to February 2007 were reviewed and each week samples were submitted for Flu detection by RT-PCR to detect the influenza activity stratified by age. The data were then correlated with Fl, u circulation, which was defined as the period while Flu was detected in samples by the virology laboratory. ILI was defined as fever associated with respiratory symptoms. Results: From May/2006 to February/2007 a total of 85,842 medical visits were recorded and 11,591(13.5%) were classified as ILI. The monthly number of visits ranged from 7,363 to 10,641 and ILI-specific visits ranged from 641 to 2037 (average = 1159 visits) per month. During Flu season ILI visits increased by more than 1SD and total visits increased by 2SD above average. When broken down by age the data showed that while the number of medical visits per month increased in all age groups, the increase was more pronounced in children younger than 5 years of age (average + 2 SD) and in those between 5 to 14 years of age (average + 1SD). People over 60 showed the lowest rates of total and ILI visits. Conclusions: Flu seasonal outbreak in Ribeirao Preto was associated with an increase in total and ILI-related medical visits, mainly in children younger than 14 years. Lower numbers of visits in the elderly maybe due to influenza vaccination targeted to this age group. Vaccination of children aged less than 2 year, as well as school-aged children will very likely reduce the numbers of ILI-related and total medical visits in the region.

Abstract P170

Spatial and Temporal Heterogeneity in Hospitalizations Associated With Influenza and Respiratory Syncytial Virus (RSV) in 10 US States, 1989-2003

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Introduction: Geographical differences in influenza and RSV-associated hospitalizations have not been quantified in the US. Further, the relative contribution of influenza and RSV to respiratory hospitalizations remains unclear, and may be confounded by spatio-temporal heterogeneity in viral activity. We sought to estimate peak timing of influenza and RSV hospitalizations, as well as to estimate rates of influenza-associated hospitalizations, controlling for this potential spatio-temporal heterogeneity in viral activity. Methods: We analyzed State Inpatient Databases (SID) from 10 states from the AHRQ's Healthcare Cost and Utilization Project (HCUP), which represents 34% of the current US population. Weekly hospitalizations with any diagnosis of pneumonia, influenza, or RSV were compiled for 1989-2003. We compared the peak timing of influenza- and RSV-specific hospitalizations across seasons, states, and by dominant influenza (sub)types (A/H3N2 vs. A/H1N1-B). Using a seasonal regression model, we estimated excess rates of pneumonia and influenza hospitalizations associated with influenza for all ages, children, and seniors. Results: Neither influenza nor RSV epidemics systematically peaked before the other in the 10 states, even when controlling for dominant influenza subtype (P=0.94). Variability in timing of influenza hospitalizations was more important between seasons than between states (61% and 10% of the variance explained by season and state, respectively). By contrast, for RSV, there was more variability between states (51%) than between seasons (13%). Seasonal estimates of hospitalization rates associated with influenza were 41 per 100,000 on average (35 for children ≤ 5 and 179 for adults ≥ 65). Conclusions: Our results suggest the presence of spatial and/or temporal heterogeneity in peak timing of influenza- and RSV-associated hospitalizations, but further research is needed to better understand these relationships. Additionally, these findings suggest that influenza burden varied substantially between years, partly explained by variations in influenza subtype dominance. Finally, RSV appears to be associated with localized epidemics, perhaps due to transmission mostly amongst a less-traveled population.

Abstract P171

Climatic Components Associated With Influenza Seasonality in Brazil

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Introduction: In temperate regions, seasonality of influenza epidemics is believed to be driven by climatologic factors, but the precise causes for its diverse pattern in other zones remains elusive. In a previous paper, we have shown that annual influenza epidemics travels from less densely populated regions near the equator to the more populated centers in the South and Southeast Brazil. Brazil is a large country encompassing the equatorial Amazon rain forest in the North, semi-arid landscapes in the Northeast, savanna climate in the Central states to subtropical climates South regions. Here, we investigate the associations of climate factors and spatial epidemic patterns. Methods: We investigated which monthly climatic variable best discriminated between the influenza epidemic and non-epidemic periods in each of the five Brazilian regions. Periods were composed of three months with highest and lower incidences respectively for each region, as based on patterns of influenza and pneumonia mortality rates from1979 to 2001 and on recent laboratory surveillance data. Monthly average precipitation and temperature were obtained from each state's capital and aggregated according to the regional divisions of Brazil. Results: Temperatures in the Southern, Southeastern and Central states were significantly (T-tests, $p<0.01$) lower during the influenza period that occurs in winter, when monthly averages 5°C below the non-influenza period. Precipitation in the Southeast and Central regions were also significantly lower (175 to 53 mm and 198 to 31mm, respectively). This differs greatly from the equatorial regions, where epidemic seasons occur earlier in the year, and precipitation was shown to be the only important climatologic component between epidemic and non-epidemic months (respectively 114 and 360 mm in the North, and 58 and 242 mm in the Northeast). Conclusions: Our results demonstrate that the climatologic conditions present during the influenza season are of very different nature in equatorial zones and regions far from the equatorial line. We propose that rainfall in the equatorial latitudes and low temperatures in the remaining territory underlies the unexpected pattern of influenza traveling as a wave from low to high densely populated regions of Brazil.

Abstract P172

Description of a Distinct Antigenic Drift Variant of Influenza A (H3N2) Circulating in Nairobi, Kenya

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Global influenza surveillance is an important public health endeavor that allows for the detection of viral antigenic drifts and shifts. Data derived from surveillance activities are also useful in informing the WHO vaccine expert group in selection of yearly vaccine strains. These data enable public health authorities to detect outbreaks and the emergence of dangerous influenza subtypes. No consistent influenza surveillance has been conducted in east and central Africa to date in contrast to the Americas, Europe, Australia and Asia where laboratories and public health agencies actively conduct surveillance. To address this shortcoming, the United States Army Medical Research Unit – Kenya (USAMRU-K), the Global Emerging Infections Surveillance and Response System (GEIS), the Kenya Medical Research Institute (KEMRI), and the Centers for Disease Control and Prevention - International Emerging Infections Program (CDC-IEIP) - Kenya recently instituted a collaboration to conduct surveillance for influenza and influenza like illnesses in Kenya. Nasopharyngeal samples were taken from individuals two months and older who presented to participating outpatient clinics with acute upper respiratory symptoms. The samples were inoculated in MDCK cells and cultures showing cytopathic effects were further characterized using serologic assays. Influenza A viruses were isolated and molecularly characterized using nucleotide sequences of the HA gene. We demonstrate for the first time the isolation of influenza A viruses of the H3N2 subtype in Kenya. We also show that these viruses are distinct antigenic variants that have drifted from the WHO recommended current 2006/2007 vaccine strain or any other vaccine strain from the past nine years and then discuss possible implications of this finding for public health.

Abstract P173

Detection of a Summer Influenza A/H1N1 Cluster Among Basic Trainees at the Marine Corps Recruit Depot, San Diego

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Background: Military recruits are at increased risk for respiratory infections associated with the physical stress and close contact of basic training. The Navy Respiratory Disease Laboratory (NRDL) at the Naval Health Research Center in San Diego conducts population-based surveillance for febrile respiratory illness (FRI) at all US military basic training centers. Specimens and clinical data are collected systematically from consenting trainees and FRI rates are calculated weekly. Although high FRI rates are common among basic trainees, influenza is a relatively rare cause within this highly vaccinated population. In August 2006, an unusual cluster of early-season influenza was detected among recruits at Marine Corps Recruit Depot San Diego (MCRD-SD). Methods: NRDL specializes in many methodologies, both classic and novel, to identify, confirm, and characterize viral pathogens, including influenza, that affect recruits. NRDL increased routine sampling at MCRD-SD. Specimens from MCRD-SD were given highest priority and were tested by molecular methods followed by culture. Sera were also drawn from 3 platoons for comparison with accession serum, including the platoon containing known influenza cases. Results: We identified an unusual cluster of influenza A/H1N1 in San Diego recruits in the summer of 2006. We were able to quickly confirm that the cluster was localized within the group and that influenza transmission had ceased. Rapid, early distribution of seasonal influenza vaccine facilitated ongoing training without disruption or further intervention. No other cases were identified after influenza vaccination was initiated in late Aug 2006. Sequence information from the recruit influenza samples was sent to CDC. Conclusion: Continued collaboration with the CDC has revealed that no other influenza A/H1N1 pathogens of the same strain are currently circulating in the United States. Although not found in the United States, this strain is seen in other parts of the world. The discovery of this unique, early-season, emerging strain of influenza A/H1N1 validates the need for active, ongoing respiratory surveillance at military training sites. In the era of increasing concern about pandemic influenza, such surveillance is vital to quickly identify and respond to public health outbreaks, both within and outside the military.

Abstract P174

Influenza Surveillance in New Zealand, 1997-2006

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The influenza surveillance in New Zealand aims at monitoring incidence and distribution of influenza, early detection of influenza epidemics and identifying the predominant circulating strains. The national influenza surveillance system includes sentinel general practice (GP) surveillance, laboratory-based surveillance, hospital admission and mortality surveillance, and vaccine coverage surveillance. The sentinel GP surveillance operates in the winter from May to September with the participation of about 80 volunteer GPs reporting influenza-like illness (ILI) weekly and providing respiratory specimens. The laboratory-based surveillance is a network of four regional hospital laboratories at Auckland, Waikato, Christchurch and Dunedin and the WHO National Influenza Centre (NIC) at ESR, Wellington, reporting all-year-round respiratory virus diagnoses largely from hospital in-patiens and out-patients. The laboratory network conducts strain surveillance for the respiratory specimens collected from sentinel GPs and hospital clinicians. Hospital admission data for influenza (ICD-10CM code 487) from the New Zealand Health Information Service's National Minimum Dataset (NMDS) forms the basis of influenza morbidity surveillance. Deaths from influenza (ICD-9CM 487) from the NMDS constitute influenza mortality surveillance. In 1997, influenza vaccination was made available free to those over 65 years of age and in 1999 free vaccination was extended to risk groups less than 65 years. The data that medical practitioners provide to Health Benefits Limited to claim reimbursement are used to estimate coverage in these two groups. This presentation will evaluate the national influenza surveillance over the last 10 years from 1997 to 2006. Various surveillance datasets will be compared and discussed.

Abstract P175

The Review of the Situation on Influenza in Kazakhstan in 2003-2006

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One of the most urgent and paramount problems of public health services is struggle with respiratory viral to infections. In this connection, the basic emphasis make on preventive maintenance of respiratory viral infections. The main task of the National reference laboratory on the control for viral infections (NRL) is epidemiological decoding of respiratory infections. In Kazakhstan epidemic season have been associated with 83617 cases of influenza during 2003-2006. Share of children before age 14 compiled 59-61%. NRL in this period carried out researches on virus isolation using cellular culture of dog kidney–MDCK, and also chicken embryos 8-9 day time. At work with chicken embryos studying strains carried out in reaction of hemagglutination. Besides virusological researches, it used serological researches. In work pair serum from patients were studied. Reaction of hem agglutination inhibition carried out by standard method using 1% suspension of hen erythrocytes. Also it is carrying out researches by a method of luminescent microscopy. Influenza for 2003-2004 had a seasonal nature and without sharply expressed epidemic flash. At inspection of the population and decoding of diseases had been allocated 41, 3% on a share of A type (H3N2), B type-33%, A type (H1N1)-25, 6%. For epidemic season 2004-2005 it had been isolated 73 strains. From it's the main role belonged to virus A (H1N1)-52%, on the second place virus A (H3N2)-34, 2%, virus B-13, 7% was. For epidemic season 2005-2006 it were isolated 5 strains: 3-type B, and 2-type A. Influenza vaccination is the primary method for disease prevention and its severe complications. In Kazakhstan annual influenza vaccination is now recommended for the following groups: persons aged >60 years; adults and children of any age with chronic disorders of pulmonary or cardiovascular systems; children of children's home; health-care workers; workers of bird's factories (farms).

Workshop Topic #2

Virus Structure/Function and Receptor Binding

Poster Abstracts

Abstract P201

Some Events in the Biogenesis of Influenza Virus Nucleocapsid Protein

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It is known that influenza virus nucleocapsid protein (NP) possesses a high efficiency of homo-oligomer formation [Ortega et al., 2000; Ye et al. 2006]. We have earlier shown that in influenza virus infected cells stable compact NP oligomers are revealed [Prokudina-Kantorovich & Semenova, 1996]. The properties of compact NP oligomers suggest that they are the final completely folded structures in NP biogenesis [Prokudina et al., 2005]. Here we report that in addition to folded compact NP oligomers the incompletely-folded NP-NP complexes, designated "NP multimers", are indicated in influenza virus infected cells. NP multimers are detected in 5% stacking gel of SDS/PAGE as retarded and loose structures, heterogeneous in size. NP multimers dissociate into monomeric subunits at +50 °C in contrast to compact NP oligomers dissociating at +80 °C. NP multimers are more sensitive to proteolysis and to detergents than NP oligomers. NP multimers possess a weak immunoreactivity to some monoclonal antibodies. Taken together, the results presented indicate that in contrast to compact NP oligomers, NP multimers are not a highly ordered structure, probably due to incomplete folding of its NP subunits. It was also shown that NP multimers appear at early stages of NP synthesis (maybe co-translationally), preceding compact NP oligomer formation. The NP multimers are partially chased into faster migrating compact NP oligomers. Thus NP multimers presumably serve to bring nascent unfolded NP molecules into close contact with each other for further oligomerization, to protect monomeric NP subunits from proteolysis, and serve as intermediates in the posttranslational folding of NP. In the course of infection, the excess NP multimers not converted into compact NP oligomers accumulate in cells, degrade, join with some unfolded cell proteins, and partially penetrate into the extracellular space. Comparative studies on association of compact NP oligomers and NP multimers with nuclear and cytoplasmic fractions were also carried out. Thus, NP multimers probably take part in both folding and misfolding NP pathways.

Abstract P202

Amino Acids 223 and 225 of H4N6 Influenza Virus HA Determine Receptor Specificity and Swine Respiratory Epithelial Cell Infectivity

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H4N6 influenza A viruses were isolated from pigs in Canada in 1999 (Karasin et al. 2000. J. Virol. 74:9322-9327). Sequence analyses revealed that these viruses were of avian lineage, but had differences at amino acids 223 and 225 of the hemagglutinin (HA) protein compared to avian H4 sequences in GenBank. The equivalent positions in the H3 influenza HA, residues 226 and 228, play major roles in determining receptor preference and species specificity of H3 influenza viruses. Comparison to H3 sequences suggested that the H4N6 viruses isolated from swine had a preference for SAα2,6Gal, rather than the typical avian virus preference for SAα2,3Gal. We examined whether amino acids 223 and 225 of this H4 virus impacted the binding preference (using a synthetic glycopolymer binding assay) or virus infectivity in primary swine respiratory epithelial cells. Viruses with four different HA sequences were created: the original virus isolated from pigs, viruses with single mutations to avian-consensus residues (L223Q or S225G), and a virus with both mutations. The virus with a single S225G mutation displayed small differences in binding and infectivity, compared to the original virus isolated from pigs. In contrast, the virus with a single L223Q mutation showed decreased affinity for SAα2,6Gal and greatly decreased infectivity in swine cells. The virus with both mutations also showed greatly decreased infectivity, and had decreased affinity for SAα2,6Gal and increased affinity for SAα2,3Gal. These data indicate that mutations at both amino acids are necessary for high affinity binding to SAα2,3Gal, and that residue 223 plays a dominant role in infectivity. To determine whether these viruses functionally utilize α2,3- or α2,6-linked sialic acids for entry, we treated the swine cells with either 2,3-specific sialidase or 2,3/6-specific neuraminidase prior to infection with each of the four H4N6 viruses. The 2,3-specific sialidase had no impact on virus infectivity, while the 2,3/6-specific neuraminidase decreased the infectivity of all viruses. Taken together, our results suggest that these H4 viruses primarily utilize SAα2,6Gal to enter primary swine respiratory epithelial cells, and that the lower infectivity of the viruses with avian-like amino acid 223 is due to a decrease in affinity for SAα2,6Gal.

Abstract P203

Heterosubtypic Cross-Neutralization of Influenza A Viruses by a Novel Hemagglutination-Inhibiting Monoclonal Antibody

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Influenza A virus hemagglutinin (HA) is the major target protein inducing antibodies that neutralize virus infectivity. Sixteen antigenically different HA subtypes (H1-H16) of influenza A viruses have been identified in nature. It is generally believed that the neutralizing antibodies to HA are not cross-reactive among HA subtypes. However, we generated a novel monoclonal antibody specific to HA, designated S139/1, which showed heterosubtypic cross-reactive neutralization and hemagglutination inhibition of influenza viruses. S139/1 was obtained by immunizing mice intranasally with intact formalin-inactivated A/Aichi/2/68 (H3N2) virus. S139/1 showed neutralization and hemagglutination inhibition activities against some strains of the H1, H2, H3 and H13 subtypes of influenza A viruses. In enzyme-linked immunosorbent assay, S139/1 showed broad specificity to many other viruses, including H1, H2, H3, H5, H9, and H13 subtypes. Six escape mutants of A/Aichi/2/68 (H3N2) were selected by S139/1, and sequence analysis of HA genes of those escape mutants revealed amino acid substitutions at position 156, 158 or 193. A molecular modeling study showed that these amino acids were located in the HA surface on the globular head and formed a conformational epitope adjacent to the receptor-binding domain of HA. Four and three escape mutants selected from A/Adachi/2/57 (H2N2) and A/WSN/33 (H1N1), respectively, all had amino acid substitutions at position 193 (H3 numbering). These results suggest that S139/1 neutralizes virus infectivity by affecting the common antigenic site shared among different subtypes of HA. The present study suggests the possibility of antibody therapy for heterosubtypic immunity against multiple subtypes of influenza A virus.

Abstract P204

Molecular Basis of Amino Acid Substitutions in the N2na Protein of Influenza A Virus During Evolution

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Background: We analyzed the molecular basis of amino acid substitutions in the N2NA protein to obtain detailed information concerning the effects of the accumulation of amino acid substitutions on the structure and function of influenza A virus glycoproteins during evolution. Methods: We introduced single-point amino acid changes into the NA protein using PCR-based random mutation or site-directed mutagenesis. The mutant NA cDNAs were inserted into pME18S vector and expressed in COS cells. Neuraminidase activity of the expressed mutant NAs was assayed. Results and Conclusions: We introduced 152 random single-point amino acid changes into the NA protein of A/Tokyo/3/67 strain. Forty-eight % of them were tolerated. Rates for the stalk and for the surface or inner portion of the head region of the NA protein were 81, 54 and 19 %, respectively. Deleterious changes causing the NA protein to stop at the Golgi/endoplasmic reticulum (ER) were thought as structural changes and were scattered throughout the protein. All of the observed amino acid changes in the head region, except for one, which introduced cysteine residues or replaced cysteine residue by another amino acid residue stopped at the Golgi/ER. On the other hand, deleterious changes causing the NA protein to move to the cell surface were thought as functional changes and were located mainly within 5 A of the center of the active site of the sialidase. In order to investigate the effect of accumulation of amino acid substitutions on the structural character of N2NA protein during evolution, the same amino acid substitutions were introduced at 23 homologous positions on N2 proteins of A/Tokyo/3/67, A/Bangkok/15/85 (H3N2) and A/Mie/1/2004 (H3N2). The results showed a shift (discordance) in tolerance at some of the positions. An increase in discordance was found to be correlated with the interval in years between virus strains and the discordance rate was estimated to be about 0.6% per year. In H3HA, it was calculated to be 0.9%. This suggests that in the NA protein, accumulation of structural changes caused by amino acid substitutions during its evolution occurs more slowly than in the HA protein.

Abstract P205

Neutralizing IgG Human Monoclonal Antibodies to Pathogenic H5N1 Viruses

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A panel of IgG_1 human monoclonal antibodies (HMAbs) identified by hemagglutination inhibition (HI) assay has been produced from peripheral B cells of an individual immunized with prototype H5N1 vaccine (A/VN/1203/04). Sequence analysis of 11 antibody clones showed three clusters of different HMAbs as represented by HMAbs designated as BF1-1, BF1-19 and BF1-10. BF1-1 and BF1-10 have distinct CDR 1, 2 and 3 regions of both heavy and light chains. Interestingly, BF1-19 has the same heavy chain as BF1-1 but the light chain of BF1-10. Antibody binding affinity, K_D, studies showed all three HMAbs ranging between 10^{-8}-10^{-9}. Preliminary HI assays were performed against a panel of H5N1 viruses from Asia, A/HK/156/97, A/Thailand/1(KAN-1)/04, A/Turkey/65-1242/06, A/Whooping Swan/Mongolia/224/05, A/Japanese White eye/HK/1038/06, A/HK/213/03, A/VN/1203/04, and A/Dk/Laos/3295/06. Using a definition of ≥ 1:8 titer as positive inhibition and with spent supernatants from human hybridomas containing 10-40 µg/ml IgG, BF1-1 and BF1-19 neutralized A/HK/156/97, A/Thailand/1(KAN-1)/04, and A/VN/1203/04. This pattern is consistent with the observation that CDR3 of the heavy chain is primarily responsible for antibody specificity. BF1-10 having a different heavy chain CDR3 neutralized the same three isolates plus A/Japanese White eye /HK/1038/06. Preliminary in vivo protection studies with homologous wild-type A/VN/1203/04 showed BF1-1 and BF1-19 at 1 mg/kg and 2.5 mg/kg providing respectively 80% and 100% protection. BF1-10 at 1 mg/kg and 2.5 mg/kg provided similar protection of 75-80%. These findings support the feasibility of isolating cross-neutralizing HMAbs as therapeutic antibodies and provide evidence that these cross-neutralizing antibodies can be elicited by H5N1 vaccination. Confirmatory and epitope mapping studies are underway with purified antibodies.

Abstract P206

The Tolerance of Influenza B Virus HA To Amino Acid Changes

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As human influenza A and B viruses (A and B virus) circulate among human beings, they both accumulate amino acid changes in their hemagglutinin (HA) molecules. Among natural isolates, the ratio of nonsynonymous mutation rate to synonymous mutation rate of A virus HA (AHA) is about 1.5, while that of B virus HA (BHA) is about 0.5. This fact has suggested greater negative selection on BHA compared to AHA. Previously we have reported that the tolerance of AHA to amino acid changes was about 50%, that is, half of the single mutations introduced into AHA in vitro did not affect the receptor binding ability of AHA. To understand if such selection is due to restrictions on the changes of amino acids in BHA, we have investigated the tolerance of BHA to amino acid changes. Using the cDNA of B/Kanagawa/73(BK73), we generated HA with PCR based random mutations and with site-specific mutations. We examined the effect of each amino acid change on the receptor binding ability of the mutant BHA as expressed on Cos cells. Among 301 BHA mutants with a single amino acid change, 202 mutations at 164 positions of BHA1 and 109 mutations at 95 positions of BHA2, respectively, were observed. Only 24 of the mutations in the BHA1 region prevented the receptor binding ability. Most these mutations were at positions close to the receptor- binding site. None of the mutations in the HA2 region affected receptor-binding ability. The fusion ability of 49 mutants was also examined. Amino acid changes at positions around the fusion peptide affected fusion ability, but most of those at the other positions had no effect. These results indicate that at least the BHA expressed in vitro is highly tolerant to amino acid changes. The low nonsynonymous mutation rate cannot be ascribed to an intolerance of BHA to amino acid changes.

Abstract P207

Genomic Structure of Highly Pathogenic Influenza H5N1 Viruses

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During the latter half of 2005 a widespread outbreak caused by influenza highly pathogenic H5N1 virus among wild and domestic birds occurred in Russia. Identification of these strains is based on a limited number of features among which hemagglutinin properties and signs of receptor specificity in the HA receptor-binding pocket are of key significance. It is quite clear that pathogenicity level is a polygenic sign and majority of individual genes of influenza A viruses contribute to pathogenicity of influenza viruses to birds, animals and humans. Therefore analysis of nucleotide sequences of the entire genome of influenza H5N1 virus isolates obtained in Kurgan region (Siberia) was conducted. The following amino acid substitutions such as T1081, D126E, H138Q, E212K, P217S were revealed in HA of A/Chicken/Kurgan/05/2005 and A/Duck/Kurgan/08/2005 influenza viruses as compared with moderately pathogenic influenza H5N1 viruses. HA receptor-binding site of Kurgan isolates was typical for avian influenza viruses and contained Glu and Gly at positions 226 and 228, respectively. Structure of the cluster of positively charged amino acid residues at the cleavage site was identical for all isolates: QGERRRKKR.According to the data of neuraminidase structure analysis NA of the H5N1 isolates tested was suggested to belong to Z genotype. High pathogenicity of avian influenza viruses were known to be associated with their ability to suppress type I interferons (IFN) activity. NS1 protein was considered to be type I IFN antagonist. NS1 gene was found to have a short deletion located in the region encoding site of dimerization and dc-RNA-binding, i.e. domain performing the most important functions. Deletion included a short fragment of the following structure 79-AIAS\V\S-85. Many strains belonging to different influenza A subtypes had Serine residue at position 83. In a number of highly pathogenic influenza H5N1 viruses Serine residue was found to be highly conservative at positions 82-83 suggesting their functional significance. We propose region 70-85 to be the region modulating NS1 protein functional activity and presumably directly involved in control of type I IFN expression mediated by siRNA-interference. It was also confirmed by existence of Gly residue at position 92 of NS1 protein.

Abstract P208

Haemagglutinin Mutations Responsible for the Binding of H5N1 Influenza A Viruses to Human-type Receptors

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Since 2003, H5N1 influenza A viruses have spread throughout many countries in Asia, infecting large numbers of poultry and humans with lethal outcome. With the spread of these viruses to Europe and Africa, concerns have been raised about the emergence of a pandemic influenza virus capable of spreading efficiently among humans. Human and avian influenza A viruses differ in their recognition of host cell receptors: the former preferentially recognize receptors with saccharides terminating in sialic acid-α2,6-galactose (SAα2,6Gal), whereas the latter prefer those ending in SAα2,3Gal. Conversion of SAα2,3Gal to SAα2,6Gal recognition is thought to be one of the changes that must occur before avian influenza viruses can replicate efficiently in humans and acquire the potential to cause a pandemic. By identifying mutations in the receptor-binding haemagglutinin (HA) molecule that enable avian H5N1 viruses to recognize human-type receptors, it may be possible to predict (and thus to increase preparedness for) the emergence of pandemic viruses. Here, we show that some H5N1 viruses isolated from humans can bind to both human and avian receptors, albeit with varied affinities, whereas those isolated from chickens and ducks only recognize avian receptors. Mutations at positions 182 and 192 independently converted the HA of H5N1 viruses that recognized the avian receptor to ones that recognized the human receptor. Analysis of the HA crystal structure from an H5N1 virus showed that the locations of these amino acids in the HA molecule are compatible with an effect on receptor binding. In addition, mutations at positions 222 and 223 consistently decreased

the HA affinities of these viruses for avian-type receptors. The amino acid changes identified here may serve as molecular markers for assessing the pandemic potential of H5N1 field isolates.

Abstract P209

A Quantitative Method to Study Cell-cell Membrane Fusion Mediated by H5 Hemagglutinin

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Hemagglutinin-mediated endocytosis is known to play an important role in the entry of influenza virus into host cells. The membrane fusion process mediated by H5 hemagglutinin of H5N1 (A/Hatay/2004/(H5N1)) had been reconstituted using recombinant vaccinia virus infected 293T cells to express H5 on the cell surface. 293T cell-cell membrane fusion was then initiated by the treatments of low pH and trypsinization. In order to study the process quantitatively, two sets of 293T cells were transfected with gene constructs of either hepatitis C virus (HCV) NS3/4A protease or a secretion-blocked EGFP-4A/4B-SEAP (4A/4B: a decapeptide substrate of NS3/4A protease; SEAP: secreted alkaline phosphatase) fusion gene. The overnight infection of H5 hemagglutinin containing vaccinia virus was performed 24 h after the pre-transfections. For the fusion process, one set of 293T cells were served as the layer cells and the other set of cells were used as overlay cells. Significant SEAP activity could be detected in the culture medium only after cell-cell fusion occurred. Cell-cell fusion provides an environment in which the protease encounters its substrate 4A/4B, thereby releasing SEAP from the fusion protein. In this report, we used a simple, sensitive and quantitative method to study the membrane fusion process by measuring the extracellular activity of SEAP.

Abstract P210

Effect of the Addition of Oligosaccharides on the Biological Activities of Influenza A Virus Hemagglutinin

E Takashita, Y Muraki, Y Matsuzaki

Influenza A/H3N2 viruses have developed an increased the number of glycosylation sites on the globular head of the hemagglutinin (HA) protein since their appearance in 1968. We have previously constructed seven mutant HAs of A/Aichi/2/68 virus with one to six glycosylation sites on the globular head, as found in natural isolates, and analyzed biological activities of the molecule. The glycosylation sites of mutant HAs correspond to representative A/H3N2 isolates (A/Victoria/3/75, A/Memphis/6/86 and A/Sydney/5/97). The results showed that mutant HAs containing three to six glycosylation sites decreased receptor binding activity but retained cell fusion activity. Here, we attempted to produce recombinant viruses possessing these mutant HAs to investigate their biological activities. Mutant viruses expressing the HAs with four to six glycosylation sites exhibited drastic decrease in efficiency of virus production.

Abstract P211

The Crystal Structure of H5N1 Neuraminidase Reveals Opportunities for Novel Drug Design

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The persistence and continuing spread of highly pathogenic H5N1 avian influenza virus as well as the high case fatality rate in humans has raised serious concerns that it may acquire a greater capacity for human-to-human transmission and spark a pandemic. The virus neuraminidase, which is essential for virus proliferation and transmission, is the target of the recently available anti-influenza drugs Tamiflu (oseltamivir) and Relenza (Zanamivir). These selective inhibitors were discovered through structure-based drug design of subtype N2 and N9 enzymes. Recent reports of the emergence of oseltamivir-resistant influenza A virus H5N1 variants with mutations N294S or H274Y in the viral neuraminidase highlight the importance of developing alternative anti-influenza drugs. The 9 NA subtypes fall into two groups, which are structurally as well as phylogenetically distinct. X-ray crystallographic structures of 3 group-1 NAs, N1, N4 & N8, showed the catalytic sites to be virtually identical but to differ from those of N2 and N9 (group-2) NAs. The major difference focuses on the conformation of the 150-loop (residues 147-152) of group-1 and the creation of a large open cavity adjacent to the active site, not present in group-2, that closes upon inhibitor binding. Further X-ray crystallographic structure determination of oseltamivir resistant H5N1 neuraminidase mutants will reveal the structural basis for differential drug resistance between the two groups and together with the exploitation of the size and location of the group-1 cavity, should provide valuable insights for the development of new anti-influenza agents.

Workshop Topic #3

Outbreak and Pre-Pandemic Response

Poster Abstracts

Abstract P301

Use of the Centers for Disease Control and Prevention (CDC) Outbreak Management System (OMS) for Investigation and Management of Outbreaks and Pre-Pandemic Response

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Background: Early identification of human cases of H5N1 infection is essential in delaying the introduction of influenza into a community. Mathematical models suggest that an emerging novel human influenza virus subtype might be containable during pandemic alert phases 3-5 through targeted use of antivirals and application of public health measures. In-depth follow-up of cases and contacts during this period is critical in assuring that appropriate isolation of symptomatic persons and quarantine of well contacts is initiated and maintained. Close contacts may be offered antiviral post-exposure prophylaxis and contacts that become symptomatic should be referred immediately for medical evaluation, treatment and isolation. CDC's OMS was designed to facilitate such investigation activities and the application chosen to accomplish this task for pandemic influenza preparedness. Objective: To describe how OMS is used to support case and contact investigations. Methods: OMS Version 1.2, SAS software (SAS Institute), and Microsoft Access are being used for data collection and analysis. Fields and related vocabulary terms were identified in the OMS core application. Supplemental data tables were created to reflect desired case surveillance data based upon the World Health Organization case investigation form. Information related to contact investigation and monitoring, specific healthcare exposures, isolation/quarantine, laboratory data, prophylaxis and treatment information were also incorporated. SAS and Microsoft Access are being used to create reports and daily investigation follow-up activities. Results: The OMS package allows information on cases and contacts to be entered. Follow-up triggers can be established, investigation activities recorded, and relationships and exposures to persons, birds/poultry, locations, and conveyances can be tracked. Data entry is logical and user friendly and integration with SAS and Access allow for reports and analyses to be conducted with ease, accuracy and precision. Conclusions: The methodology described allows for cases and contacts to be tracked during pandemic alert period. Lessons learned from Severe Acute Respiratory Syndrome (SARS) were incorporated, as well as experience gained in working with OMS for tuberculosis. OMS helps ensure that identified infectious persons do not get lost to follow-up. Creation of a standardized package allows structured data collection for analysis and communication across jurisdictions.

Abstract P302

UHN Pandemic Planning Project

S Roberts

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Executive Summary: The University Health Network (UHN) is made up of the Toronto General Hospital (TGH), the Toronto Western Hospital (TWH) and the Princess Margaret Hospital (PMH). As the largest Toronto Hospital providing Acute Care Services it is the main Hospitals that will provide front-line services and be impacted by the onset of a Pandemic Influenza outbreak. The Shared Information Management Services (SIMS) is a Partnership comprised of information management and information technology (IM/IT) services across several healthcare organizations in Toronto, Canada, to create a shared technology service that supports the health-care information needs of the respective organizations. This partnership creates a single information management group, focusing on electronically improving the coordination of health-care services. The Project Management Office (PMO) provides project management expertise to SIMS and oversees project tracking and approval functions to ensure that all project implementation efforts are timely, appropriate and financially responsible. The UHN Pandemic Influenza Task Force approached SIMS to provide IM/IT solutions to support their initiatives. The three (3) areas of focus were Project 1: Anti-virals Distribution and Dispensing, Project 2: Human Resources Redeployment and Project 3: Patient Management and Triage. The unavailability of resources from SIMS Technical Teams and PMO, also budget constraints required creative solutions to minimize extending the use of resources on this Project. Therefore leveraging on existing systems was vital to the buy-in of these key stakeholders. We identified IT systems solutions for Projects 1 and 2 as follows: 1. Anti-virals Distribution and Dispensing - upgrade current GE Centricity Pharmacy system: (a) Additional new Facility/Clinic to be explicitly used by Occupational Health and Safety (OHS) to track and monitor Anti-virals Dispensing to Staff across multiple sites and locations; (b) Centricity Import functionality and processing of the Security Card Access File to facilitate Staff authentication using UHN Badge ID (unique card number) as the Medical Record Number (MRN#); (c) Ongoing reporting and use for Infection Prevention and Control events and annual Flu Shot Campaign by Occupational Health and Safety (OHS) Nurses. 2. Human Resources (HR) Redeployment - upgrade current Nursing Resources Team Scheduling System (NRT): (a) Migrate system to Corporate Intranet servers for HP Support and Maintenance; (b) Upgrade and expand NRT system for use by the UHN Staff Redeployment Centre (RDC); (c) Ongoing reporting and use for redeployment of Staff and daily NRT assignments by HR and Nursing Information Systems (NIS) respectively. 3. Patient Management and Triage - Ministry of

Health and Long Term Care (MOHLTC) reporting requirements not defined. Working Group decision to use current UHN Emergency Department processes. During the Project Lifecycle, the management of: stakeholder expectations, relationship management, communications, negotiating clarity of scope, involvement of resources, discreet leadership and direction were critical success factors requiring constant vigilance and effort to keep all aspects of the Project moving forward. Successes January 31 - February 01, 2007: 1. Upgraded Centricity Pharmacy application with the capability to import UHN badge information for 11,000 staff, dispense drugs throughout UHN from 18 kiosks and report on the distribution of 600,000 anti-viral drugs. 2. Expanded the architecture and upgraded the Nursing Resources Team application to capture, monitor, track and facilitate the redeployment of 11,000 Employees and Staff throughout UHN.

Abstract P303

Food Markets With Live Birds as Source of Avian Influenza

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It has been reported that 5 patients with H5N1 infection but no history of exposure to sick or dead birds before the onset of the disease in People Republic of China. Further investigations found that 4 of these 5 patients had visited live poultry about 1-2 weeks before the disease onset. To understand the possible role of the poultry markets in transmission of H5N1 avian influenza to human, we investigated 9 markets in Guangzhou, where one of these patients had visited. Only 1 of 79 wire cages samples but none of 94 anal swabs from live birds was positive for HPAI H5N1 from the markets. Phylogenetic analysis suggested that viral hemagglutinin (HA) and neuramidase (NA) genes were very close to those identified from the patient in revolutionary relationships. One of 110 persons in the poultry business at the markets had neutralizing antibody against H5N1 virus strain whose titers against A/Hong Kong/486/97/H5N1 and A/Vietnam/1194/04/H5N1 were 1,280 and 640, respectively. This study suggests that food markets with live poultry may be a source for avian influenza infection in which healthy live birds may carry the virus. The practice of selling live birds directly to consumers in food markets should be discouraged in areas currently experiencing influenza outbreaks among birds.

Abstract P304

Global Response; Early International Stockpile Efforts

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Background: This Poster will discuss findings from our work with three International Influenza stockpiles in 2005-2006. Technical assistance was provided for stockpiling efforts in three countries. Each situation focused on different needs with unique strategies. While recognizing the complexities associated with the global response to a Pandemic are significant, the author will highlight the differences between each of these examples; National, Regional, and Sub-regional stockpiles. This poster will assess each stockpile's strengths and weaknesses. Discussion will include: Identifying items for stockpiling; Distribution plans; Authority/Chain of Command; and International Coordination. Methods: An analysis will be conducted on the findings from the work done in Thailand, Vietnam, and Kenya. Our review will include: 1) specific strategies employed; 2) scope of materials; 3) funding; and 4) geographical coverage. Conclusion: Stockpiling is a program, not a purchase. Stockpiling is an important component of the strategy for containment and control to reduce the threat to human health posed by viruses with pandemic potential. A collaborative approach is necessary to build sustainable influenza response capacity.

Abstract P305

Zoonotic Influenza: Agricultural Workers at High Risk of Infection

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A series of recent cross-sectional studies have demonstrated that persons whose occupations involve exposure to swine and poultry may be at increased risk of zoonotic influenza virus infection. For instance, US swine confinement workers had an increased odds (OR=30.3, 95% CI-3.8-243.5) of having elevated antibodies against swine H1N1 influenza virus compared to non-swine exposed controls. Similarly, US veterinarians who reported working with poultry or waterfowl had an increased odds (OR=17.7, 95% CI=2.3-Infinity) of having elevated antibody against H7 avian influenza virus

compared to non-poultry exposed veterinarian controls. A recent large prospective study of 805 US swine workers has corroborated these studies with swine-exposed subjects (OR=54.9, 95% CI=13.0-232.6) and their non-swine exposed spouses (OR=28.2, 95% CI=6.1-130.1) having an increased adjusted odds of elevated antibody to swine H1N1 influenza virus compared to non-exposed controls. The swine-exposed workers also had prospective evidence of swine influenza virus infection through self-reported influenza-like illness data, comparisons of enrollment and follow-up sera, and the isolation of a reassortant swine H1N1 virus from an ill swine farmer. Such occupations may serve as a bridging population for influenza virus spread between animals and man. Recent influenza modeling data have demonstrated that the intense occupational exposures of humans working in swine or poultry confinement buildings could facilitate the generation of novel influenza viruses, as well as greatly accelerate human influenza epidemics. This presentation will review such research and posit that swine and poultry workers should be considered for priority receipt of pandemic vaccines and antivirals.

Abstract P306

Barriers to the State and Local Public Heatlh Management of Pandemic Influenza

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Since 2001, the United States has dedicated significant financial and personnel resources preparing the public health system for the next bio-emergency, including pandemic influenza. However, pandemic influenza 'fatigue' is spreading across the nation, resulting in less focused attention to planning efforts on the part of both the medical community and the public. Despite significant progress made in pandemic planning, considerable challenges remain in our collaborative response to an infectious disease crisis, including: the nonspecific influenza like illness (ILI) clinical syndrome and resultant issues in diagnosis; the excessive number of clinical specimens anticipated to be submitted for laboratory diagnostics; shortages or unavailability of laboratory reagents; surge capacity of laboratory personnel and equipment and inadequacies in the clinical specimen transport system. Specific problem areas requiring continued attention include real time access to rapid diagnostic laboratory tests; standardized surveillance strategies for the severe end of the spectrum of ILI infection including hospitalized patients and deaths; adherence to vaccine priority group recommendations; shortages/distribution problems in vaccine supplies; implementing antiviral treatment and prophylaxis recommendations and the public health management of such a crisis, including isolation, quarantine and other community mitigation strategies. Other anticipated problems include issues with risk communication; adequate essential services/first responder staffing and public cooperation and understanding of public health recommendations. Although there are many reasons to applaud the hard work dedicated to planning efforts to date, response to other crisis, including natural disasters within the recent past, suggest that the system may not fare as well as one would hope in the face of an influenza pandemic. Many of the unresolved problems are generic in scope, and should be considered in dual preparedness planning efforts. Pandemic planning involves collaboration with new partners, with the potential for more attention focused on the control of seasonal influenza and an improved response to other public health emergencies.

Abstract P307

Development Challenges for Oseltamivir in Pandemic Influenza

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Oseltamivir (Tamiflu), an oral neuraminidase inhibitor, has been stockpiled by around 80 governments to assist the management of an influenza pandemic. It inhibits viral replication and significantly reduces both the duration and severity of seasonal influenza symptoms and the emergence of secondary complications, especially when administered early. Oseltamivir is effective against all influenza A and B strains so far tested in preclinical studies, including H5N1 (avian) influenza, and WHO guidelines strongly recommend oseltamivir for the treatment of individuals with avian influenza infection and the prophylaxis of their close contacts. To ensure the availability of oseltamivir in a pandemic, stockpiling at global and regional levels (WHO administered) and at a national level is necessary. However, this requires an amount of oseltamivir that is several orders of magnitude greater than that provided by the manufacturing capacity originally established to fulfill the seasonal demand. The drug's license holder, F. Hoffmann-La Roche Ltd, has significantly increased its own manufacturing capacity and has also appointed manufacturing partners to rapidly increase production capacity, thereby providing a stockpiling supply for the WHO and local governments. In this way, the production capacity for oseltamivir has been expanded from an average annual capacity of approximately 8 million treatment courses (1999–2003) to approximately 400 million treatments per annum. Roche is on track to fulfill all orders received from the WHO and local governments for pandemic stockpiles by the end of Q1 2007. Current dosing recommendations for oseltamivir are based on efficacy and

safety data collected during seasonal outbreaks only. Since data on the treatment of clinical cases of H5N1 with oseltamivir are currently limited, further studies are crucial to ensure that the treatment and prophylaxis regimens for oseltamivir are optimal for control of the eventual pandemic strain. These studies will aim to produce models that can rapidly predict what changes in dosing regimens are needed for a newly emerging pandemic strain, by assessing its virulence and pathogenicity and its susceptibility to oseltamivir at different dose levels and durations. These models will be validated and refined using clinical data systematically collected from individuals infected with H5N1 and treated with oseltamivir. Outcomes will rapidly indicate what changes in dosing regimens, if any, are needed for a newly emerging pandemic strain. The processes for collecting and reporting safety data on oseltamivir during a pandemic are also defined in an internal pharmacovigilance plan. The possible emergence of resistance of the pandemic strain to oseltamivir will be closely monitored and the activity of oseltamivir when used in combination with other drugs (e.g. amantadine) will be explored. New pediatric formulations of oseltamivir are also under development, such as extemporaneous preparations and smaller capsules. Details of these ongoing development activities will be presented in more detail.

Abstract P308

Laboratory Surveillance in Russia at Phase 3 of Influenza Pandemic

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Epizootic of influenza A (H5N1) in Russia, Ukraine, Azerbaijan and many countries of South East Asia, Europe, Near East, Africa continued to be reported. This infection has become enzootic in certain areas of the world. Human infection resulting from direct contact with infected birds continued to be registered in affected countries. Separate clusters human – to human transmission were revealed in Indonesia where the highest case fatality rate was registered. Current global situation determined by WHO as Phase 3 (Pandemic Alert) could be evaluated as growing public health threat. Necessity to monitor the situation closely using both local and regional epidemiological analysis and laboratory surveillance has appeared. Laboratory tests for influenza A(H5N1) surveillance in Russia include virus isolation (BSL-3 condition), direct immunofluorescent assay with subtype specific monoclonal antibodies to HA1 of H5N1 virus and RT-PCR with following genome sequencing. Hemagglutination inhibition and microneutralisation test has been used in serological surveillance for avian influenza by detection of antibodies in sera from the people of high risk groups.

Abstract P309

Preparing for Human Cases of Avian Influenza in Canada

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Goal/objective: With the goal of increasing preparedness for outbreaks of avian influenza (AI) in Canada, the Public Health Agency of Canada (PHAC) developed a guidance document in collaboration with the Canadian Food Inspection Agency (CFIA) which could be used at the time of an AI outbreak, but also in advance of an outbreak to inform policy decisions and specific preparedness activities. Methods: Collaboration between animal and human health authorities was vital to the development of recommendations and securing supplies aimed at streamlining the response to any future outbreaks of AI in Canada. The guidance document, developed as a result of this collaborative process and engagement of the Pandemic Influenza Committee, is focused on the human health issues related to domestic outbreaks of AI and captures lessons learned from the H7N3 outbreak in the province of British Columbia. The document includes recommended actions for any future outbreaks using a risk assessment approach and is intended to improve public health readiness across all Canadian jurisdictions. Results: The guidance document, Human Health Issues Related to Avian Influenza in Canada, was posted on the PHAC website in 2006 and promoted to partners and stakeholders during education sessions addressing AI response activities. The document includes the roles and responsibilities of various responders, identifies response activities for a number of potential avian influenza threats, and provides practical tools aimed at fostering an efficient and rapid response. This collaborative effort resulted in: enhanced preparedness for surveillance of human cases, a recommendation for annual influenza immunization of CFIA workers (in line with the National Advisory Committee on Immunization recommendation for annual immunization of cullers); creation of a central antiviral stockpile, increased stocks of personal protective equipment and other supplies, and improved communication amongst partners to support a rapid response to AI in Canada. Conclusions: These activities have improved the level of preparedness in Canada. The process of developing the guidance document has resulted in implementation of important proactive recommendations and enhanced federal infrastructure to support outbreak response. This process has also fostered ongoing discussions regarding challenging issues and identified gaps that can now be the focus of next steps.

Abstract P310

A Review of the Scientific Evidence on Modes of Transmission of Influenza

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Background: Pandemic planning for infection control in health care settings, in particular the use of Personal Protective Equipment health care workers should be based in how influenza is transmitted. Despite having a longstanding presence in our population, significant controversy remains whether influenza is transmitted through the droplet or through the aerosol route. Purpose: This research investigates the relative importance of the droplet and aerosol routes of transmission of influenza. Methods: The scientific evidence on routes of transmission was examined along two lines of inquiry: (1) A systematic review of the scientific literature on aerosol spread of influenza. A search strategy was developed and executed in order to identify all published English language studies that provided some suggestion of spread of influenza via the aerosol route. Studies were categorized by whether they came from an observational or experimental setting and assessed according to pre-established criteria. (2) Analysis of the long term spread of influenza in our populations. Patterns of spread of influenza were compared to varicella, a well-established aerosol-transmitted virus with respect to the two main indicators of aerosol spread infection: high attack rates and transmission in the absence of close contact. Results: Twelve studies that provided some suggestion of spread of influenza via the aerosol route were identified and evaluated. Key limitations of these studies in establishing aerosol transmission were large inconsistencies between experimental settings and natural transmission, and a failure to exclude transmission via droplet route. Household attack rates of influenza, estimated from six antiviral randomized control trials ranged from 14-19% and contrast sharply to those observed for varicella [61-100%]. While transmission of varicella in absence of close contact has been documented in the hospital setting, this hallmark characteristic of aerosol-spread infection has not been observed for influenza. Conclusions: Taken together, this evidence provides no proof that influenza is transmitted through aerosols and demonstrates transmission more consistent with the droplet route. The infection control measures for aerosol transmission of influenza, including the use of N95 respirators, are not supported by the scientific evidence.

Abstract P311

Public Health Response to an Outbreak of Low Pathogenicity H7N3 Avian Influenza in UK, Including an Associated Case of Human Conjunctivitis

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On 26 April 2006, veterinary authorities in the United Kingdom (UK) reported an outbreak of avian influenza type A/H7 among a housed poultry flock in Dereham, Norfolk. All birds on the farm were culled, and restrictions were applied. The virus was subsequently confirmed as low pathogenic avian influenza (LPAI) H7N3 virus. Two further free range poultry farms showed serological evidence of previous infection with H7N3 and these flocks were also culled. Precautionary action taken at an early stage, consisted of risk assessment of human exposure, monitoring of exposed individuals and offer of interventions to prevent and treat human infection, prevent further spread and limit the possibility of viral reassortment. These included prophylaxis and treatment with oseltamivir and offer of seasonal influenza vaccine where indicated. Testing for serological evidence of exposure to influenza A/H7N3 is now completed. One human case of conjunctivitis connected with the infected premises was confirmed due to influenza A/H7N3. The patient was treated with oseltamivir and, along with one close contact, was followed up for the next 7 days. Neither developed new symptoms. A number of other people associated with the incident developed eye symptoms and influenza like illness (ILI); all were followed up for evidence of influenza A/H7N3 infection where indicated. None were positive. We will describe the public health response to a local incident which had significant national ramifications, and detail how the different divisions of the Health Protection Agency (at local and national levels), the UK Department for the Environment, Food and Rural Affairs (Defra), the UK State Veterinary Service and the UK Department of Health worked together to manage the incident. The implications and lessons identified will be presented.

Abstract P312

Serological Surveillance of Low Pathogenic Avian Influenza (H5N2) Antibodies Among Poultry-related Workers in Taiwan During 2005-2006

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Since 2003, highly pathogenic avian influenza (HPAI) H5N1 viruses have been widespread and endemic in many countries, especially Southeast Asian including China, Indonesia, Vietnam and Thailand and also have caused about 60% mortality of human cases in the world. There had some evidences to show that avian influenza viruses led to asymptomatic infection in humans by unintentional contacts during daily work exposures. Moreover, occupational exposure was well-known as a potential risk factor for acquiring the infection by avian influenza viruses since the first human highly pathogenic avian influenza outbreak in Hong Kong in 1997. To evaluate the possible transmission of low pathogenic avian influenza (LPAI) viruses from domestic poultry to human, a serological surveillance was performed in Taiwan. We collected total 308 serum samples from 299 poultry-related workers (206 males and 93 females, 9 paired sera) from October of 2005 to April of 2006. A modified hemagglutinin inhibition (HI) test described by Meijer was used to measure the HI antibodies against the low pathogenic avian influenza (H5N2) virus among poultry-related workers, including live-bird market workers. Questionnaire including demographic information, exposure history, travel history, protection measures and flu vaccination was delivered. Our results showed that 60 out of 308 sera had their serotiters above 1:40 against the Taiwan's low pathogenic avian influenza (H5N2) virus, and the seroprevalence rate of these poultry-related workers were 19.48%. All positive samples will be confirmed by micro-neutralization assay and/or western blot and the work is in progress. In conclusion, this is the first report that we found high seroprevalence rate of low pathogenic avian influenza (H5N2) virus in poultry-related workers in Taiwan, very different results from our 2003-2004 findings that all the tested samples including those who involved to slaughter many chickens infected with the 2003-2004's LPAI H5N2 and those who dealt with red-face ducks infected with HPAI H5N1 smuggling from China to Kinmen islet in Taiwan area were all seronegative. Therefore, continuous serological monitoring the possible inter-species transmission in high risk populations who are working at interface between animals and humans is very crucial in public health.

Abstract P313

Development of a Real-time Database for Tracking the Emerging Epidemiology of H5N1 in Humans

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If the current threat to human health from highly pathogenic avian influenza (HPAI) A/H5N1 progresses from WHO pandemic alert phase 3 through Phases 4 and 5 to a pandemic, there will be a need to rapidly identify cases and describe the changing epidemiology of the virus in humans. In order to meet these challenges we have developed a publicly funded, UK-wide, web-enabled data capture system using ASP.NET and SQL Server. The UK avian influenza database (AIDB) is designed for operation by local public health units and collation of these data by national centres in each of the four countries within the UK. Accurate and timely daily summary information can then be fed to central government in a coordinated manner. Selected data will also be used to drive real-time modelling of an emerging pandemic in the early stages of WHO alert phase 6. The database captures information such as: patient details; presenting illness; epidemiological history; medical history; clinical condition; travel and transport history; interaction with health services; diagnostic test results; antiviral therapy; and linked details of close human contacts. Additionally, this is not just a tool for capturing relevant epidemiological information but the report generating functions will enable case management and follow up on a daily basis. An environmental section of the database has also been developed, which will provide a means of capturing the details of persons potentially exposed during the response to outbreaks of avian influenza in birds, e.g. the recent HPAI A/H5N1 outbreak in turkeys in Suffolk, UK. This may well be the first operational system of its kind, specifically designed for capturing epidemiological data on avian influenza in humans. It has the potential for wider deployment outside the UK. The system will be presented and described.

Abstract P314

Case Investigation of Suspected Human Cases of Avian Influenza in Tieviessou, Grend Lahou - Cote d'Ivoire

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Background: The growing epizootic and increasing number of human cases of Avian Influenza A (H5N1) has caused worldwide concern about a pandemic. Ivory Coast experienced its first case of H5N1 in poultry in April 2006. On 6 December 2006, clinicians at PHCI (Plantations et Huileries de Cote d'Ivoire)-Tieviessou observed abnormally high rates of patients presenting to the clinic with influenza-like symptoms (ILI).Patients' history revealed the occurrence of deaths among chickens in the village during the previous month. On 8 December 2006, the Medical Practitioner at PHCI informed the animal health authorities in Abidjan about the reported chicken die-offs. One epidemiologist from CDC-Ivory Coast, one epidemiologist from INHP, and one laboratorian from Instiut Pasteur went to Tieviessou on 9 December 2006 to investigate. Methods: We interviewed the health workers of the PHCI medical care unit and consulted the medical record book in order to identify patients with ILI. We collected throat and nasal swabs from all patients who consulted with ILI on the day of the investigation, and from some of the patients who had consulted with ILI on the previous day. Samples were sent to the Institut Pasteur laboratory and tested for Avian Influenza by ELISA and PCR. Additionally, we assessed the state of backyard poultry in the village. Results: In all, 9 patients with ILI were identified in the hospital. Oral and nasal swabs were collected from all 9 patients. One sample tested positive for Influenza A but negative for H5, and one sample tested positive for influenza B according to ELISA. After PCR, we had 6 samples positive for the M gene. The village investigation revealed that at least 3 backyard poultry had died. Villagers were instructed to immediately report any cases of sick or dead chickens to the animal health authorities and to avoid eating sick or dead chickens. Conclusion: Avian Influenza is and remains a great challenge in Ivory Coast and in other African poor resource settings with poor human and animal surveillance systems. The deployment of a multisectorial rapid response team can be an effective approach to investigate suspected outbreaks in humans and animals.

Abstract P315

Serology, Pathogenicity and Molecular Characterization of the H5N1 Influenza Virus Isolated from the Human Case in Shenzhen

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Background: Highly pathogenic avian influenza (HPAI) virus H5N1 has not only spread rapidly in domestic poultry, but also crossed the species barrier and transmitted to mammals, including humans HPAI H5N1virus caused outbreak wave in poultry in China during the season of 2005 to 2006, resulting in total of 22 cases of Human infections in 14 provinces of China. In this study, Serology, pathogenicity and molecular characterization analysis of the H5N1 virus isolated from the human case in Shenzhen were conducted. Methods: The changes of virus copies in tracheal aspirate specimens and the level of antibody of the infected man were tested by real-time RT-PCR and microneutralization, during the treatment period. The antigenic ratios of the H5N1 isolates were performed by HI assay. All the eight segments also had been sequenced. Results: the HI titration of antibody in sera increased from 1:40 to 1:160 during the first three weeks from onset, then, decreased to 1:80. At the same time, the virus copies decreased from2.30E+06 to 6.55E+03. Compared to the two representative H5N1viruses (A/Anhui/1/2005, A/chick/LNHS-JHL/23/2005), both of the antigenic ratios to this H5N1 isolates were 1.4. According to the sequences analysis, a new potential glycosylation site emerged at the Asn 154 residues in HA molecule. In PB2 molecule the Glu 627 Lys mutation had been detected. Conclusions: the pathogenic and serologic tests conformed the relationship between the antibody titration and virus copies of the infected man. Some minor changes were detected in the H5N1 isolates, compared with the strains isolated from Vietnam, Thailand, Indonesia and Hong Kong in 1997.

Abstract P316

NAMRU-3 Outbreak Support Capacity and Response

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The U.S. Naval Medical Research Unit No. 3 (NAMRU-3) serves as the World Health Organization-Eastern Mediterranean Region Influenza Reference Laboratory. As such, NAMRU-3 has developed outbreak support platforms to project diagnostic capacity to countries within Africa, the Middle East, and the Central Asian Republics. As a member of the World Health Organization Global Outbreak Alert and Response Network, NAMRU-3 is continually poised to provide support to those countries in need. NAMRU-3 is comprised of diverse scientific staff (i.e, physicians, veterinarians, epidemiologists, microbiologists, etc) which allows for a country-specific response based on individual scenarios. In addition to personnel, NAMRU-3 deploys diagnostic supplies, personal protective equipment, and the Idaho Technology Incorporated Ruggedized Advanced Pathogen Identification Device (RAPID) real-time polymerase chain reaction (RT-PCR) platform as it has proven reliable in harsh environments. From January 2006 through February 2007, NAMRU-3 has supported 16 outbreak investigations with varying levels of support. Most noteworthy was a three week response mission in Azerbaijan beginning in March 2006 resulting in the rapid identification of five positive H5N1 cases as judged by RT-PCR. In addition to avian influenza, NAMRU-3 provides on site diagnostics for seasonal influenza outbreaks such as was experienced in Jordan in November of 2006. Over the course of two months, NAMRU-3 trained Jordanians and collected 138 specimens resulting in 24 (17%) influenza isolates all typed as Influenza B (B/OHIO/01/2005-LIKE). NAMRU-3 is positioned centrally in a region of the world that has limited advances in terms of laboratory diagnostic infrastructure. Nevertheless, there is a clear need for support as highly pathogenic avian influenza continues to devastate the poultry industry, pose a health risk to exposed humans, and elevate the chances of development into an eventual pandemic strain. By providing outbreak support, NAMRU-3 has been able to readily identify the etiological agent while simultaneously providing training, biosafety upgrades, assessments of preparedness, and specimen collection kits. These initiatives are geared to initiate sustainable surveillance systems utilizing NAMRU-3 as a regional reference lab. Efforts such as these should serve as a model for other capable laboratories worldwide.

Abstract P317

FLUSECURE Towards Sufficiency of Pandemic Influenza Vaccines in the EU

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FLUSECURE represents a network of 10 European public health institutions, with the aim to support an efficient production chain of sufficient pandemic influenza vaccines in collaboration with the European vaccine manufacturers. The project primarily focuses on shortening the lead time necessary for the production of pandemic influenza vaccines in case of a pandemic. The major objectives of this network include: establishment of a library of new vaccine seed stocks; rapid development and production; cross protection studies; comparative adjuvants testing and vaccine formulation optimization; development of new correlates of protection; development of a trial network of clinical study centers throughout Europe. This project has been made possible by grants from DG-SANCO and contributions from each of the participating European member states.

Abstract P318

Influenza Vaccine Production and Usage on a Global Scale

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Each year over 300 million doses of influenza vaccine are produced for seasonal influenza prophylaxis, most of the vaccine being used in the developed world. A number of studies have been carried out over recent year tracking the policy of nations towards influenza vaccination and the use of vaccine by countries. Most of this work has concentrated on developed and rapidly emergent economies, little is known about those less affluent nations that are already struggling with war, famine, HIV, TB and Malaria. In these poor countries the majority of the population are below 30 years of age and have little or no antibodies to potential emergent influenza viruses. The presentation will give the early results of work we are carrying out in trying to quantify policies, procedures and use, with regard to influenza and pneumococcal vaccine, in the developing world and report on preparations, if any, they may be making to minimise the impact of a potential pandemic.

Abstract P319

Preventive Action By Influenza Vaccination Campaigns for All workers in Poultry Farms in Cuba

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Background: The emergence of new H5N1 in Hong Kong in 1997, plus H7 N7 in the Netherlands has also been reported. Concern has been raised regarding the possibility that a pandemic influenza strain may emerge through human-avian gene reassortment within workers directly involved in culling operations, who may become simultaneously infected with a human influenza virus strain and an avian influenza virus strain. The trivalent influenza vaccine immunization may prevent the infection of these individuals (staff working directly in poultry farms) with human influenza strains, thereby reducing the potential for human-avian reassortment of genes should they become coinfected with avian influenza during the culling operation. For this reason in our country, the Ministry of Health increased the immunization against influenza in all workers from 6 poultry farms in different cities of Cuba. Materials and Methods: This study was carried out during the 2004-2005 season. All workers were vaccinated (formulation recommended by WHO for this season). The search was done by antibody titration before vaccination and 1 month after by hemagglutination inhibition test (HI)with inactivated antigens H3, H1, B and AH5, AH7 and AH9 too. Statistical analysis was made using the Student´s test. Results: Protecting antibody titres (≥40) detected for the subtype H3N2 and H1N1 were between 22% and 34% before vaccination and were 75%; 94% afterwards. For type B 11% (before) and 49% (after) vaccine. On the other hand, neither workers had antibody titer against the flu as AH5, AH7 and AH9. GMTs obtained before and after vaccination showed a significant difference ($p<0,01$) for the study strain. Conclusion: The vaccine (one dose) achieved satisfactory levels of antibodies for the strains H3N2, H1N1 and type B. Future studies are needed.

Abstract P320

Severe Acute Respiratory Syndrome By Influenza AH1N1 With Complications and Deaths in Non-Vaccinated Elderly Population Against the Impact of Effectiveness of Influenza Vaccine in Elderly Home Care in Holguín City. Cuba

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Background: From June to July, 2001 a severe outbreak of acute respiratory illness occurred in the city of Holguín (eastern part of Cuba). It was detected by the active surveillance system. In this paper, we focused our attention on the emergence of this outbreak of influenza A H1N1 and its impact on elderly population. The outbreak was very severe between people non-vaccinated with old age (≥ 70 years). The patients presenting with serious complications and a poor evolution, were more likely to die. However, neither acute cases for flu were detected in the elderly home care, where all personnel was vaccinated. Methods: Indirect immunofluorescence; Immunoperoxidase staining; and molecular methods were used. Culture was in MDCK cells and chicken eggs and hemagglutination inhibition was used for antigenic characterization as well as serologic studies. Results: Fourteen cases (82,4%) were positive by IFI; ten cases(66,7%) were positive with IPS.Type and subtype A(H1N1) were demonstrated by RT-PCR, too. In culture, influenza virus was isolated from 23,52 % of the collected samples. Four hemagglutinating agents were characterized antigenically and genetically related to the reference strain A/New Caledonia/20/99 (H1N1). In the community, the serological studies by IH showed low levels of antibody for the strains AH1N1(2,9%). However, in the elderly home care the vaccination(one dose) achieved satisfactory levels of antibodies(more than 95 %). These findings suggest a significant vaccine protection against A/New Caledonia/20/99(H1N1). Conclusions: This study shows that vaccination is the key measure for preventing influenza and for reducing impact of epidemic.

Abstract P321

Evidence for Avian Influenza A Infections Among Iowa's Agricultural Workers

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In 2004 we enrolled 803 Agricultural Health Study (AHS) cohort members in a 2-year prospective study of zoonotic influenza transmission. The study involved the collection of sera and animal exposure data upon enrollment and at 12 and 24 months. In 2006, we also collected similar cross-sectional data and sera from 66 University of Iowa employees and students (university controls) who denied any poultry exposures. Sera were studied with microneutralization assays against H4, H5, H6, H7, and H9 avian influenza viruses and hemagglutinin inhibition assays against 3 human influenza viruses. Participants (n=869) were 56.7% male and had a mean age of 54.6 yrs. Many participants had poultry exposures: 58.2% reported some exposure to birds either through poultry production, hunting, or handling poultry sometime in their lifetimes; 16.1% had worked with poultry since the year 2000; and 15.3% reporting hunting wild game birds. Bivariate and multivariable modeling was used to examine potential risk factors for association with elevated enrollment sera antibody titers against the individual viruses. In multivariable modeling age, gender, receipt of influenza vaccines, and elevated antibodies to human influenza viruses were included when statistically important. By proportional odds modeling, subjects who self-reported hunting wild birds or recently working with poultry had elevated adjusted odds of increased antibody titer against avian H7 virus (OR=2.8,95%CI 1.2-6.5; OR=2.5,95%CI=1.1-5.8) While titers were too sparsely distributed to run proportional odds modeling for H5 virus, a traditional exact logistical regression model for titers ≥ 1:10 revealed that AHS members were at increased odds (OR=9.2; 95%CI=1.6-infinity) of elevated titers compared to university controls. None of the poultry exposures were clearly associated with elevated antibody titers to H4, H6, or H9 viruses. Examining the serial serum samples revealed little evidence of an increase in antibody titer over time for any of the viruses. Hunting and poultry exposure may be important risk factors for avian influenza virus infection among agricultural workers.

Abstract P322

Rapid Community Vaccine Distribution Targeting Hard-to-Reach Populations in New York City

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Annual influenza vaccination rates in the U.S. among underserved racial/ethnic minority groups and other hard-to-reach (HTR) populations living in urban areas (such as undocumented immigrants, homebound elderly, substance users) has been disappointing. In the event of an influenza pandemic, gaining early and rapid access to immunize these groups, especially those in high risk categories, will be a challenge. Effective programs to rapidly vaccinate HTR populations are urgently needed. Project VIVA, a four-year community-based intervention, aimed to deliver vaccines rapidly within the East Harlem and South Bronx neighborhoods of New York City (NYC). The project was also designed to test the feasibility of a rapid distribution protocol that could be utilized in the event of an emergency. Public health nurses and outreach workers did community outreach education (with a survey) and then returned to offer free influenza vaccinations door-to-door and on street corners over ten days in October, 2005. Reactions to the project were positive in the local communities and participants formed long lines to receive the vaccine. Of 1,668 participants screened, 99% (1,648) received vaccine; 58% of the vaccine was distributed on street corners. Among those vaccinated, the mean age was 46; 52% were female, 76% Hispanic/Latino and 77% reported income of <$9,600/yr. Overall, 47% were members of a HTR population and 38% were medically-indicated to receive influenza vaccine. Compared to those surveyed during the baseline assessment (n = 1,077), those vaccinated were more likely to be: undocumented immigrants (p<0.0001), homebound elderly (p<0.0001) or uninsured (p<0.0001), but were less likely to be homeless (p=0.03), or current substance users (p<0.0001). Factors key to project success were: selection of staff with complementary research skills and firsthand knowledge of the communities, community involvement in project development and implementation, ongoing community outreach, and prioritizing street-based distribution. To prepare urban communities for a possible influenza pandemic, instituting annual mass vaccination efforts in non-traditional settings is recommended. These results demonstrate that vaccination can feasibly be delivered to members of hard-to-reach populations through a community-based approach.

Abstract P323

Preparing Responders for Avian/ Pandemic Influenza: An Update on the CDC Influenza Division's International and Domestic Rapid Response Training Activities

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Background: The circulation of avian influenza A (H5N1) among domestic and wild birds in Asia, Africa, and Europe has highlighted the critical need to train Rapid Response Teams (RRT) of public health practitioners from countries affected by these outbreaks. Methods: CDC's method to address this need focuses on developing training material and providing training courses to three different audiences: 1) international public health responders, 2) domestic public health responders within U.S. States and Territories, and 3) CDC internal staff. Human and veterinary epidemiologists are included in the training. Results: In July 2006, the CDC's International Emerging Infections Program in Thailand, together with WHO and regional ministries of health, unveiled the first ever Rapid Response Training curriculum to 14 countries. As a result, over 1,000 local Rapid Response Teams have been formed in Thailand, Cambodia, Indonesia, Vietnam and Burma using locally modified versions of this CDC-sponsored curriculum. CDC's Influenza Division and WHO staff have also completed additional trainings in Egypt, India, Kenya and Senegal. As a result, local RRTs are now being trained in over 30 countries in Africa and Asia. Within the United States, the CDC's Influenza Division provided $2 million dollars to the Council of State and Territorial Epidemiologists to co-sponsor three regional "Train the Trainer" meetings using a domestically adapted version of the RRT curriculum. These funds will be used to train local RRTs within the U.S. States and Territories during the summer of 2007. To enhance internal CDC surge capacity over 300 CDC internal staff and EIS Officers have also received standardized training. This number will increase to over 500 by January, 2008. Conclusions: International authorities agree that early detection and response are the keys to containing or slowing the spread avian and pandemic influenza. Thousands of local-level RRTs have received standardized instruction in case investigation, case management, infection control, national and international reporting procedures, and interventions to contain a pandemic worldwide. This has also had the ancillary benefit of improving coordination between animal and human health agencies domestically and internationally, and has created a coordinated RRT infrastructure that can help better respond to all infectious diseases.

Abstract P324

Review of China Pandemic Preparedness Plan

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Background: The threat of a human pandemic of influenza has prompted development of national preparedness plans. In September 2005, the Ministry of Health, China completed and issued the first draft of the China pandemic preparedness plan. In December 2006, the plan was reviewed by an international team as a preliminary step to updating it. Methods: Several assessment tools, such as the WHO Influenza Pandemic Planning Checklist and the European CDC's Assessment Tool for National Pandemic Influenza Preparedness were used. The plan was also compared to other national and community pandemic plans for content and organization. Results: Definitions for pandemic phases were not completely consistent with those defined by the World Health Organization. Although most of the basic aspects of pandemic alert and pandemic phase responses are covered, details on how the plan would be operationalized was lacking. Gaps were identified in risk communications; plans for stockpiling of personal protective equipment, laboratory reagents, antivirals, and other supplies; and plans for maintenance of essential services. Notably, the plan did not appear to be based on a formal risk assessment or estimates of the impact of a pandemic on disease burden and health and other essential services in China. Several areas of research were identified which would improve the accuracy of projections of pandemic impact. Conclusion: Pandemic planning is a dynamic and ongoing process requiring regular review and can benefit by the inclusion of international evaluators. A comprehensive pandemic preparedness plan should actively involve multiple government sectors and be based on risk assessment and projections of impact of control measures. Many of the assumptions used in these projections are based on data acquired in western Europe and North America and may not apply to Asia, indicating the need for further in-country research.

Abstract P325

Development of New Candidate H5N1 Vaccine Virus for Pre-pandemic Vaccine

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A new candidate H5N1 vaccine was developed by reverse genetics to meet the need for immunization against clade 2.3 highly pathogenic avian influenza viruses currently circulating in birds. Reassortant virus Anhui/01/2005(H5N1)-PR8-IBCDC-RG5 (Anhui/PR8) was produced in compliance with Good Laboratory Practice (GLP) specifications. The virus contains 6 internal genes from A/Puerto Rico/8/34 (PR8) and the NA and modified HA genes from A/Anhui/01/2005(H5N1). The reassortant virus was rescued by transfection of plasmid DNAs into certified Vero cells. The HA and NA genes of rescued virus are identical to those of the parental virus except for the modified HA cleavage site where 4 basic amino acid residues were removed. The reassortant virus was trypsin-dependent for the formation of plaques in chicken embryo fibroblast (CEF) cells. The reassortant virus was also evaluated in mammalian (ferrets) and avian (chickens) animal models and was confirmed to be non-pathogenic. Anhui/PR8 is suitable as a pre-pandemic vaccine candidate for subsequent clinical trials in humans.

Abstract P326

Human Antibody Response to Avian Influenza A (H7N3) Virus During an Outbreak in Poultry in Norfolk, United Kingdom

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In April 2006, an outbreak of low pathogenic avian influenza A H7N3 (Eurasian lineage) virus occurred in three poultry farms in the UK. Infection of poultry was confirmed on two farms by real time RT-PCR, virus culture and standard pathogenicity tests in vivo. Infection on the other farm was confirmed only by the presence of H7 antibody in poultry. As a result, a one kilometre radius restriction zone was set up around each farm and epidemiological tracing and surveillance carried out. No additional infected premises were identified. Virus identical in the haemagglutinin (HA) gene to one of the H7N3 poultry viruses was also isolated from four separate nasopharyngeal, throat and eye swabs of a single human index case who presented with conjunctivitis. A serological follow-up of exposed individuals was carried out, focussing on individuals potentially exposed to H7N3, either prior to identification of the infected facilities or during the clean-up process. Particular attention was paid to 10 individuals who presented with clinical respiratory illness within 7 days of possible exposure. Serological analysis on serum, taken after 28 days post-exposure, was performed using a combination of microneutralisation (MN), haemagglutination inhibition (HI) and Western blotting (WB) tests. Low levels of antibodies against H7N3 virus were detected in the convalescent serum of the index case by all three techniques. We found no evidence of seroconversion in 41 other available convalescent sera, and no evidence of H7 antibodies in several other individuals presenting with either conjunctivitis or mild respiratory symptoms at the time of the poultry outbreak. An avian paramyxovirus type 1 (Newcastle Disease Virus) was isolated from conjunctival swabs from one case of conjunctivitis, and serological rises to human seasonal influenza were also identified. This investigation suggests a low risk of transmission of H7N3 to humans, but highlights the need for surveillance of people working in close contact with poultry.

Abstract P327

Emerging Issues in the Management of the Surveillance Dataset During a Highly Pathogenic Avian Influenza A/H5N1 Outbreak in Suffolk, UK

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On 3rd February 2007, an outbreak of highly pathogenic H5N1 Avian Influenza was confirmed in a poultry farm. An essential public health intervention was to identify, describe and record all persons potentially exposed, to appropriately target actions. This was the responsibility of the local Incident Management

Team (IMT), comprising the Health Protection Agency (HPA) and local public health partners. Demographic and clinical data were collected on potentially exposed persons being offered oseltamivir prophylaxis. Initial data were recorded on a paper proforma at designated clinics, before being entered electronically onto a database. Period and level of exposure of exposed persons were sought from their employers. The dataset was used to: identify and notify exposed persons of dates and times of vaccination clinics; notify health care practitioners of those exposed; identify exposed persons requiring repeat prophylaxis; and identify those exposed before confirmation of H5N1 for follow up by telephone for 7 days. The dataset also generated timely information for national and IMT outbreak meetings. An algorithm was developed by the HPA Centre for Infections, to aid risk stratification of exposed persons in case they developed symptoms. Fewer than 50 exposed persons were anticipated at the onset of the incident but by the end of it, 482 people had been offered and received prophylaxis, and had been offered seasonal influenza vaccination. Multiple sources of information arriving piecemeal, the need to take appropriate action quickly and the requirement for up-to-date situation reports, required strong data management and highlighted the importance of accurate and readily accessible data. Emerging issues unique to this outbreak required rapid and clear response by the IMT.

Abstract P328

Investigation of Suspect Human Cases of Avian Flu in Ivory Coast From February to March 2007

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Context: The discovery of avian influenza pockets related to virus A (H5N1) in Asia sparked the installation of early warning systems to contain a possible epidemic. In this context the unexplained human cases of flu-like illness and fatal acute respiratory infection (ARI) in locales experiencing mortality of poultry evoked a strong suspicion of infection by highly pathogenic virus A (H5N1). In Ivory Coast deaths by (ARI) coincided with dies-offs on traditional poultry farms in the localities of Diobala and Facobly, found in the north and the west of the country, during February and March 2007. Investigations were carried out in these two locations. Method: The cases were actively researched by interviewing the community and reviewing the health consultation registers. This made it possible to identify the suspect cases that were sampled for blood and nasopharyngeal swabs. At the laboratory systematic review on the nasopharyngeal swabs for the M genes of the flu virus by a conventional PCR was completed. In the event of positive, a PCR multiplexing for H5, N1 was carried out. In the event of negative of this PCR, the samples were inoculated on cell line MDCK. The research of the VRS, hMPV, coronavirus 0C43, 229E, influenza C and of Parainfluenza was carried out on the negative sample for the gene M. Results: In the locality of Diobala, 15 nasopharyngeal swabs were analyzed finding two cases of infection by coronavirus human OC43 and cases of infection by influenza virus C. In Facobly on 4 samples taken and analyzed, two positive samples have gene M. the PCR multiplexing H5, N1 was negative. Conclusion: The monitoring of the influenza constitutes a priority today. We should not however forget respiratory emergence again viruses with an increased virulence.

Abstract P329

Plan for Pandemics: A Training Programme for Educators

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Background and Objective: In January 2006, the Department of Health of the Generalitat of Catalonia approved an action plan against possible pandemics in Catalonia, a region located in the northeast of Spain with more than seven million inhabitants. Based on this plan, and with the participation of several working groups of the Scientific Advisory Council, protocols for prevention of nosocomial infection, laboratory diagnoses, use of antiviral drugs, prevention in workers exposed to animals, activities in the scenario of human cases in phase 3 and in phases 4 and 5, and emergency responses were drawn up and a programme for the training of health care centre staff was elaborated. The objective of the Training Programme for Educators was to bring forth elements in order to increase the knowledge and skills of health care centre staff with respect to the Plan for Pandemics. Material and methods: A workshop on 20-hour preparedness and response to a pandemic aimed at technicians of the Epidemiological Surveillance Units of the Department of Health was carried out. A consensual agreement was reached on the materials to be delivered (Power Point and explanatory text) by a working group of the Scientific Advisory Council. Slides and accompanying text were selected for two training sessions of

one and two hours, respectively with the heads of the various territorial units of Epidemiological Surveillance. Results: In October 2006, 16 training sessions of two hours for educators were convened. These were attended by representatives of 142 health centers and long-term care facilities (80% of those invited). Lectures were given by two epidemiologists, one from a territorial unit and the other from central services. According to the complete information about sessions received by February 2007, the persons attending the aforementioned training sessions had, in turn, imparted training sessions in at least 94 health centers (53% of total health centers). Conclusions: The organization of a training programme for educators on the Plan for Pandemics, including drawing up and delivery of visual material for presentations facilitates carrying out training sessions and, thereby, the preparation of health centre staff.

Abstract P330

High Yield Reassortants of Influenza Type B Viruses: NP Gene Obtained From High Yield Donor

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One of the critical steps in producing the annual influenza vaccine is to create high yield (hy) seed viruses by reassortment. Hy reassortants of type A influenza viruses have been successfully created by our laboratory, and previously by Dr. Edwin Kilbourne's laboratory for many years (as well as other laboratories worldwide). However, no hy B reassortants could be developed for vaccine production. In the past three years, we have created eight hy reassortants of influenza type B viruses for the current circulating strains. They include NYMC's BX-3 (B/Hong Kong/1434/2002), BX-5a (B/Jilin/20/2003), BX-7 (B/Jiangsu/10/2003), BX-11 (B/Florida/7/2004), BX-15 (B/Hawaii/33/2004), BX-15a (B/Hawaii/33/2004), BX-17 (B/Malaysia/2506/2004), BX-19 (B/Ohio/01/2005). The CDC found that all reassortants were antigenically indistinguishable to the wild type (wt) parent. All reassortants were also sent to the major influenza vaccine manufacturers for consideration for use in vaccine production. In general, hy reassortants increase the virus yield by approximately 2 fold (range 0.6-16 fold). In this study, we analyzed gene composition of all hy B reassortants by RFLP analysis. All hy reassortants obtained the genes for the surface antigens (HA and NA) from their corresponding wt virus. However, in contrast to hy reassortants which require the M gene for high yield, all eight hy B reassortants obtained the NP gene from the hy donor strain (B/Lee/40). The parental source for the remaining genes varied among the hy B reassortants. The results indicate that the NP gene may be important in producing high yield growth in type B reassortant viruses.

Abstract P331

Challenges and Opportunities Arising From Cooperation Programmes Developed to Support African and Middle-Eastern Countries in the Management of the H5N1 Epidemic

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Prior to the introduction of H5N1 in the African continent, the Food and Agriculture Organization of the UN (FAO) had foreseen a series of training courses (TCP – Technical Cooperation Programmes) in Africa and in the Middle-East to improve preparedness and build capacity in areas that had never experienced such a disease. In fact, with the exception of two self limiting outbreaks in the Republic of South Africa, HPAI had never affected Africa or the Middle East. The TCP's were held in 7 regions and hosted over fifty laboratory staff from developing countries. During 2006, 22 working weeks were spent abroad by IZSVe laboratory staff in the framework of the programmes. Training was delivered to recipients with variable levels of resources, expertise, skill and dedication operating under completely different conditions. However, personal contacts between IZSVe staff and scientists belonging to recipient countries were established and resulted in a very positive outcome. This enabled the timely diagnosis of the first H5N1 outbreak in Africa, and subsequent confirmations in other countries. One of the most relevant achievements was that the training exercise increased the awareness, of developing countries as key-players in the crisis and resulted in the availability of strains for the international scientific community. Notwithstanding the overall international effort in supporting these countries, since the affected countries were lacking experience and resources, expectations on immediate implementation of diagnostic protocols could not be fulfilled by all recipient countries. This is also partly due to the fact that reliable, simple, high throughput diagnostic tests, applicable under local conditions still require improvement. Major constraints identified were the language barrier, variability in the levels of equipment of the hosting laboratory and the infrastructure required to service and manage sophisticated laboratory equipment. It appears clear

that the resolution of certain difficulties is beyond the scope of these specific programmes, however, certain identified impediments could resolved at an international level. These include, the facilitation of international transportation of suspected specimens – both at a carrier and customs level, the availability of validated rapid tests of suitable sensitivity and ensuring the availability of diagnostic reagents for serological and molecular diagnosis in the regions. Given the threat that H5N1 represents for the poultry population of Africa and the human health implications resulting from the loss of a major protein source and the pandemic potential it is imperative that a continuous effort is made to maintain the awareness and trust in scientists actively involved in veterinary diagnosis in developing countries. The international community should promote networking and joint surveillance as these are the primary components of a coordinated action against H5N1on a global scale.

Abstract P332

Phylogeny and Molecular Changes of H5N1 HPAI Viruses Circulating in African Poultry

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In early 2006 following its westward spread from East Asia, highly pathogenic avian influenza (HPAI) H5N1 subtype viruses were detected in poultry in Africa. Since then, 26 human cases have been reported in this continent (12th March 2007). Among the activities carried out by the OIE/FAO Reference Laboratory for avian influenza at the IZSVe, we received and analysed about 1,000 samples from birds collected in 13 African countries. The presence of H5N1 HPAI virus was confirmed in samples submitted from 6 of these countries. Representative isolates were characterised, fully sequenced and compared. Phylogenetically, all African isolates are closely related to the viruses that have been circulating in birds throughout Europe, Russia and Middle East since late 2005 and cluster separately from those circulating in poultry in the East Asian countries. Available sequences from the viruses isolated from African human cases in Egypt and Djibouti also fall in the same Euro-African lineage. Within this genetic lineage, minor sublineages could be identified and, in some cases, grouping correlated to the geographical origin of the isolates. Interestingly, unique amino acid changes in the haemagglutinin (HA) molecule were identified in viruses circulating in a given country or geographical area, i.e. for Sudan R329G; Egypt P239S and Ivory Coast S4G. Deduced amino acid sequence analysis revealed other interesting features which are believed to have an effect on human health implications. All African isolates from birds showed the PB2 627K mutation, related to enhanced replication in non-avian species. Furthermore, one isolate (A/duck/Egypt/452-1/2006) revealed mutation M230I in the HA gene, which was also identified from isolates obtained in human cases in the same country. This mutation is located in the receptor binding domain and may influence receptor binding affinity. Isolate A/chicken/Egypt/452-2/2007 exhibited NP V33I mutation, characteristically observed in all the H5N1 human isolates. At the time of writing, no known mutations related to adamantadine resistance have been observed in any of the African viruses obtained from birds. The results of our efforts have generated data on the genetic characteristics of African isolates, including those which are believed to influence the human health implications of this infection. Continuous support of African affected countries in diagnosing AI infections in the animal reservoir is essential to provide the international scientific community with relevant information to manage the pandemic potential and the animal health implications of this infection.

Abstract P333

Argentina's Preparations to Face Up With a Probable Influenza Pandemic

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Introduction: However we aren't able to predict when the next Influenza Pandemic would start and its severity, we can affirm that the level of preparedness of the countries will determine the seriousness of the impact. For this reason, Argentina evaluates its Public Health Norms from its "Strategic Action Plan for the Pandemic Influenza and SARS" through Simulacrums. Argentina was the first country of America in developing this kind of exercises and had realized 4 field drills from December 2005 to nowadays testing different possibilities. Objectives: To know the degree of preparedness of the Public Health Services against an eventual Influenza Pandemic; To determine corrective measures. Background: The province selection was determined by: Local Preparedness Plan develop, Emergency

Committee in activity (COE), hospital response, professional capacitation, multisectorial organisms collaboration, social communication. Setting selection: Commercial exchange services, tourism, border transit; Sanitary response resourses; Tourist center, 10 national and international daily flights. Results: On August 11th of 2006, in Puerto Iguazú, Misiones, a Simulacrum of Pandemic Influenza in Phase IV of WHO took place. International, national and local authorities were involved. The exercise started in Puerto Iguazu's Airport and continued in the local hospital. Initial Hypothesis: WHO Pandemic Phase 4: Flight arrival with 90 passengers from an endemic avian influenza country; Flight authority's notification of a passenger with respiratory symptoms in the plane; Implementation of the "Strategic Action Plan for the Pandemic Influenza and SARS"; Notification to: Dirección de Sanidad de Fronteras, Epidemiología, Emergencias Sanitarias, Migraciones; COES activation; Transfer of all the cases to the Hospital; Bordering countries communication, MERCOSUR and international organizations (PAHO); Triage setting, isolation, diagnosis and treatment in the local Hospital. Conclusions: Though field drills we are able to identify: Relevance of the preparedness plans; Weakness and strengthness of the affected population; Importance of the multidisciplinary work; Importance of the local organization and the specific roles of the actors.

Abstract P334

Canada's National Surveillance Strategy for Pandemic Influenza and Emerging Respiratory Infections Response

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Background: Lessons learned from SARS and avian influenza (H5N1) involving humans have underscored the importance of strengthening surveillance infrastructure for early detection and ongoing monitoring of emerging respiratory infections (ERI). Canada's pandemic and ERI surveillance strategy has focused on developing capacity for responding to outbreaks of severe respiratory illness (SRI) and implementation of pandemic plans. Methods: To develop Canada's national pandemic and ERI surveillance strategy a formal evaluation of the routine influenza surveillance system (FluWatch) was conducted, an SRI protocol was created, and the surveillance annex of the Canadian Pandemic Influenza Plan was developed in collaboration with Provincial and Territorial (PT) stakeholders. To assess the feasibility of the pandemic and ERI surveillance strategy, current PT surveillance plans were reviewed; minimum national data elements and reporting processes were drafted; potential data collection sites were discussed; and capacity building for front line health care professionals was planned. Results: A 3-fold early warning strategy was developed linking together routine influenza surveillance, enhanced recommendations for front line vigilance specific to evolving pandemic alert levels, and hospital-based surveillance for SRI-not yet diagnosed (SRI-NYD). Surveillance guidelines and data management tools were developed to support the strategy. Regular risk assessments are included in the early warning strategy and the 'Early Warning and Reporting' on-line curriculum targeted to front line physicians aims to build surveillance capacity. Gaps in the implementation processes were described and details for the revision of the surveillance annex were proposed. Conclusion: The development of Canada's pandemic and ERI surveillance strategy has provided an opportunity to build on current infrastructure and establish and reinforce communication links between primary care providers and public health and laboratory professionals at local/provincial/territorial and federal levels. The system is not yet seamless; ongoing consultations and evaluation of the system will be needed to ensure plans are implemented, accepted and tested and performance barriers are identified.

Abstract P335

A Cross Border Initiative for Addressing Surveillance and Laboratory Issues Related to Avian and Pandemic Influenza in North America

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Introduction: A Laboratory and Surveillance Technical Working Group was formed in 2006 between public health departments in Canada, Mexico and the United States to provide technical support for avian and pandemic influenza preparedness under the tri-national Security and Prosperity Partnership (SPP) initiative. The group aims to address laboratory, surveillance and epidemiological issues and considerations related to the development of a comprehensive, coordinated and evidence-based approach to plan for and respond to an influenza pandemic and human health issues associated with the introduction of H5N1 avian influenza virus into North America. Methods: The group is achieving key objectives through a broad set of activities, including: operational procedures and

tools for the communication of laboratory, surveillance and epidemiological information across borders; sharing H5N1 virus case definitions and surveillance approaches; exchanging information and collaborating on joint preparedness and response activities for slowing the introduction and spread of H5N1 virus into the three countries; sharing laboratory protocols and information reporting procedures; identifying areas of technical and mutual assistance, including a review of Federal-Province/Federal-State and State-Local interactions; and, reviewing the real time application of epidemiologic and laboratory information to inform and evaluate vaccine and antiviral use. Results: Communication through identified points of contact has been established and key issues are being addressed, such as facilitating the sharing of critical information and enhancing the transportation of specimens and materials across borders. Key input has been provided into the development of the North American Plan for Avian and Pandemic Influenza, to strengthen cross-border planning and response efforts. Conclusions: The work of this multi-national group will strengthen the capacity in North America to detect influenza strains of human pandemic potential and will help address the very high demand for real time epidemiologic and laboratory information needed to respond to an outbreak of pandemic influenza. This North American approach to pandemic influenza preparedness serves as a model for addressing cross-border mutual assistance arrangements, protocols, and/or agreements for responding to public health emergencies of international significance of common interest.

Abstract P336

Report on Case of Human Avian Influenza in Shenzhen, China: Application of Field Epidemiology

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Background: Shenzhen, very closed with Hongkong, has conducted an effective surveillance system for influenza. The Ministry of Health (MOH) of People's Republic of China required all local health departments to report and manage each human Highly Pathogenic Avian Influenza (HPAI) case according to Emerging Public Health Events Ordinance (EPHEO). The authors defined of filed epidemiology as: application of epidemiology theories and methods assimilating theories in communication, public relation, and science of decision-making. This paper explicated investigation on the first human HPAI in the city of Shenzhen, P.R.China to clarify the application of field epidemiology in the whole process. Objectives: To analyze characteristics of human avian flu case and to explore effects of field epidemiology application in the investigation. Methods: An emergency response system was started up to follow the probable human HAPI case detected first by the "Undefined Pneumonia Surveillance System of Shenzhen". Public health professionals administered several epidemiologic investigations and gave all contacts of the patient 7-day-long medical observation for temporally related influenzalike illness. Real-time Polymerase Chain Reaction (PCR) assay was applied to test respiratory tract samples and/or throat swabs of the patient and all his contacts specific for the hemagglutinin gene of influenza A H5N1. Hemagglutination inhibition assays and H5 antibody titer tests were carried out for serum samples from the 8th, 14th, 21st, 30th, and 60th day of disease course. H_5N_1 virus was isolated from the patient's respiratory tract samples by viral culture and genotyping for HA and NA fragments were fulfilled. Activities and strategies such as media response, notification in the public, communications with multiple related sectors, social participation and information exchange with Hong Kong and Macao were involved in field control and management. Results: The patient was male, 31 years old, with an occupation as a truck driver for one factory, and had been residing in Shenzhen for 7 years. Starting from influenza-like illness, the patients received treatment on the 4th day of the commence from a clinic and on the 6th day from a regular hospital. On the 8th day of the disease course, he was confirmed by Shenzhen Center for Disease Control & Prevention as human avian flu case and was then transferred to Intensive Care Unit (ICU). On the 83rd day of commence, the patients was healed and released from the hospital. The patients had mainly been driving in Pearl River Triangle Region of Guangdong Province. There was no significant contact history of doubtable transmission factors before the illness. The patient and five family members lived together, but no family member was affected. A small food market, which was under formal supervision, located near his residence. The authors tested 35 swabs from live birds and bird's coops in the market for H5 nucleic acid and all were negative. No fowl with abnormal death was proved in an 8-kilometer-range radius area near the patient's residence. During the 7-day-long medical quarantine periods, 4 contacts had fevers (above 38^{o}C) and 2 were tested to be positive for influenza (type B). No contact had positive results for H5N1. The influenza H5N1 virus isolated for the case was named as A/Guangdong/02/2006(H_5N_1). Characteristics of genotyping included the receptor specificity and the connecting peptide between HA1 and HA2 were revealed from avian influenza origin. Analysis on virus load showed that on the 4th week of the course virus load was almost negative. Microneutranization revealed the emergence of serum neutranization antibodies on the 15th day of commence, the peak (1:160) in the 4th week, and the decrease (1:40) on the 83rd day. Investigation process and control measures were released to the general public through the media. The case was reported via internet by the

"Chinese Undefined Pneumonia Surveillance System" soon after the probable case was diagnosed by the hospital. Soon after the laboratory confirmation, information was released to the society, as well as Hong Kong Center for Health Protection. Local Departments of Agricultures, Industries & Business, and Entry-Exit Inspection & Quarantine Bureau together with the Public Health Department put up combined actions. Shenzhen Center for Disease Control & Prevention initiated computer-assited telephone survey to investigate attitudes and knowledge of residents in town, revealing that positive atmosphere dominated and no panic existed. Conclusions: Rapid laboratory diagnose of the virus was the key for successful treatment and survival result of the case. Still, the pathogen was from birds resources. No human-to-human transmission was observed, however infection source was unclear. Field epidemiology could offer methods for public health events response.

Abstract P337

Asymptomatic Infection With Avian Influenza A/H5N1 in Korea During 2003-2004 Outbreaks

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Background: From December 2003 to March 2004, highly pathogenic avian influenza (HPAI) H5N1 viruses caused 19 outbreaks in 7 provinces in Korea. All poultry within 3 KM of infected farms were culled. And seasonal vaccination and prophylactic antivirals were given to those who participated in the culling. Considering there were no reported patients or no significant increase of influenza-like illness (ILI) in all affected provinces, evaluation for the possibility of human infection by the exposure was executed. We retrospectively performed serological analyses targeting high-risk groups during the outbreaks. Methods: We tested 4,108 sera from 2,820 persons by microneutralization recommended by the WHO. Because of the cytotoxicity against MDCK cells, we analyzed 3,448 sera from 2,512 persons. A/Chicken/Korea/ES/03 (H5N1) was kindly provided by the National Veterinary Research and Quarantine Service, which was isolated during the outbreak. Sera are positive by microneutralization if anti-H5 titers of ≥80 of single sera or ≥4-fold changes in paired sera in at least two independent assays. All sera tested positive by microneutralization were confirmed by Hemagglutination Inhibition (HI) using horse RBCs and the Western blot with purified baculovirus-expressed hemagglutinin (HA) protein derived from A/Vietnam/1203/2004 (H5N1) virus. Results: Nine (0.36%) of 2,512 persons were seropositive with the neutralizing titer ranging from 1:80 to 1:640. As of the results, 5 persons were seroconverted and 4 were positive in single sera obtained 3 weeks after exposure. In the HI assays, only two were positive, but all nine persons showed specific reactivity to H5 HA with Western blot. Therefore, all microneutralization-positive sera were confirmed to be positive against A/H5N1 by the WHO case definition. Conclusions: Among nine seropositives confirmed by this serosurvey, no one developed any ILI or fever during the monitoring periods after last exposure, and was considered to be asymptomatic infection. In conclusion, the serologic evidence for infection in persons who joined in the culling demonstrates the possibility that avian influenza viruses might infect humans. Therefore, it needs more study to understand the risk factors in culling operations.

Abstract P338

Molecular Characteristics of Swine Influenza A(H1N1) Viruses Isolated From Humans and Animals

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Now the world awaits the next influenza virus pandemic that is concerned as an inevitable event. Therefore, efforts of researchers from different countries go in search for features pointing to the emergence of the next pandemic strain and look for its possible source. Different authors suggest H1N1-"swine-like" and H2N2 influenza A viruses to be potentially pandemic pathogens. Similar "swine-like" H1N1 viruses together with H3N2 initiated seasonal increase of influenza virus sickness rate in humans in Almaty, Kazakhstan in 1984. Isolation of "swine-like" H1N1 viruses was confirmed by positive seroconversion. The cause of "swine-like" viruses emergence in human population in Almaty region still is not known. Taking into account the facts of emerging of pandemic influenza A type virus strains in China, the possibility of the virus penetration from the East China provinces from birds to pigs and then to humans in Almaty regions is rather high. The first case of "swine like" virus isolation in 1983 from human is associated with lethal influenza infection of animal caretaker, who had contacted pigs both at home and meat-processing factory, where he worked. A part of Almaty H1N1 "swine-

like" isolates were obtained from patients with physiological (pregnancy miscarriages, innate encephalopathies and pneumonias in newborns from mothers with virusemy) or acquired immunodefficiencies. The three Almaty "swine-like" viruses were isolated from lung and trachea pieces of 46, 47 and 65 years old patients, died of rapid abdominal form of influenza with simultaneous cardiovascular and respiratory lesion. However, it is quite difficult to determine evolutionary links and origins of the Almaty isolates. At this moment, due to absence of data on primary sequences of earlier strains, it is hard to prove and disprove these two hypotheses about the origins of the pandemic viruses and the role that circulation of Almaty isolates might play in onset of new pandemic strains. This again confirms the hypothesis of re-emergence of "swine like" strains in human and swine populations and the question occurs about the natural reservoir and mechanisms of such long term persistence of H1N1 "swine like" human influenza virus.

Abstract P339

Public Health Aspects of Influenza A/ H5N1 in the EU in 2005/2006

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In late summer and autumn 2005, the threat from influenza A/H5N1 to the European Union (EU) became acute following the rapid westward spread of infection from South East Asia towards EU borders - predominately through wild birds. It posed a particular challenge for the new EU public health agency, the European Centre for Disease Prevention and Control (ECDC), which had only opened in May 2005. One of its first tasks was therefore to provide a portfolio of tools to guide EU public health policy and practise against this emerging threat. This included the development of risk assessments, the identification of at-risk groups, and guidance for risk mitigation strategies for people potentially exposed. Other challenges included the shifting of surveillance to allow the rapid identification of the emerging pathogen in humans, and to ensure that the public received timely and considered information in the face of considerable media confusion. During spring 2006, the threat of infection became a reality within EU bird populations, with 13 EU Member States reporting either infections in wild birds or poultry outbreaks. Due to the efforts of veterinary teams, all cases were rapidly contained and infection did not become established in birds. This, together with the work of ECDC and EU public health professionals considerably reduced any risks to human populations, and to date no human H5N1 infections have been seen in the EU. The increased profile of influenza had a positive impact of providing impetus for the development of plans against potential influenza pandemic, delivering improvements in preparedness. However, there has been public and political confusion on the risks posed by avian influenza compared with a future pandemic, or even ordinary seasonal influenza, and this has created some difficulties and deflected from the broad planning for a pandemic, irrespective of the viral type. There are several lessons from the experience of ECDC in responding to the public health threat posed by H5N1 within the EU in 2006 which would be of benefit to other industrialised countries that may also have to face H5N1 or similar highly pathogenic zoonootic infection in the future.

Workshop Topic #4

Replication and Assembly

Poster Abstracts

Abstract P401

An Adenoviral Vector-Mediated Reverse Genetics System for Influenza A Virus Generation

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The artificial generation technology of influenza A virus entirely from cloned cDNA in plasmid-transfected cells, the so-called "plasmid-based reverse genetics system", represents a seminal advance for the entire field of virology. Above all, the development of vaccine reference viruses for highly pathogenic influenza viruses, including the currently circulating H5N1 subtype, relies entirely on this technology because the cleavage site of the hemagglutinin requires genetic modification from a virulent- to avirulent-type. Since at least eight plasmids are transfected into one cell for virus generation, the major limiting factor for this plasmid-based system is the transfection efficiency of the target cell. In general, 293T cells, which are readily transfected with plasmids, have been used for plasmid-based reverse genetics systems. However, 293T cells cannot be used for the development of human vaccine reference viruses because they are not validated for vaccine production. African green monkey kidney (Vero) cells, which have been used for the vaccine production of rabies and polio viruses, are the recommended cell line for the development of influenza vaccine reference viruses by the WHO, but these cells have a low transfection efficiency. It is, therefore difficult to generate infectious viruses by using plasmid-based systems in Vero cells, although some success has been achieved. To address these limitations, we established a reverse genetics system using the well-established technique of adenovirus type 5-based gene transfer. An E1 and E3-deleted replication-incompetent adenoviral vector (AdV) possessing the cDNAs of viral genes under the control of the human RNA polymerase I (PolI) promoter and the mouse PolI terminator, efficiently induced viral RNA synthesis and led to a high virus yield in Vero cells. These results suggest that an AdV-mediated reverse genetics system would be valuable for the production of vaccine reference viruses in pandemic situations.

Abstract P402

How to Retain the Type B Influenza Hemagglutinin 196/197 Glycosylation Site That is Lost During Virus Growth in Eggs

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Chicken embryonated eggs are used to grow influenza viruses and manufacture influenza vaccines. However, recent influenza B strains from both antigenic lineages do not grow well in eggs and adaptation of virus in eggs usually results in the loss of the N-linked glycosylation site in the hemagglutinin (HA) protein at positions of 196 or 197. The loss of the glycosylation site can affect vaccine antigenicity and immunogenicity. To determine the effect of the 196/197 glycosylation site on virus growth in eggs and to find a solution to retain the 196/197 glycosylation site in the vaccine strains, 6:2 reassortant influenza B vaccines were generated by reverse genetics. Recombinant viruses were generated using two plasmids encoding the HA and NA from wild type influenza B strains isolated between 2003 and 2005 and the six plasmids encoding the internal genes from the cold adapted B/Ann Arbor/1/66 master donor virus used in the production of the type B vaccine component of the live attenuated FluMist® vaccines. Paired recombinant B strains, with or without the 196/197 glycosylation site, were obtained and analyzed. In contrast to production in eggs, the glycosylation site was retained in MDCK cells. We identified one virus that was able to grow in eggs and retained the glycosylation at position 196/197. From this virus we identified an amino acid residue that stabilizes the 196/197 glycosylation site. Modified type B influenza viruses containing the stabilizing amino acid residue were generated. These modified viruses retained both the 196/197 glycosylation site after growth on eggs and their antigenicity. The finding of an amino acid residue that stabilizes 196/197 glycosylation has significant implication in influenza B vaccine manufacture.

Abstract P403

An Amino Acid on Influenza C Virus M1 Protein Affects the Virion Morphology by Altering the Membrane Affinity of the Protein

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Background: The M1 protein of influenza A virus is a driving force for virus budding and involved in virion morphology. For influenza C virus, we previously showed that an amino acid at the residue 24 of the M1 protein is involved in the morphology of the virus-like particles (VLPs); expression of M1 having Ala or Thr at the residue 24 together with the other viral components leads to filamentous or spherical VLP formation, respectively. In this study, using reverse genetics, we attempted to investigate the role of the residue in virus replication and morphology and to detect any differences in virus-infected cells. Methods: Recombinant C/Ann Arbor/1/50 (rWT) and a mutant recombinant virus (rMG96A), the former of which has Ala and the latter of which has Thr at the residue 24 of the M1 protein, respectively, were generated by reverse genetics. Virion morphology was examined by electron microscopy. Growth kinetics and localization of viral proteins were analyzed using HMV-II cells. Membrane-associated viral proteins in the virus-infected cells were detected by membrane floatation analysis. Results: 1) The number of the nucleotides of C/Ann Arbor/1/50 consensus sequence was 12,906. 2) The rWT grew a little more efficiently than rMG96A (2.1×10^5 vs. 4.6×10^4 $TCID_{50}$/ml). 3) The rWT exhibited filamentous and rod-like shape, while the morphology of rMG96A exhibited spherical. 4) No significant differences were observed in the infected cells with respect to viral protein accumulation and distribution of the viral proteins. 5) In the rWT-infected cells, approximately 90% of M1 protein was found to be associated with plasma membranes, whereas the proportion of M1 in the rMG96A-infected cells was 60%, although the distribution of HE and NP proteins exhibited no significant difference. Conclusion: An amino acid (Ala/Thr) at the residue 24 of the influenza C virus M1 protein is involved in virion morphology by altering the affinity of M1 to the plasma membrane.

Abstract P404

Adenoviral Expression and Purification of the Influenza A Virus Polymerase Complex

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The RNA-dependent RNA polymerase of influenza A virus consists of three subunits PB1, PB2 and PA. The three subunits assemble in the nucleus to a functional complex in which the N-terminal region of PB1 interacts with the C-terminal region of PA and the C-terminal region of PB1 interacts with the N-terminal region of PB2; no direct interactions between PB2 and PA have been reported. Previous studies of the three-protein polymerase (3P) complex have relied upon over-expression of the individual subunit proteins in yeast, insect cells (using baculovirus vectors) or mammalian cells (using either vaccinia virus vectors or transient transfection of 293T cells with plasmid DNA constructs). Expression systems using non-mammalian host cells are suboptimal due to trace contamination by nucleases or due to the lack of host cell factors that participate in complex formation and/or polymerase function. The current mammalian cell systems are mainly characterized by low expression of recombinant protein. In order to address these concerns, we have developed an adenoviral expression system for the influenza A virus (A/WSN/33) polymerase complex. Adenoviruses were created for each individual subunit and mammalian host cells were then co-infected simultaneously with all three viruses and the 3P complex was isolated from the nucleus of infected cells. A TAP tag consisting of a thrombin cleavage site, a 6xHis affinity tag, a tobacco etch virus (TEV) cleavage site and a tandem IgG binding domain was inserted at the C-terminus of PA; this served as a tag to facilitate the purification of the nuclear 3P complex. The formation of the 3P complex was verified by immunoblot analysis of the affinity purified proteins using antibodies specific for each of the polymerase subunits, as well as by SDS-PAGE and subsequent silver staining of the separated proteins. Functional testing of the enzymatic activity of the purified 3P-complex from human and avian influenza virus strains is under way. It is expected that this vector system will permit robust expression of the 3P complex in a wide variety of mammalian cell types, in a functionally active form.

Abstract P405

Characterization of Matrix Protein Interactions Involved in Influenza Morphology

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Influenza A virions can be spherical or filamentous depending on strain. The influenza virus matrix protein (M1) is a critical determinant of virion morphology and also has an important role in viral assembly. Published studies with A/Udorn/72 and either A/WSN/33 (WSN) or A/Victoria/3/75 have identified several M1 residues involved in virion morphology, including 41, 95, 204, and/or 218, depending on the study. M1 may also be involved in the host range restriction of influenza A viruses, since human strain recombinants containing an avian M gene are attenuated in humans and non-human primates. We characterized WSN and A/Aichi/68 (Aichi) virion morphology and filament formation in MDCK and chicken DF-1 cells. Both viruses produced spherical virions and did not form filaments as reported previously. However, infection with the recombinant virus WSN-AichiM, which contains the complete Aichi M gene, or the virus WSN-AichiM1, which contains a chimeric M segment expressing Aichi M1 and WSN M2, produced long cellular-associated filaments in MDCK and DF-1 cells. Because infection with Aichi did not induce filament formations in infected cells, it is likely that an interaction of Aichi M1 with WSN protein(s) is involved in the filament formation observed. To identify residues involved in the filament formation of WSN-AichiM1, we mutated 4 residues (115, 121, 137, and 218) in the Aichi M1 protein. These M1 residues are uniquely conserved among human or avian strains. Aichi M1 has all 4 human virus-conserved residues. WSN M1 has 3 of the conserved human residues, but residue 218 is threonine, which is seen in most avian isolates. We rescued WSN-AichiM1Q virus in which all 4 conserved human residues in the Aichi M1 gene were mutated to their avian equivalents. This virus did not induce long filament formations in MDCK and DF-1, suggesting that some of the conserved residues are involved in the interaction of M1 with WSN protein(s) required for spherical virion formation. Our results suggest that interaction of M1 with other viral protein(s) affects virion formation. Further characterization of the protein-protein interactions involved in viral assembly and egress is underway.

Workshop Topic #5

Developments in Diagnostic and Serologic Techniques

Poster Abstracts

Abstract P501

Influenza A and B Detection and Subtyping by RT-PCR-RFLP During 2001-2003 Laboratory Surveillance in Sao Paulo, Brazil

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Influenza viruses circulate worldwide mainly during seasonal epidemics of respiratory illness. Differential diagnosis of other respiratory viruses require more rapid and accurate influenza assays for guide the surveillance, prevention, containment and treatment of related illness. The current predominant influenza virus circulating among humans are H1N1 and H3N2 subtypes of influenza A and influenza B, but infections with highly pathogenic subtypes has been documented as causing outbreaks associated with severe infections and mortality. Laboratory diagnosis of influenza has extended the role in the prepandemic stages. One strategy includes the development of the RT-PCR assay that can be used first to detect all influenza viruses and subsequently can screens a pandemic strain rapidly. The purpose of present study was to apply a RT-PCR-RFLP for detection and subtyping of Influenza virus in samples received during 2001 to 2003 in a university virology laboratory in Sao Paulo, Brazil. Methods: Four hundred and twelve stored samples were submitted to RNA extraction by QIAamp Viral RNA extraction kit (Qiagen, Valencia, CA), according to the instructions of the manufacturer. Amplification of viral gene was accomplished by using of a duplex two-step reverse transcriptase RT-PCR protocol. The primers used in this study for detection of influenza A target a segment of the influenza M-protein gene (nucleotide position 149 and 847) were: MF, 5´-CTCATGGAATGGCTAAAGACA-3´; MR, 5´-CGATCAAG/TAAT-CCACAATATC-3´. The primers used for amplification of influenza B target a part of the influenza NS gene (nucleotide position 37 and 145) were: NSF, 5´-ACAAATTGAGGTGGGTCCG-3´; NSR, 5'-GGCAAGTGCACCAGCAGAAT-AACT-3'. Duplex RT-PCR was performed by adding 3mL of extracted RNA to a mixture containing 2.5mM each of the deoxynucleoside triphosphates (dNTPs) dATP, dCTP, dTTP, dGTP, and 100µM of the DTT, 4µL of 5× first-strand buffer (Invitrogen, Brazil), 8µL of water, 1µL of each forward primer (MF and NSF) at a concentration of 0.5µg/µL. One hundred units of M-MLV reverse transcriptase (Invitrogen, Brazil) and 15U of RNase inhibitor (Invitrogen, Brazil) were then added, and the mixture was incubated at 42°C for 60min. Subsequent PCR amplification of cDNA was accomplished in the same tube through the addition of a second mixture containing an additional 2.5mM dNTPs, 2.5U of Taq DNA polymerase (Invitrogen, Brazil), 2.5mM $MgCl_2$, 1µL of the each reverse primer (MR and NSR) at a concentration of 0.5µg/µL, and enough water to give a final reaction mixture of 50µL. The optimized profile in the thermal cycler (MJ Research Watertown, Massachusetts, USA) was a initial step of 95°C for 3min, followed by 39 cycles from denaturation at 94°C for 30sec, annealing at 56°C for 30sec, and synthesis at 72°C for 90sec. Amplification was completed with prolonged synthesis at 72°C for 10 min. Positive controls (Influenza A/NewCaledonia/20/99 H1N1, Influenza A/Panama/2007/99 H3N2, and Influenza B/HongKong/33/01) and negative control (water for resuspension of the samples) were included in each series. Amplicons were visualized by ethidium bromide staining following electrophoresis on a 2% agarose gel. The two amplicons of influenza A and B were easily distinguished by their molecular weights of 698 and 108bp, respectively. When Influenza A was identified, 10µL of unpurified PCR products were digested with HindIII and ScaI enzymes (New England Biolabs, Berrly, USA) in two separate reactions, according to the instructions of the manufacturer. Digested fragments were visualized by ethidium bromide staining following electrophoresis on a 2% agarose gel. The amplicon cleaved by HindIII indicate Influenza A subtype H3N2 and by ScaI indicate Influenza A subtype H1N1. Results: Of the 412 specimens tested, 79 were positive for influenza by duplex RT-PCR, 47 for influenza A and 32 for influenza B. Of the 47 samples positive for influenza A the digested amplicon pattern indicate 12 H1N1 (25.5%) and 35 H3N2 (74.5%). Annual incidence demonstrated alternance of type and subtype prevalence during study period: 58% influenza A in 2001, with predominance of H1N1 (66%); 57% influenza B in 2002, with H3N2 starting the season; and 88% influenza A H3N2 in 2003. Discussion: Duplex RT-PCR-RFLP applied in our study permited to detect influenza A and B from clinical samples easily and fast. This protocol consists in the adaptation of two pair primers of distinct studies for application in the same reaction in duplex RT-PCR format. This primers were designed for be complementary to highly conserved genes viral resulting in all influenza strains amplification, including other animal influenza A. According to initial study (Cooper, 2000), this primers for influenza A could detect H5N1 in human outbreak during 1997-1998. An analysis of primers sequence from M-gene influenza A in Mega 3.1 software (ClustalW) shown complementary for strains H5N1 (DQ835316, EF124795, DQ334771) of recent outbreaks, in additions to H3N2 and H1N1. In one step more, all samples influenza A positive could be subtyped in the present protocol. This RFLP initially described by CDC Influenza Branch as simple genotyping method capable of distinguishing the internal genes of human H1N1, H3N2, and avian H5N1 influenza A virus can be used to screen virus in laboratory surveillance. According with RFLP patterns from clinical isolated, this rapid and simple screen method can determine if influenza A identified is a current strain circulating (H3N2 or H1N1) or not. From a public health perspective, the RT-PCR-RFLP method is straightforward and can be performed by laboratories that do not have ability do directly sequence virus isolates. The great limitation of this protocol is that nontypeable isolates need a further analysis in conventional methods for characterization of influenza viruses. Conclusion: The duplex RT-PCR-RFLP

described here demonstrated to be applicable in influenza screening in laboratory surveillance, distinguish different influenza circulating strains and could be used as first test for elucidate suspected cases in pre-pandemic phases.

Abstract P502

MChip: A Diagnostic Microarray for Influenza Virus Identification, Subtyping, and Surveillance

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The MChip is a diagnostic microarray based on the single matrix (M) gene segment of influenza A capable of inferring antigenic subtype with automated data interpretation via a neural network. The MChip was developed at the University of Colorado at Boulder in conjunction with the Centers for Disease Control and Prevention and has been tested extensively on hundreds of samples. The presentation will introduce the salient features of this low-density microarray and subsequently present results from a series of studies aimed at characterizing the response of the MChip to a wide variety of influenza viruses and patient samples. First, studies of human influenza A/H3N2 and H1N1 viruses and performance comparisons to viral isolation, RT-PCR, and QuickVue A+B (Quidel Corp., San Diego, CA) will be presented. Second, the analysis of 43 distinct A/H5N1 samples from varied geographic locations and host species (2003-2006) that resulted in correct identification 24 "unknown" samples will be discussed. The MChip has also been characterized with well-known older viruses including A/Brevig Mission/1/1918 and a variety of other H1N1 viruses from the early to mid 1900's. Ideas for continued improvements to the microarray assay and future directions will then be discussed. It is our hope that MChip and associated molecular diagnostic technologies will assist in the continued and increased surveillance of influenza viruses at both local and worldwide levels.

Abstract P503

A Simple Immuno Blot Assay for Rapid Detection of H5 Avian Influenza Virus

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A kit for rapid detection of avian influenza virus of the H5 subtype is developed. This kits is based on a simple immuno blot assay using a monoclonal antibody that reacts specifically with the HA protein of the H5 virus. The detection limit of this kit is around 0.1 HA unit of the H5 virus. The specificity and sensitivity of the kit was determined using trachea and cloacae samples collected from chickens experimentally infected with H5 and H6 viruses. The results show that this kit could differentiate H5-infected chickens from H6-infected and the non-infected chickens. Moreover, this kit has a sensitivity of 76% and a specificity of 100% when using the results of "Flu Detect test strip" (Symbiotics) as the reference. The result showed that the immuno blot kit developed in this study has the potential to be used in the field.

Abstract P504

Detection of Minute Changes in Influenza Genome Fragments by Multitemperature Single-Strand Conformational Polymorphism (MSSCP) Technique

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Single-stranded DNA fragments attain a number of conformational forms which may be separated by electrophoresis in native polyacrylamide gels giving a characteristic pattern of electrophoretic bands. Minute sequence changes (e.g. point mutations) may have significant effect on electrophoretic pattern of single-stranded DNA. Changes of gel temperature during electrophoresis increase the sensitivity of mutation detection in PCR products; this technique, a modification of SSCP technique, was named MSSCP (where M stands for "multitemperature"). This method modified in our laboratories was applied for characterization of influenza A cDNA fragments. A series of primers were synthesized after the comparison of the hemagglutinin, neuraminidase and PB2 gene sequences of different origin. PCR reactions were run using these primers and the products

were denatured. Single-stranded DNA fragments were subjected to MSSCP electrophoresis where, after silver staining, they gave characteristic ssDNA band patterns. This technique was applied to analyse hemagglutinin, neuraminidase and PB2 gene fragments from recent isolates of avian flu. Minor differences within a serotype were detected which makes the MSSCP technique a valuable tool for quick preliminary characterization of influenza variants.

Abstract P505

Evaluation of the MChip With Historic Influenza A (H1N1) Viruses

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The rapidly evolving nature of the influenza virus presents unique challenges in disease surveillance. The matrix and hemagglutinin (HA) genes of the influenza A virus are known to co-evolve, with the mutations in the M gene evolving at a lower rate than the HA. The high degree of conservation of the M gene along with it being a very reliable target for PCR amplification makes it a desirable target in the development of a diagnostic assay. The MChip, a low-density microarray consisting of 15 distinct oligonucleotides complementary to only the matrix (M) gene segment of influenza A viruses, has been shown to be an effective tool in the identification of influenza A viruses and differentiation between the H1, H3 and H5 subtype. Since sequences for the microarray were designed using recently circulating viruses (1998-2005) and influenza A viruses isolated from human and animal species isolated in the past 89 years are available, we wanted to test the robustness of the assay with a range of historical influenza A (H1N1) viruses from a number of different species. 16 influenza A (H1N1) viruses collected between 1918 and 1980 and from avian, human and swine sources were evaluated using the MChip. All viruses were detected by the M-chip and when an artificial neural network was trained with temporally related H1N1 viruses, all 16 isolates were assigned an accurate subtype. The three capture sequences previously thought to be a determinant of the H5 subtype bound with several of the older H1N1 viruses and two of those isolates were from non-human sources. Several historic H1N1 viruses had positive signals with capture sequences designed to be sensitive to avian influenza viruses, confirming their proposed avian ancestry. This rapid, point-of-care diagnostic for subtyping is robust enough to identify virus strains that circulated as long as 89 years ago and can be a useful tool in the surveillance of influenza A viruses in both the human and animal populations.

Abstract P506

Evaluation of the Quidel QuickVue Influenza A+B Kit in Detecting Avian and Swine Influenza Virus

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Introduction: Many viruses are responsible for both mild and severe respiratory infections. Of them influenza is responsible for the most severe illnesses in most age groups. Case History: On June 29, 2006 the Respiratory Virus Section of the Institute Adolfo Lutz, São Paulo, Brazil, received a postmortem blood sample from a 03-year–old female child from Ribeirão Preto city, State of São Paulo, Southeast region of Brazil. According to her parents, on June 24, 2006, she presented mild influenza–like symptoms and good health and has died suddenly on June 26. We are requested for investigating adenovirus and enterovirus. Virus isolation attempts: For virus isolation attempts the clinical sample was inoculated in the Hep-2 and MDCK cell cultures. RNA Extraction: RNA was isolated from the blood sample by using the "QIAMP VIRAL RNA MINI KIT" (QIAGEN, Santa Clarita, California, USA) according to the manufacturer's instructions. RT-PCR- Influenza virus detection: Universal Type A, Universal Type B and specific primers to H1, H3 and H5 influenza virus strains were kindly provided by the Centers Disease Control and Prevention (CDC) Georgia. Primers for enterovirus group detection were also including. Phylogenetic analysis: The sequences were assembled, aligned, and edited using DNAStar (Madison, WI) and BioEdit version 5.0.6. Results: Influenza of type A subtype H1 was specifically identified by RT-PCR performed with RNA extracted from the blood specimen. Regarding virus isolation attempts, no viral replication was observed in the cell cultures. During the autopsy, the main gross finding was the heavy lungs, with marked beefy appearance caused by a diffuse edema. Formalin-fixed paraffin-embedded sections of the lungs showed, at the H&E stain, diffuse alveolar damage by mononuclear cells, and marked fibrinous exudates in the air spaces. Although only postmortem blood sample was under investigation, due to the rapid evolution to death, influenza virus RNA detected in the blood highlights the rare occurrence of acute fatal influenza pneumonia. Discussion and Conclusion: This investigation brought out the first documented case of fatal pneumonia in our experience due to influenza virus infection. Phylogenetic analysis of HA1 gene sequence indicated that this strain is referred to Clade 1 viruses.

Abstract P507

Surveillance of Influenza During the Epidemic Season 2006/2007: Comparison of Near Patient Test Quickvue With Immunoperoxidase Staining and Viral Culture for the Detection of Influenza A (H3N2) Viruses

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Objective: Routine virological influenza surveillance is usually based on cell cultures as the „gold standard" in testing; this procedure provides both the most accurate diagnosis and the opportunity for further study of the virus. Nevertheless, it takes at least 2 – 3 days and usually a week or longer in practice. Laboratory diagnosis of influenza using „near patients tests" is suitable for rapid antigen detection (15-20 minutes) and provides very early information on virus circulation and/or a possible influenza outbreak. Material and Methods: During the epidemic season 2006/2007, the QuickVue Influenza A+B test (Quidel Corporation, USA) was used in parallel with the indirect immunoperoxidase assay (IPA) and virus isolation. Clinical specimens (nasopharyngeal swabs) from influenza-like illness were collected through the national surveillance network. OuickVue: The test was carried out according to the kit procedure manual. Virus isolation: Samples were inoculated in parallels onto Madin–Darby Canine Kidney - MDCK cells (Institut Pasteur, Paris, France) in 96-well multiwell plates (GAMA Group, Czech Republic) and centrifuged 15 min at 3500 rpm. After incubation for 2-3 days at 35.5 °C in 5% CO_2 atmosphere, infection was confirmed by IPA and tissue culture fluid was observed for the haemagglutination (with 0.5% suspension of guinea pig red blood cells). Monoclonal antibodies (CDC Atlanta, USA), swine anti-mouse polyclonal antibodies with conjugated horseradish peroxidase (Sevapharma, Czech Republic) and 3-amino-9-ethylcarbazole substrate (Sigma) were used for IPA. Results: All of the 454 specimens (collected from October 1, 2006 to February 23, 2007) were tested by the QuickVue, IPA and cell culture. None of 200 samples collected before the influenza epidemic (by January 4, 2007) appeared influenza positive in any test. The first unambiguously positive results were detected in the 2nd calendar week, whereas the total ARI/ILI morbidity in this period of time was at the seasonal level, with only a local gentle increase in morbidity being observed. Using the QuickVue test, the first cases of flu A in the Czech Republic were detected as early as 7 days before the rise in morbidity and 10 days before the positive isolation. Altogether 254 samples were tested from the 2nd to 7th calendar week when epidemic activity of influenza A viruses was observed. Eighty-nine (35%) of these specimens were positive for influenza A by IPA, influenza virus A (H3N2) was isolated in 78 (30.7%) specimens and 60 (23.6%) specimens tested positive for influenza A in the QuickVue test. IPA served as a "gold standard" since it allows clear detection of virus-infected cells. Compared with IPA, the QuickVue test showed a sensitivity of 67.4% (60/89) and a specificity of 99.0% (200/202). The positive predictive value (PPV) was 96.8% while the negative predictive value (NPV) achieved 87.3%. Conclusions: Sensitivity of the QuickVue test was generally lower than that of cell culture or IPA, but the major point of interest is the speed with which information about circulation of influenza viruses was obtained. Confirmation of the QuickVue results by IPA or cell culture was achieved within the next 2 to 10 days. Although not perfect, near patient tests can play an important role in the early identification and ongoing monitoring of influenza viruses and annual epidemics. The rapid detection of influenza virus is important for effective anti-viral therapy, prescription of antibiotics, exclusion of influenza-like pathogens and early institution of measures to limit the spread of infection, particularly in nursing homes, hospitals etc. Furthermore, after the SARS epidemic, rapid diagnosis is more important than ever before. On the other hand, IPA providing results within a reasonable interval is a very sensitive tool for the diagnosis of influenza virus, comparable in sensitivity to PCR. Combination of the rapid tests with the conventional methods proved highly effective in practice but has the disadvantage of being costly, and posing limitations to the use directly in the GP practice.

Abstract P508

Development of a Diagnostic Assay and Confirmatory Methods for Detection of Influenza H5N1 Infection in Humans, and Application Through the Creation of a UK H5 Laboratory Network

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Increasing diversity among influenza H5N1 viruses has resulted in the need for sensitive and specific diagnostic assays, fully validated for the detection of H5 viruses belonging to all HA clades and subclades, in particular recently circulating clade 2 H5N1 viruses. A real-time one-step TaqMan RT-PCR assay specific for the detection of influenza A H5 viruses from clades

1, 1′, 2 and 3 has been developed. The H5 real-time assay was shown to be highly sensitive, detecting <1PFU of H5 virus from each of the HA clades. Specificity of the H5 RT-PCR for influenza A H5 viruses was demonstrated using influenza A viruses of different subtypes, clinical samples containing influenza A H1N1, H3N2, H5N1 and influenza B viruses, and other respiratory viruses. Confirmatory assays validated for the verification of H5 virus infections included a real-time RT-PCR pyrosequencing assay, a restriction enzyme digestion assay, and a quadriplex real-time RT-PCR assay capable of simultaneously detecting influenza A and B virus, avian H5 subtype and an internal control (MS2). A network of proficient regional laboratories providing on-call for rapid detection/confirmation of H5N1 infections using the validated assays was established in October 2005. The quality of diagnostic testing has been supported by the provision of quality assured, quantitated, positive control material, National Standard Operating Procedures, training programs and proficiency testing. Diagnostic testing for H5 avian influenza using an algorithm to provide robust differential diagnosis has been performed on returning travellers to the UK, from H5N1 affected regions, or in potentially exposed poultry workers. Although no positive cases were detected, the diagnosis of influenza A H3N2 infection in 50-60% of cases analysed highlights the importance of differential diagnosis in suspect avian influenza patients, particularly when seasonal influenza viruses may be circulating.

Abstract P509

Development of Competitive ELISA for Determination of Antibody Response to Influenza A (H5) Virus in Human Sera

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Virus neutralization test (VNT) was determined as more sensitive than hemagglutination inhibition test in determination of antibodies (Abs) to A(H5N1) virus but need in application of infectious virus was appeared. This restrict use of VNT in practical laboratories. A competitive ELISA (c-ELISA) using A(H5) subtype specific neutralizing monoclonal antibody (H5-MAb) produced in our laboratory was evaluated in detection of H5-specific Abs. c-ELISA was designed by simultaneous addition of sera and H5-MAb to A(H5N1) virus-coated wells. Percent of inhibition was detected as reduction in OD450 obtained with MAb alone. A threshold value of 30% inhibition was adopted for routine analysis in detection of H5-positive sera. The specificity of c-ELISA was proved in investigation of sera from both A(H1N1) infected patients and volunteers immunized with seasonal influenza vaccine. Percent of inhibition varied in the ranges 15,6±8,4 in postinfection or postvaccination sera. Paired sera from 60 A(H5N1) vaccinated volunteers were tested by c-ELISA and VNT. The conversions of H5-specific Abs were detected by both tests in vaccinated people. Geometric mean titer in postvaccination sera was estimated by VNT as 1/151. Percent of inhibition was determined as 56,9±15,9% in the same sera by c-ELISA. A significant correlation was observed between levels of inhibition registered in c-ELISA and VNT-Abs titers (r=0,5,p<0,001). Thus, c-ELISA could to be evaluated as sensitive, specific and simple test in determination of Abs to A(H5N1) virus and useful in determination of virus transmission in humans.

Abstract P510

Is Influenza A Rendered Non-viable Following Treatment With RNA Isolation Kit Lysis/Binding Buffers?

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As government agencies and health care providers around the world escalate surveillance for highly pathogenic avian influenza (HPAI) viruses the number of isolates warranting typing could rapidly overwhelm the capacity of BSL-3 facilities. Many agencies having opted for the ease and speed of real-time PCR will need to isolate viral RNA from a large number of potential HPAI samples. Unfortunately high throughput sample processing protocols are more compatible with lower level containment facilities. The ability to inactivate the virus at earlier steps in the RNA isolation, enabling their removal from the BSL-3 facility, will greatly improve the throughput capacity of typing laboratories. To remove samples from a BSL-3 facility a team must demonstrate that either the virus is not a highly pathogenic strain or show, without questionable doubt, that there are no active virus particles present. While many viral RNA isolation kits allude to the ability of their lysis/binding buffers to inactivate virus, to our knowledge no published data exist demonstrating that influenza A virus is indeed inactivated by these reagents. In this study we tested the lysis/binding reagents from two popular viral isolation kits, the QIAgen QIAamp viral RNA Mini Kit and the Ambion MagMAX -96 Viral RNA Isolation Kit, for their ability to inactivate prototypical examples of 5 of the most prevalent HA subtypes of avian influenza A (H1, H3, H4, H5 and H6) and three common vaccine strains of human influenza A (H1N1; A/New Caldonia/20/99, H3N2; A/Nanchang/933/95, and H3N2; A/Panama/2007/99. The presence of cytocidic compounds in the lysis buffers of both kits was overcome by

repeated dilution and centrifugal ultrafiltration of lysis buffer treated sample. Inactivation of virus following treatment with lysis reagents was demonstrated by non-viability in culture after two passages (no CPE or indication of virus after immunohistochimical staining). These data suggest that influenza A specimens treated with the Ambion and QIAgen lysis buffers would be safe to remove from BSL-3.

Abstract P511

Rapid Multiplex Nested-PCR for the Detection of Avian Influenza and Other Respiratory Viruses

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Introduction: Specific diagnosis of respiratory tract infections relies on laboratory investigation. This study developed and evaluated five groups of multiplex nested-PCR assays that could simultaneously detect 20 different respiratory pathogens. Materials and Methods: The five groups of multiplex nested-PCRs were: Group 1 comprised of influenza virus (Flu) groups A and B and subtypes H1N1, H3N2, H5N1-specific primers. Group 2 comprised of parainfluenza virus (PIV) types 1, 2, 3, and 4. Group 3 comprised of human respiratory syncytial viruses (hRSV) A and B, human rhinoviruses (hRV) and human enteroviruses (hEV). Group 4 comprised of human coronaviruses (HCoV-229E, HCoV-OC43, SARS-CoV), and human metapneumoviruses (hMPV). Group 5 comprised of Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila and human adenoviruses (ADV). These multiplex nested-PCRs adopted the Fast PCR (within 35 minutes) technology, and were evaluated with 303 clinical specimens. Results: The results show that these multiplex nested-PCR assays were specific and more sensitive (100-1000 fold) than conventional methods. Among the 303 clinical specimens tested, the multiplex nested-PCR achieved an overall positive rate of 48.5% (95% CI = 42.9-54.1%), which was significantly higher than that of virus isolation (20.1%, 95% CI = 15.6–24.6%), and that of immunofluorescence assay (13.5%, 95% CI = 9.7–17.4%). The improved sensitivity was partly due to the higher sensitivity of multiplex nested-PCR compared to conventional methods in detecting cultivatable viruses. Moreover, the ability of the multiplex nested-PCR in detecting non-cultivatable viruses, particularly rhinoviruses, coronaviruses OC43 and metapneumoviruses, contributed another major part of the gain (15.6%) in the overall positive rate. In addition, this multiplex nested-PCR could also detect multiple infections (7/303, 3.2%) and simultaneously subtype influenza A viruses H1, H3, and H3. Conclusion: The rapid multiplex nested-PCR assays can improve the diagnostic yield for respiratory infections, which allows prompt therapeutic interventions and infection control measures to be taken.

Abstract P512

Development of New Monoclonal Antibodies to Influenza A (H5N1) Virus

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Development of monoclonal antibodies (MAbs) for investigation of avian H5 virus evolution, design of rapid specific and sensitive diagnostic test and creation of target medical product for human treatment was the task of our investigation. Influenza viruses A/Vietnam/1194/04 (vaccine strain NIBRG-14) (H5N1) or A/duck/Pottsdam/1402-6/86 (H5N2) were used for immunization of mice and following hybridomas preparing and cloning. As result of these investigations six MAbs directed according to western blot analysis to HA1 molecule of influenza H5N1 virus and two MAbs to HA1 of influenza H5N2 virus were obtained. Besides, two MAbs directed to NP determinants (NP–MAbs) were developed. Six HA1–MAbs to H5N1 virus were directed to epitope common for different H5 strains and interacted with influenza A(H5N1), A(H5N2) and A(H5N3) viruses in ELISA and immunofluorescent analysis (IFA). Three of these MAbs were found to be highly reactive in hemagglutination inhibition (up to 1:40000 and higher) and neutralization test. In contrast HA1–MAbs to H5N2 virus interacted in ELISA test and IFA with gomologous virus exclusively and were non reactive with other H5 viruses. NP–MAbs were broadly reactive with all investigated subtypes of human, swine and avian influenza A viruses both in ELISA and IFA tests. Thus, obtained MAbs to hemagglutinin of influenza A(H5N1) with virus neutralizing activity as highly reactive reagents could be used for humanization procedure with the aim of development of preparation for prevention and treatment of H5N1 infection in humans. Additionally, broadly reactive MAbs to virus NP and subtype specific HA1-MAbs are prospective both for design of rapid diagnostic test and for use in ELISA – microneutralization test.

Abstract P513

Evaluation of the Specificity of a Modified Haemagglutination Inhibition Assay Using Horse Erythrocytes

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Haemagglutination-inhibition (HI) is a simple gold standard method used to evaluate the immunogenicity of influenza vaccines. However, "classic" HI tests using turkey or chicken erythrocytes are insensitive for detection of antibody responses to avian influenza haemagglutinin after vaccination or natural infection. Alteration of the HI assay by substitution with horse erythrocytes with higher α2,3Gal receptor content has shown improved detection of antibody to avian H5 in human sera following vaccination with various pandemic H5 trial vaccines. We demonstrate the validity of the modified HA assay in assessing post vaccination antibody responses. We evaluated human serum from a UK national cohort and pre and post seasonal influenza vaccine sera from healthy adults and elderly using modified and conventional HI as well as microneutralisation (MN) to validate specificity, sensitivity and precision of the detection of post vaccination H5 antibody. Two age stratified panels containing UK sera from 2003 and 2004 (approx. 850 sera each) representing age groups from 1 to 84 years were examined. Results show highly specific H5 antibody detection and low cross-reactivity of the HI reaction using horse erythrocytes with antibodies to human influenza. We analysed 3437 sera and found antibodies reactive to H5 influenza (A/Vietnam/1194/2004 reverse genetics virus NIBRG14) using HI horse in 3.7% of these sera overall. In the age groups under 60 years (N = 2498), 0.8% of sera showed antibody reactivity. Higher frequency of antibody reactivity to H5 (10.1%) was found in subjects aged >60 (N=702). Overall, 2.6% of sera in the >60 goup were also positive for H5 antibody by MN compared to 0.1% MN in <60 group. As H5 influenza has not been endemic in the UK, it is highly unlikely that these results represent evidence of exposure to H5 which increases with age. It is possible that elderly individuals with measurable pre existing antibody to H5 have a form of useful heterosubtypic immunity which may enhance protection to novel subtypes of influenza. Such individuals may respond differently to a primary course of H5 vaccine.

Abstract P514

Towards the Development of a Commercial Assay for H5N1

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Standardized diagnostic reagents are not presently available for detection of Avian Influenza H5N1 despite the numerous research RT-PCR assays that have been described and used for detection. Distribution of the FDA-cleared "Influenza A/H5 Virus Real-time RT-PCR Primer and Probe Set" developed by the CDC is limited to laboratories designated as the Laboratory Response Network. Our goal is to develop, produce, and register with the appropriate regulatory agencies, standardized PCR-based diagnostic reagents to detect Avian Influenza Virus H5N1, Influenza A, and Influenza B. The availability of robust, stable, manufactured reagents would ensure that an assay with consistent sensitivity and specificity is utilized at different testing laboratories. The prototype 1-step RT-PCR multiplex Avian Flu TaqMan® assay presented here employs five different primer-pair/probe sets, two for H5N1, and one each for pan-Influenza A, pan-Influenza B, and an internal control. The influenza primers and probes were designed by the CDC and include the FDA-cleared set for H5N1. Each target probe is labeled with a spectrally unique fluorescent reporter molecule to allow simultaneous detection in a single well. The internal control is an Armored RNA® (aRNA) that is co-extracted and amplified with the specimen and is used to monitor sample extraction and amplification efficiency. RNA transcripts from a positive control that contains the targeted regions of H5, Influenza A, and Influenza B were used for assay development. The assay integrates sample extraction, master mix preparation, and amplification on the m2000 (Abbott Molecular) to provide automated high throughput capability. Influenza particles are extracted on the m2000 using reagents that have been developed for HIV and HCV viral RNA extraction. Multiplex amplification of the positive control transcript demonstrates that the assay has 10 to 100 copy sensitivity for each H5, pan-Influenza A, and pan-Influenza B with the 250 copies of spiked aRNA detected at each level of the positive control tested. The extraction reagents, the aRNA, and many of the RT-PCR components are currently used in manufactured and regulatory-cleared kits and will serve as the basis for development of a standardized multiplex assay on the m2000 platform that is amenable for targeted surveillance or widespread routine testing.

Abstract P515

The MDCK 33016-PF Cell-Line is Not Only Suitable for the Production of Cell-Based Influenza Vaccine But is Also an Ideal Substrate for Influenza Virus Isolation

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Background: Isolation of the human influenza virus from clinical samples using hen eggs is problematic: relatively poor virus yields are achieved due to the low susceptibility and reproducibility of eggs for the virus. Currently, as there is no alternative accepted cell-based system, hen eggs continue to be used to isolate reference strains for the manufacture of influenza vaccine. Novartis Vaccines has recently established a suspension cell-line (MDCK 33016-PF) for the manufacture of a new cell-based influenza subunit vaccine (flu cell culture [FCC] vaccine). MDCK 33016-PF is derived from the Madin-Darby canine kidney (MDCK) cell-line, which is an established substrate for the isolation and replication of the influenza virus from clinical specimens. Although the MDCK 33016-PF cell-line is currently used to propagate viral strains isolated from hen eggs, the opportunity to propagate strains isolated from cell systems would be desirable, making influenza vaccine production independent of hen eggs. The objective of this study was to investigate whether the MDCK 33016-PF cell-line is a suitable substrate for the isolation and replication of wild-type influenza strains. Methods: Clinical specimens (nasal or throat swabs) containing influenza A and B virus subtypes were obtained from children and adults during the 2006-2007 Northern hemisphere influenza season. The susceptibility and reliability of the MDCK 33016-PF cell-line for isolation and replication of the influenza virus was compared with the established MDCK CCL 34 cell-line and hen eggs by determination of hemagglutinin (HA) titers, polymerase chain reaction (PCR) and virus titration. The accuracy of replication was assessed by sequencing of the HA gene in the original material and in isolates obtained from the second passage. Virus titers obtained from isolates grown in suspension MDCK 33016-PF cells were compared with those from MDCK 33016-PF cells adhered on plates. Results: Preliminary results indicate that the isolation capacity of the MDCK 33016-PF cell-line is at least as good as that of the established MDCK CCL 34 cell-line, but much greater than that of hen eggs. After passage of virus samples in MDCK 33016-PF cells, amino acid substitutions were identified in only a few isolates. In contrast, nearly all egg-passaged viruses contained one or more amino acid exchanges, predominately in the HA1 gene. Furthermore, higher virus yields, of at least one log level, were found following replication of isolates in suspension MDCK 33016-PF cells compared with adhered MDCK 33016-PF cells. Conclusions: These data demonstrate that the MDCK 33016-PF suspension cell-line is an ideal system for the isolation and replication of wild-type influenza strains, as it offers a greater isolation capacity compared with hen eggs. Furthermore, due to the high replication accuracy, the use of cell-based isolates for the production of a human influenza vaccine may lead to a more authentic vaccine. Improved match between the circulating wild-type strains and those contained in the vaccine should offer greater protection against influenza for the vaccinee. Further studies are ongoing.

Abstract P516

The Speed and Reliability of the Antigen Detection Kit Capilia Ful A, B When Used in a Clinical Setting

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Introduction: High reliability has been widely reported for commercial antigen detection kits now commonly used for the diagnosis of influenza infection in Japan. In this study, we studied the speed of the kit and the relationship between the speed and its diagnostic reliability, in a clinical setting. We also did an experimental study of the relationship between the speed and the viral concentration. Materials and Methods: Physicians at 13 clinics belonging to the Influenza Study Group of the Japan Physicians Association participated in the study. Patients with influenza-like symptoms were enrolled from 10 December 2005 through 30 March 2006. Patients were diagnosed as influenza A, influenza B, or not infected with influenza by the commercial antigen detection kit, Capilia FluA+B (Tauns, Japan), with throat swabs, nasal swabs, or nasal aspirates. The time to appearance of a positive line was measured from the time of specimen application and was classified to three groups; 0-3 min, 3 - 15min and over 15 min. Virus isolation was done by standard methods using Madin-Darby canine kidney cells. PCR was done for samples negative by virus isolation. Serially diluted laboratory prepared influenza A/H1N1, A/H3N2 and B virus antigens were used as test specimens. The time to positive was measured for each concentration of a viral antigen. Results: A total of 192 patients were enrolled; 120 patients with influenza

A and 72 patients without influenza, confirmed by virus isolation and PCR. The appearance time of the positive line was measured in seconds for 34 patients with influenza A. The sensitivity was 95.0%, specificity was 87.5%, predictive value for a positive test was 92.7%, predictive value for a negative test was 91.3% and accuracy was 92.2%. The time to positive was 0 - 3 minutes in 76.3%, 3 - 15 minutes in 20.2% and over 15 minutes in 3.5% of the positive cases. The positive predictive value was 96.7% in 90 patients (0 - 3 minutes), 82.1% in 28 patients (3 - 15 minutes), and 80% in 5 patients (over 15 minutes). The time to appearance of a positive line became longer as the antigen concentration decreased. Similar, linear relationships between the viral antigen concentration and the time to appearance of a positive line were obtained for the A/H1N1, A/H3N2, and B viruses. Discussion: The high reliability of the antigen detection kit, Capilia Flu A+B, was confirmed by use of clinical specimens. Furthermore, the outstanding speed at which the kit is able to make a diagnosis was shown. The diagnosis by the antigen detection kit was highly reliable, especially for cases diagnosed within three minutes. The results showing that the time to appearance of a positive line is shorter for specimens with a high antigen concentration may important, which would make this parameter a useful clinical indicator of the viral load.

Abstract P517

Serological Assays to Detect Antibody to Avian H5N1 Influenza Viruses in Humans

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Since late 2003, avian H5N1 viruses have re-emerged in humans as a consequence of epizootic spread throughout Asia, Europe and Africa. Viruses that have caused human disease are comprised of multiple antigenically and genetically distinct H5N1 clades and subclades. Serological assays that are sensitive and specific for the detection of antibodies to the H5N1 viruses in human sera may be used for diagnosis of infection and are essential for seroepidemiological studies that evaluate the frequency and risk of human infection with avian influenza viruses in populations with exposure to domestic or wild birds, or H5N1 virus-infected patients. While the microneutralization (MN) assay remains the gold standard for detecting antibodies to H5N1 viruses, a simpler, modified hemagglutination-inhibition (HI) assay that uses inactivated virus and is more sensitive than the traditional HI assay has been established. This modified HI uses horse erythrocytes that possess predominantly α 2-3 linkage sialic acid receptors to improve the sensitivity of detecting anti-H5 antibody in human sera. We have examined the relative sensitivity and specificity of the modified HI in comparison with the MN assay. Although the two assays show comparable sensitivity for clade 1 and 2 H5N1 viruses, the modified HI assay exhibited reduced specificity compared to the MN assay for clade 1 viruses. Additional factors that can influence the sensitivity and specificity of the modified HI are currently under investigation. For a patient meeting the clinical and epidemiological criteria for a suspected H5N1 case, current WHO guidelines require the detection of a four-fold or greater rise in neutralizing antibody titer between paired acute and convalescent sera, with the convalescent sera achieving a titer of 1:80 or higher for serodiagnosis of H5N1 infection. When only a single serum sample is available, a neutralizing antibody titer of 1:80 or higher in a serum sample collected 14 or more days after symptom onset and confirmatory positive result by a second assay is required. The latter standard should also be employed to establish criteria for positivity when evaluating single serum samples in seroepidemiological studies that evaluate risk and frequency of H5N1 virus infection in human populations.

Abstract P518

Use of Pseudotyped Particles Expressing Influenza A/Viet Nam/1194/2004 Hemagglutinin in Immunological Assays

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The influenza envelope glycoprotein hemagglutinin (HA) is the major target of protective antibodies. A correlate of protection has been established by using the hemagglutination inhibition (HI) assays, with titers predicting protection in humans. However, since the hemagglutination inhibition (HI) test resulted relatively insensitive for measuring antibody responses to the recently avian influenza viruses, there is an urgent need of new systems that can be easily used to screen human sera. The micro-neutralization represents a valid alternative to HI test, however this assay requires Biosafety Level 3 laboratories, and obviously the accessibility to the whole virus. Recently, the pseudotyped particles have been a widely used tool to express viral glycoproteins. They are based on a process known as "pseudotyping", in which lentiviral or retroviral core proteins (Gag) may interact with glycoproteins derived from other enveloped viruses, such as HA from Influenza, and

assemble into infectious particles. The resulting particles are called "pseudotypes". Lentiviral vector particles pseudotyped with Influenza A/Viet Nam/1194/2004 HA were prepared and characterized for their susceptibility to neutralizing antibodies by using a luciferase reporter gene. Preliminary results showed that animal sera against the avian influenza virus H5N1 were able to neutralize pseudotypes bearing the homologous HA, in contrast to the pre-immune sera, which had no effect. The application of pseudoparticles in immunological assays (such as neutralization, ELISA, hemagglutination inhibition) using sera from vaccinated volunteers will be showed. The pseudotype-based neutralization assay is promptly adaptable to a high-throughput format for the evaluation of vaccine efficacy; furthermore, using plasmids encoding for any other HA sequence, a panel of pseudotypes expressing HA from different viral strains could be generated and used to evaluate cross-protective antibodies.

Abstract P519

Rapid Selection of Reassortant Live Attenuated Influenza B Vaccine Strain

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Live attenuated influenza vaccine can be generated by reassortment between attenuated donor strain and virulent wild type viruses. The reassortant viruses inherit the two RNA segments encoding HA and NA surface glycoproteins from the epidemic wild type virus and the six internal RNA segments (PB2, PB1, PA, NP, M and NS) from an attenuated donor strain. The annual production schedule for influenza vaccine necessitates rapid generation and characterization of vaccine strains within narrow window of time frame. Most importantly, the identification of reassortant with desired 6:2 RNA constellation is time-consuming and labor-intensive, and therefore, efficient screening system for reassortant is essential. In this work, a rapid and simple multiplex RT-PCR system was developed to identify the origin of the genome segments. The PCR primers were designed for specific amplification of RNAs from the cold-adapted donor strain, B/Lee/40 ca virus, by multiple sequence alignment of variable region. The system was adapted and optimized with 8 independent wild type influenza B viruses. The one-step RT-PCR method is sensitive and efficient for routine identification of reassortant vaccine viruses of the desired 6:2 RNA constellation carrying the attenuation property of donor virus and immunogenicity of wild type virus. This method could be practically implemented as preliminary genotyping of reassortant live influenza vaccine strains.

Abstract P520

Evaluation of Six Rapid Tests in Detecting Human Influenza in a Paediatric Population

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A large number of rapid influenza detection tests are now available that are simple to use, quick (generate a result in 15 minutes) and can be performed outside the laboratory. The rapid tests have been shown to alter the management of a patient's illness, detect outbreaks in institutions and may play a role in pandemic influenza control. While previous evaluations of influenza rapid tests have reported good specificity, but poorer sensitivity, few studies have compared several rapid tests head-to-head even though the sensitivity of the same test can be highly variable between studies (due to differences in specimen type, influenza type/subtype circulating, patient age and the 'gold standard' test used for comparison). In this study however, we have conducted a head-to-head comparison of six influenza rapid tests for their ability to detect influenza A and B from 177 clinical specimens taken from mostly paediatric patients between June and October 2006 and compared these results with laboratory detection methods for influenza (immunofluorescence, cell culture and real-time RT-PCR). Compared with cell culture, five of the rapid tests (Binax Now Influenza A&B, Directigen EZ Flu A+B, Denka Seiken Quick Ex-Flu, Fujirebio Espline Influenza A&B-N, and Quidel QuickVue Influenza A+B Test) demonstrated a similar influenza A sensitivity of 67-71% and a specificity of 99-100%. However one rapid test (Rockeby Influenza A antigen test) had a significantly lower influenza A sensitivity of only 10% (with a specificity of 100%). For the five kits that detected influenza B antigen, sensitivity was considerably lower than that seen for influenza A (sensitivity for all the kits was 30%), although as the number of influenza B specimens tested in the evaluation was small, this figure may be somewhat reduced. As the use of these tests is likely to increase in the future, both for seasonal influenza and in the event of a pandemic situation, it is necessary to continue to evaluate, in a clinical setting, any new tests for influenza available on the market.

Abstract P521

Evaluation of the Actim Influenza A & B Test for the Rapid Identification of Influenza Viruses

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Swift identification of influenza outbreaks, accurate patient cohorting, and proper use of antiviral medication require a rapid and reliable identification of the etiologic agent. Influenza point-of-care tests can provide a result within a matter of 15 to 30 minutes, and thus fill the gap between clinical diagnosis and sophisticated but more time-consuming tests used in the specialized diagnostic laboratory. We have thoroughly evaluated the Actim Influenza A & B test (Medix Biochemica, Kauniainen, Finland) and compared it to a highly sensitive multiplex real-time PCR method[1] for the detection of influenza viruses in clinical specimens. During the influenza season 2005/2006 nasal swabs were collected from 752 patients presenting with influenza-like symptoms at three private medical centers in the Helsinki area. The swabs were placed in the elution buffer supplied with the test kit. One-hundred microliters of the eluted material was diluted 1:2 in RNAlater (Applied Biosystems, Helsinki, Finland) and stored frozen before PCR testing. The Actim test was performed immediately, and interpreted by an experienced technician. For PCR testing, RNA was extracted from the samples, reverse transcribed using random hexamer primers and influenza virus-specific gene segments were amplified. One-hundred and ninety-four samples were found positive for influenza A and 156 for influenza B by PCR. For influenza A, sensitivity and specificity of the point-of-care test were 70 % and 99 %. The corresponding numbers for influenza B were 58 % and 98 %. Estimated from the Ct value obtained by PCR, false-negative samples contained significantly less virus material than true-positive samples and more time had elapsed between onset of symptoms and collecting of samples. The Actim Influenza A & B test is easy to perform, to read, and to interpret, and it reliably detects both influenza A and B viruses during the first days of illness, i.e., when the patient may benefit from specific antiviral treatment.[1]Templeton et al. J. Clin. Microbiol. 2004; 42:1564-1569.

Abstract P522

Qualification of the Hemagglutination and Microneutralization Assays for High Throughput Screening of Sera for Neutralizing Antibodies Against an H5 Hemagglutinin

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Influenza infects hundreds of thousands of people every year, causing approximately 40,000 deaths in the United States alone. Outbreaks of avian H5N1 influenza virus strains have resulted in human mortality rates of >50% and the continued spread of these increases the likelihood of an influenza pandemic. Vaccines designed to prevent avian influenza infection in humans are being developed and tested using different end-point assays. Here we present qualification data on two such assays, the Hemagglutination Inhibition (HAI) and Microneutralization (MN) assay. The HAI assay measures the levels of anti-influenza antibodies in the sera of vaccinated subjects, based on the ability of the sera to inhibit binding of the virus hemagglutinin to sialic acid residues on red blood cells. Assay controls were monitored on 905 plates analyzed on 33 different days. Plate failures were less than 2% (failure defined as a standard deviation value > 2-fold the plate median value). No correlation (N=5) between the reagent source (equine red blood cells) and the median values of the positive or negative control sera was found. Precision, intermediate precision, linearity, range, robustness, and assay specificity are discussed. The MN assay measures neutralizing antibody based on the ability of the same sera to inhibit virus nucleocapsid protein synthesis (replication inhibition). Similar to the hemagglutination assay, less than 3% of the 457 assay plates analyzed on 17 different days failed to meet statistical pass criteria. Finally, we monitored signal-to-noise ratios and Z-values to demonstrate adaptability of these assays to automation and high throughput screening.

Abstract P523

Evaluation of Rapid Influenza Diagnostic Tests to Detect Influenza A Viruses With Pandemic Potential

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Background: The emergence of human infections with novel influenza A viruses is an urgent global public health problem because of the potential for an influenza pandemic if the novel viruses acquire the ability for sustained human-to-human transmission. Prompt diagnosis of human infection with novel avian influenza A viruses is critical to implement control interventions and will be especially important for clinic management during the early stage of the next influenza pandemic. Currently there are 13 rapid influenza diagnostic tests (RIDTs) commercially available in the U.S. Although studies have assessed the accuracy of RIDTs to detect infections with human influenza viruses, there are limited data about the ability of RIDTs to detect infections with avian influenza A viruses of pandemic potential. Methods: We assessed the comparative sensitivity of eight commercially available RIDTs to detect avian and human influenza A viruses. The sensitivities of RIDTs to detect serial dilutions of highly pathogenic avian influenza A(H5N1) viruses and currently circulating human influenza A(H1N1) and A(H3N2) viruses were compared. Six avian influenza A(H5N1) viruses from the genetic clades 1 and 2 isolated from human and avian specimens, two human A(H3N2) and two human A(H1N1) viruses were tested. An EID50 was determined for each virus to determine the dilution of virus sample required to yield a fifty-percent positive result. Results: All eight RIDTs assessed in this study had similar sensitivities to detect serial dilutions of human and avian influenza A viruses, including highly pathogenic H5N1 viruses. All but one RIDT performed consistently with both clades of highly pathogenic avian influenza A(H5N1) viruses. The detection level of the RIDTs averaged seven logs lower than the average EID50 for avian influenza A(H5N1) viruses. Overall, there were no differences in the ability of RIDTs to detect serial dilutions of A (H5N1) viruses compared to A(H3N2) and A(H1N1) viruses. Conclusion: Although currently available RIDTs do not distinguish between influenza A virus subtypes, the RIDTs assessed in this study were able to detect highly pathogenic avian influenza A (H5N1) viruses. Such tests may be useful for clinical management during an influenza pandemic.

Abstract P524

The Development and Application of Real-Time NASBA Technology for Detection of Avian Influenza H5N1 Virus

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It is not easy for credible detection of avian influenza (AI) in clinical samples because of the low virus titer. A nucleic acid sequence based amplification (NASBA) assay in combination with a molecular beacon was developed for the real-time detection and quantification of avian influenza H5N1 virus. The highly conserved regions of HA and NA genes were targeted. Specially, NA target is H5N1 specific. The sensitivity of NASBA H5 and N1 were tested with 10-fold dilutions of viral RNA and transcription RNA in vitro, the dilutions of virus were down to $10^{-1}TCID_{50}$ or 10^{-3} hemagglutination units (HAU), and the dilutions of transcription RNA in vitro is 10 copies/μl. The sensitivity is similar as Real-time RT-PCR assay. The specificity of the assay was demonstrated by testing with other subtype influenza viruses collected at different years such as H1N1, H3N2 and H9N2, only H5N1 viruses were positive. The H5N1 NASBA assay were applied for H5N1 patient diagnosis in China, 17 specimens from 8 H5N1 patients confirmed in 2005 by virus isolation were all tested positive by this assay; and a serial respiratory samples were collected from one confirmed H5N1 patient after confirmation of infection and also after treatment, the results showed that the samples collected at 8d, 9d and 13d were H5N1 positive, and the samples collected at 14 d, 20 d and 27d were negative. The results were accordant with the patient's clinic symptom. Compared with real-time PCR, the sensitivity of NASBA assay is similar, but is more easily and faster. The NASBA technology based assay we have developed here will provide a convenient diagnosis method for H5N1 detection.

Abstract P525

Virological Assessment and Usefulness of Rapid Diagnostic Kits for Influenza

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Background: As a result of the widespread adoption of rapid diagnostic tests the accuracy of influenza diagnosis has improved, and treatment with anti-influenza drugs has become routine in Japan. In the 2006/2007 season 15 different types of rapid diagnostic kits were released on the market in Japan, and about 20 million tests were used. Objectives: We measured the detection limits of 15 different kits by using virus strains whose viral infectivity titers had been measured by plaque assay and performed a comparative assessment with virus isolation and RT-PCR. We also investigated the distribution of viral loads according to clinical specimen, and we assessed the usefulness of the rapid diagnostic kits. Methods: Seven different vaccine virus strains and AH1N2 strain isolated in Yokohama were used. Sensitivity among the rapid diagnostic kits, virus isolation, and RT-PCR were compared. In addition, viral loads in clinical specimens (gargle fluid, pharyngeal swabs, nasopharyngeal swabs, nasopharyngeal aspirates) were plaque assayed according to type and date of collection. Results: The detection limit was 2.8 to 8.3 pfu/test for isolation culture and nested RT-PCR and $2.5x10^3$ to $3.2x10^5$ pfu/test for the rapid diagnostic kits. There were differences of 10 fold to 50 fold for the same virus strain, and there were differences of up to about 20 fold for the same product due to differences in virus strain. In addition, detection sensitivity tended to be lower for type B strains than for type A strains. The distribution of virus loads in the clinical specimens showed that more virus loads were contained in the nasal specimens than in the pharyngeal specimens. Conclusions: Virus loads of 10^3 pfu/test or more are needed for rapid diagnostic kits, and when the virus load is low or specimen collection is not adequate, the results become false negative. It is important to collect specimens that contain as much a virus load as possible.

Abstract P526

Development of the Rapid Diagnosis Test for Avian Influenza Viruses

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The outbreak of a pandemic influenza derived from a mutated avian influenza virus has been an apprehensive issue in the world. When the pandemic influenza broke out, a rapid diagnosis test by immunochromatography should be indispensable to distinguish the pandemic from the seasonal influenza and other febrile illness. To establish the rapid diagnosis test a monoclonal antibody, 4E3, which reacts with an epitope located in 1/3 of N-terminus of NP of A/Turkey/Ontario/7732/66 (H5N9) (immunogen) was produced and used for both solid-phase and colored latex-conjugated antibody of an immunochromatography (sandwich method). This rapid test detected at least $4x10^5$ focus forming unit (FFU)/ml of A/Crow/Kyoto/04 (H5N1) in 10 min, and it detected $3.4x10^5$, $5.3x10^5$, $1.1x10^5$ FFU/ml of an H5N2, H5N3, H5N9 virus, respectively. It also reacted with 15 strains (H3-H15) of avian influenza viruses (AIV) except H5 viruses. On the other hand it neither reacted with 19 human influenza viruses (A and B type), 22 other respiratory viruses or 24 bacteria. Furthermore, the rapid test detected all 10 strains of AIV (H5N1, Z type, clade 1) isolated from 10 patients in 2004 in Thailand and it also detected 1 of 1 strain of AIV (H5N1, probably clade 2-1) isolated from one patient, 2 of 2 strains of H5N1 viruses isolated from 2 ill pigs, and 7 of 7 strains of H5N1 viruses isolated from 7 dead chickens in 2006 in Indonesia. At present H5N1 viral load at nasopharyngeal region in patients with AIV infections seems not high as in case of seasonal influenza. However, viral load of the presumed pandemic influenza virus in the nasopharyngeal region which is very contagious among humans must be high enough as in the case of seasonal influenza. These results reveal that this rapid diagnosis test has enough sensitivity, specificity, stability for antigen recognition and rapid performance. This test should display great ability as a powerful tool for the rapid containment and triage especially in the early-phase of the pandemic influenza.

Abstract P527

Clincal Evaluation of Rapid Diagnostic Tests for the Detection of Influenza Viruses–What Are the Conditions That Influence Their Performance?

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Background: Rapid and accurate diagnosis is very important for the control of influenza. In Japan, commercially available rapid antigen detection tests have been increasingly used 1998: more than ten million tests are conducted every year. These tests obviate the need for other tests, prevent unnecessary antibiotic use, and are also useful for the appropriate use of antiviral agents. Objectives: We evaluate the performance of four commercially available rapid test kits: Espline Influenza A&B-N(Fuji Rebio), rapid Testa FLU Stick (Daiich Pure Chemicals), QuickVue Influenza Test (Quidel) and Clearview Exact Influenza A&B(Inverness medical), and examined the conditions that might influence the rate of detection of influenza viruses with these kits. Methods: Three types of fresh respiratory specimens (pharyngeal swabs, nasopharyngeal swabs and nasopharyngeal aspirates) were collected from patients with influenza-like illness during the influenza A and B epidemics in 2004/2005.The performance of the rapid tests were compared with the results of viral isolation in cell culture and nested RT-PCR. Results: Of 3000 respiratory specimens collected from the patients, 60% were positive for the virus in cell culture. As compared with the results of the cell culture. the sensitivity of detection using the kits was 67-94% for the type A virus and 65-89% for the type B virus, and the specificity was more than 94% for all the kits. Greater sensitivity was obtained when nasopharyngeal specimens were used, as compared to that when pharyngeal swabs were used. The sensitivity of detection was higher for H3N2 than for the type B virus. The sensitivity was lower when the test was conducted within 12 hours from the onset of the illness than when it was conducted over 12 hours after the onset of the illness. The sensitivity was also lower when the patients had low-grade fever than when they had high-grade fever. Conclusion: We conclude that the rapid diagnostic tests are easy to use and have high sensitivity and specificity, especially for H3N2. We have to consider the type of specimen collected and the condition of the patients when attempting to diagnose influenza using these tests.

Abstract P528

Novel Point of Care (POC) Multianalyte Assay for Influenza Virus Types A and B and for Seasonal and Potentially Pandemic Subtypes of Type A Virus

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Background: Rapid POC diagnostics for Influenza have generally acceptable specificity. However, they lack sensitivity when compared with laboratory assays. The Centers for Disease Control & Prevention (CDC) is funding the development of next generation POC assays intended for the rapid diagnoses of individuals with influenza-like illness. Nanogen provides a summary of its assay design and early phase proof-of-concept. Method and Materials: The multianalyte POC assay under development, in collaboration with HX Diagnostics, reduces background while improving the sensitivity and specificity of the assay. The technology employs a fluorescence detection system. The fluorophore is coupled with a capture system that enables the homogeneous binding of multiple monoclonal reagents to specific analytes, and captures the complex onto specific test lines. Pyranosyl RNA (pRNA) is a series of different oligonucleotides conjugated to different monoclonal antibodies. A complementary pRNA strand is bound to a nitrocellulose membrane to capture the appropriate monoclonal antibody-analyte complex. A simple reader collects patient and assay data to report the test results. In order to demonstrate improved sensitivity with this technical approach, a dilution curve was developed using Influenza A subtype (A Texas 1/77) and Influenza B (Hong Kong 5/72). Each point on the curve was tested with 5 replicates. When the leading POC assay was used to test each dilution, the assay was non-reactive. Conclusion: Type A and Type B analytes show at least two logs improvement in sensitivity for a simple and rapid POC assay compared to leading POC Influenza assay. Nanogen acknowledges support from the U.S. Department of Health & Human Services (HHS) through the CDC, and the Office of the Assistant Secretary for Preparedness and Response (ASPR) for advanced product development under Contract No. HHS200-2007-19345

Abstract P529

Mounting And Validation Of Immunoperoxidase Method for Detection of Influenza Virus in Lung Tissue of Balb/C Mice Embedded in Paraffin

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Background: By significance of Influenza virus like agents producers of a high mortality, associated to pneumonia, labs requires faster and less bothersome methods alowing detection of viral antigen from clinical samples. Materials and Methods: Within these samples those from necropsies preserved in paraffin, occupy a key place. In our paper, we introduced and validated immunoperoxidase technique used to detect antigens (HA and NP) in lung tissue of Balb/C mice experimentally infected and embedded in paraffin, using monoclonal antibodies against hemagglutinin and nucleoprotein. Results: Infective dose (DIE_{50}), and intranasal route were satisfactory. Clinical symptoms detected were: hair bristling, anorexia, weight loss, slight nasal discharge and grouping. Clinical picture corresponded with interstitial pneumonia detected by hematoxilin-eosin technique and immunoperoxidase. By means of optical microscope we visualize immunoreaction in cytoplasm of brown cells infected, indicating positivity of this test. There weren't non-specific in infected tissues neither in controls. Conclusions: Method was sensible, specific, and fast, allowing also retrospective analysis in human tissues of epidemiologically and clinically significant samples, occasionally it is a very important feature.

Abstract P530

Multianalyte Point of Care (POC) Assay For Influenza Virus Types A and B and For Seasonal or Potentially Pandemic Subtypes of Type A Virus

Huimiao Ren, Roy Chung, Roger Lankford, Wenli Hung, Dan Weaver, Sheetal Patel, Li-Wen Huang, Richard Egan

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Background: Currently there are no POC assays that can also provide subtyping information. With the emergence of H5N1 the potential is growing for a pandemic subtype of Type A influenza. Nanogen is developing an assay to simultaneously detect Type A, Type B, seasonal subtypes H1N1 and H3N2, as well as H5N1. This will provide necessary diagnostic data while providing important time sensitive epidemiological data to the public health sector. A summary is as follows. Method and Materials: The POC assay under development, in collaboration with HX Diagnostics, is based on a nitrocellulose lateral flow system that enables high sensitivity detection of multiple analytes. The technology employs a fluorophore coupled with a capture system that enables the homogeneous capture and detection of multiple individual analytes. A simple reader collects patient and assay data and reports the test results. An experiment was conducted to confirm the ability of the assay to detect multiple analytes in a single assay. Monoclonal antibodies (MAb) specific for Type A and Type B were striped while, separately, two types of pRNA were striped at the positions corresponding to MAb specific for H1 or H5 HA antigen. Examples of Flu B, and H1 and H5 subtypes of Type A virus were tested. Discussion/Conclusion: The multianalyte POC lateral flow assay can distinguish between Type A & Type B influenza and specific subtypes of Type A. This new technology will enable users to screen symptomatic patients for Influenza and determine if Type A is a seasonal or pandemic strain providing important clinical information. Nanogen acknowledges support from the U.S. Department of Health & Human Services (HHS) through the CDC, and the Office of the Assistant Secretary for Preparedness and Response (ASPR) for advanced product development under Contract No. HHS200-2007-19345

Abstract P531

Detection of Avian Influenza A/H5/N1/ H7 Viral Isolates With Highly Sensitive, Specific and Inclusive Real-Time RT-PCR Assays in Lyophilized Format

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Background: Real-time reverse transcription-polymerase chain reaction (RT-PCR) based tests were demonstrated to rapidly and accurately detect viruses such as influenza and SARS. In response to potential avian Influenza A, H5N1 H7 outbreaks, we developed highly sensitive and specific real-time RT-PCR assays for detection of influenza A, H5N1 and H7 viruses for use by public health officials and researchers in detecting, evaluating and managing the spread of influenza viruses. Methods: We have developed and validated automated TaqMan assay design algorithms and pipeline for accurate and efficient detection of target nucleic acid sequences. We utilized these algorithms to generate two sets of assays to specifically detect the majority (> 95%) of recently identified avian influenza isolates: i) influenza A strains and ii) subtype H5 strains. The designs involved amplification of multiple regions to enable broad strain coverage and increase the probability of detecting evolving viral strains. Applied Biosystems 7500/ 7500Fast/7300/7000/7900 Real-Time PCR systems were used to demonstrate performance of the Influenza tests. Tests are designed in dried-down format where total reaction mix (One-step RT-PCR reagents) was lyophilized. Results: High inclusivity of the assays was demonstrated by thorough in silico analysis and confirmed using a broad range of avian influenza isolates in validation studies conducted in several influenza testing laboratories globally. The assays demonstrated high efficiency and sensitivity (10-100 copies per reaction) with a broad range of influenza A strains. Conclusions: Enhanced inclusivity and sensitivity of the assays will increase the probability of detecting newly emerging strains that can result from genetic drift within the influenza A H5N1 and H7 subtypes. Our bioinformatics algorithms and assay designs can be rapidly optimized to include new sequence information as it becomes available for newly emerging, highly pathogenic influenza isolates. For research use only. Not for use in diagnostics. Not for sale in the US.

Abstract P532

Clinical Validation of a Molecular Method for the Simultanous Detection Of 11 Respiratory Viruses Causing Influenza-Like Illness

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Introduction: Since numerous viruses can be clinically indistinguishable from influenza, Genaco Biomedical Inc. developed a target enriched multiplex PCR, with xMAP suspension array detection, for 11 viral respiratory pathogens in a single reaction. Supplied as a ready-made kit, ResPlex II targets influenza A and B, RSV A and B, parainfluenza virus 1-4, hMPV, enterovirus and rhinovirus. Our study evaluated the performance of this assay on 159 negative respiratory specimens spiked with up to three viruses. Additionally, more than 100 specimens from patients presenting with influence-like illness (ILI) were tested. Method: Previously negative respiratory specimens were spiked with known virus at, or near, the limit of detection for each target, which has been determined to be <100 gene copies/reaction. Spiked samples were extracted using the bioMérieux easyMAG extractor and reverse transcribed with the Bio-Rad iScript cDNA synthesis kit. PCR was performed with the ResPlex II kit using Qiagen Multiplex buffer supplemented with PCR buffer and HotStarTaq. PCR products were hybridized to ResPlex II bead mix and labeled for xMAP detection on a Bio-Rad Bio-Plex. Specimens collected from patients with ILI were tested in the same manner. Results: Of the 229 expected virus detections in the 159 spiked samples, 212 (93%) were detected by ResPlex II. Additionally, 66 infections not previously identified by conventional culture were detected, 54 of which were confirmed by an alternate molecular method. For the unconfirmed additional infections, no suitable optimized alternate assay was available in this laboratory. 14 (6%) of the samples that tested negative on the ResPlex II panel but positive by real-time PCR had Ct values >35, indicating less than 50 gene copies/reaction. Test data on the ILI specimens will be presented. Conclusion: The ResPlex II assay provides a rapid and sensitive technique for the detection of influenza virus as well as numerous other respiratory agents which may mimic influenza infection. When applied to the specimens in this study, additional agents were detected, along with previously unknown multiple infections. Our work illustrates the power of ResPlex II to effectively detect multiple viruses with comparable speed and sensitivity to single target real-time PCR.

Abstract P533

Detection and Characterization of Human Influenza Virus by Bioplex Bead Suspension Array System

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Background: Human influenza viruses cause seasonal and sporadic outbreaks of respiratory disease. However, there are many other viral and bacterial etiological agents that also cause influenza-like illness. Therefore, it is important to be able to accurately diagnose the cause of illness in order to respond appropriately. Real-time reverse transcriptase PCR (rRT-PCR) provides a highly specific and rapid method of virus detection. However, the implementation of this method may be limited due to logistical reasons such as equipment cost or availability. Furthermore, rRT-PCR is restricted in its ability to detect multiple targets in a single reaction. In order to address these limitations, we investigated the feasibility of adapting the use of established diagnostic rRT-PCR primers and probes designed for detection and characterization of human influenza to a multiplexed suspension bead array system. Methods: Multiple oligonucleotide primer sets designed for universal detection of type A and type B influenza as well as characterization of seasonal human H1 andH3 influenza viruses by rRT-PCR were used to amplify specific gene targets from total RNA extracted from grown viral material. Amplified RT-PCR products were subsequently analyzed by hybridization to target-specific oligonucleotide probes coupled to fluorescence-labeled polystyrene beads (BioPlex Suspension Array System). Positive control materials were designed by synthesizing oligonucleotides that were complementary to the detection probe sequences. Influenza virus from specimens or controls was detected by an rRT-PCR protocol which has been established at the Centers for Disease Control and Prevention. Results: 1) Oligonucleotide labeled bead hybridization assays demonstrated limits of detection between 5 and 14 pg/µl of total RNA, or between 10 and 100 fmol control oligonucleotides. 2) Multiplexed analysis of RT-PCR amplified products did not change the detection limits of the assay. Conclusion: The BioPlex suspension array system for detection and characterization of human influenza demonstrated reaction sensitivity and specificity comparable to established rRT-PCR methods. Oligonucleotide primers and probes specifically designed for rRT-PCR can be easily adjusted to be used in a multiplexed bead-based assay for the typing and subtyping of influenza virus.

Abstract P534

The Important Role of Molecular Diagnostics in the Control of Avian Influenza

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The year 2007 will mark 10 years since H5N1 was first reported in humans in 1997. Once considered to infect only birds, it has crossed the species barrier and each new case of human infection raises the risk of a mutation leading to human-to-human transmission, and putting millions of lives at risk. Early diagnosis is important to safeguard against avian influenza. A nucleic acid sequence-based amplification (NASBA) technique was developed to detect avian influenza A subtype H5 (NASBA-H5) from various distinct lineages of influenza virus in different geographic locations and times. No false positive signals were observed with other viral pathogens of influenza A virues of subtypes other than H5 as well as other clinically relevant pathogens. The assay also has similar sensitivity with virus isolation. Our NASBA-H5 assay is in use to assist monitoring programmes for H5 influenza in the poultry industry in China and Japan. We have also tested the system on human clinical specimens and it can identify the H5 subtype at a level of 10^{-9} dilution using a virus culture with titer $10^{8}TCID_{50}$/ml. We modified the original electro-chemiluminescence (ECL) detection method to enzyme-linked oligonucleotide capture (EOC) method to allow its use in remote test sites. The EOC method utilizes ELISA plate reader, making it cost-effective. As the end-product of NASBA reaction is RNA, which tends to be unstable under normal environmental conditions, the possibility of carryover contamination of equipment from previous experiments is minimised. Moreover, standardization between different laboratories is possible as NASBA is an isothermal process. In a crisis of global importance, standardized testing utilization will facilitate a concerted effort to deter a catastrophic outbreak of avian influenza. The rapid assay can generate results within a few hours and may be a suitable alternative for routine screening. With the help of advanced biotechnology diagnostic tools, culling of poultry and human loses can be minimized.

Abstract P535

Realtime RTPCR Assays for Universal Detection of N1 Subtype and Discrimination of HPAI H5N1 Genotype Neuraminidase Genes of Influenza A Viruses

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Type A influenza viruses are divided into subtypes based on antigenic differences in their surface hemagglutinin (HA) and neuraminidase (NA) glycoproteins. There are nine known NA subtypes (N1~N9) that have been identified. N1 NA genes are found in seasonal human H1N1 as well as a number of animal virus subtypes including swine H1N1 and highly pathogenic avian (HPAI) H5N1 influenza that occasionally infect humans causing mild to fatal respiratory disease. Because influenza viruses are capable of genetic reassortment, it is important to be able to distinguish between human and animal genotypes of N1 NA in order to detect a reassortment event. In this study, real-time reverse transcription PCR (rRT-PCR) assays were optimized using primer/probe sets designed for 1) universal detection of all known N1 subtype NA genes, and 2) specific discrimination of NA genes of HPAI H5N1 genotype. Primer/probe sets were designed based on available nucleotide sequence data of 1,698 NA gene sequences. The universal N1 rRT-PCR primer/probe set was shown to detect NA genes of all N1 subtype viruses tested, including HPAI H5N1, seasonal human H1N1, PR8 H1N1, human pandemic 1918 H1N1, North American Swine H1N1, avian H1N1 and avian H7N1 viruses. Nonspecific reactivity was not observed when RNA from other type A subtypes (N2, N3, N4, N5, N6, N7, N8, N9), FluB or nasal wash collected from uninfected humans was tested. The HPAI N1 genotype specific rRT-PCR primers/probe set was shown to specifically detect the NA gene of a number of HPAI H5N1 influenza viruses tested and did not demonstrated nonspecific reactivity against NA genes of other N1 genotypes or other influenza virus NA subtypes tested. The rRTPCR assays described here can serve as effective tools for rapid subtype characterization of the N1 NA gene of human and animal influenza type A viruses as well as to discriminate HPAI N1 NA genes. The combined use of these assays will allow for the detection of a reassortment event involving the HPAI N1 NA gene in human and/or avian influenza viruses.

Abstract P536

Diagnostic Preparedness: Development and Use of Influenza Diagnostic Tests for Seasonal and Pandemic Influenza Response

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Background: Diagnostic tests for detecting influenza viruses are important for seasonal influenza surveillance and patient management. During a pandemic, laboratory tests will be used to guide implementation of non-pharmaceutical interventions such as school closures, to monitor pandemic spread and impact, and to guide management of cases and contacts. To meet this challenge, improved rapid influenza tests and guidance on their use during a pandemic are needed. Methods: We evaluated current and emerging diagnostic technologies and identified settings where greatest improvement in influenza detection was needed. We then solicited proposals to develop point-of-care tests for outpatient use (e.g., clinics, emergency departments) to differentiate seasonal from avian or other novel influenza strains. Proposals were independently reviewed and ranked. To further define the use of these and other influenza tests prior to, and during a pandemic, we reviewed published literature, consulted subject matter experts, and developed recommendations. Further engagement and input from clinical, laboratory, and public health partners is planned. Results: Among proposals received, four developers were chosen. Awards were made in December, 2006; approval and commercial availability are anticipated by 2009-10. Novel approaches for using reverse transcriptase polymerase chain reaction and immunoassays with high sensitivity and specificity are being pursued. A further description of progress in the development of these point-of-care tests will be presented. Preliminary guidance is being prepared and will provide recommendations for use of current and planned diagnostic tests in different settings (public health and referral laboratories, hospitals, clinics, and other outpatient sites such as ports of entry) for each of the defined WHO phases and U.S. stages of a pandemic. Recommendations will address 1) the need for enhanced testing to detect cases early during pandemic spread in a community, 2) the transition to targeted test usage as cases become more frequent, and 3) the criteria for use of testing to confirm the resolution of the pandemic wave. A description of these recommendations and proposals for other test development will be presented. Conclusions: Advanced development of commercial influenza diagnostic tests and guidance for use of various tests for surveillance and case management represent important steps for enhancing pandemic preparedness.

Workshop Topic #6

Animal Influenza Ecology

Poster Abstracts

Abstract P601

Detection of Influenza Virus in Wild Birds by RT-Nested PCR in West and East Slovakia

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Avian influenza viruses are zoonotic agents recognized as a continuing threat to both veterinary and human public health. Wild aquatic birds are considered to be natural reservoirs of all 16 haemagglutinin and 9 neuraminidase subtypes of influenza A viruses. Two of dominant European north-south and east-west birds' migratory routes pass across Slovakia. Several studies have been done concerning the role of birds as distributors of several pathogenic agents. In most cases, the routine testing of samples obtained from wild birds will find some influenza viruses. The vast majority of these viruses cause no harm to their carriers. In April 2006, a total of 33 wild birds were caught in National Park (NP) - Senianske ponds in east Slovakia. This NP represents one of the most significant locality for breeding and resting water birds in Slovakia. In June 2006, a total of 42 wild birds of 11 free living species were caught in NP - Parizske mociare Marsh, one of the largest wetlands in west Slovakia, located near to the villages Gbelce and Nova Vieska. Tracheal and cloacal swabs were examined for the presence of influenza virus by RT- nested PCR. In east Slovakia, 13 samples obtained from trachea and 11 samples obtained from cloaca were found positive for influenza virus. Total 18 birds, what represents 54%, were found positive for influenza virus in east Slovakia. The influenza virus was detected in 8 individuals in cloacal swabs, and in six causes in tracheal swabs obtained in Jun. Total 11 birds, what represents 26%, was found positive for influenza virus in west Slovakia. This research was supported by Slovak Research and Development Agency (the contract No. APVV-51-004105 and APVT-11-040502).

Abstract P602

Serological Evidence for the Avian Influenza Subtype H9 Virus in Domestic Birds in Nepal

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Surveillance for the Avian Influenza A virus was conducted in Nepal from January 2006 to December 2006 by collecting 930 samples from domestic birds in 28 districts of the country. Out of 930 samples, 558 were serum and 372 were cloacal, tracheal and tissue. Serum samples were tested by conducting C-ELISA and HI to detect antibody against Influenza A subtype H5, H7 and H9 virus. All tested sera were found negative for H5 and H7 subtype however, 3.94% of tested samples were found positive for the presence of subtype H9 virus of Avian Influenza virus in poultry. Meanwhile 372 swab and tissue samples were subjected to rapid antigen detection test, virus isolation and RT/PCR test which were found negative for Avian Influenza virus and viral antigen. These results suggest that Nepal is free from bird flu till to date.

Abstract P603

SCHOLARSHIP International Scholarship

Influenza Subtype H3 Circulation in Fattering Pigs of Argentina

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Pigs are important hosts in the ecology of the influenza viruses because they are susceptible to the infection by avian and mammalian influenza strains, including the human ones, for that reason they have been proposed possible "mixing vessel" for events of reasortment between human and bird viruses. In the United States for more than 80 years, the classic subtype H1N1 was the only one associated with influenza respiratory disease in pigs and since 1998 the subtype H3N2 has been associated to respiratory symptoms and/or reproductive disorders, whereas in East Europe this subtype was detected from 1984. The objective of this work was to investigate the seroprevalence of influenza A subtype

H3N2 antibodies in fattering pig farms of Argentina. From 2000 to 2002, 490 serum samples of fattering pigs with 13 to 23 weeks of life were collected from 19 farms of Buenos Aires (10), Córdoba (4) and Santa Fe (5) provinces. Sera were treated with RDE and adsorbed with turkey red blood cells. The hemagglutination inhibition test was made using an antigen prepared with A/Sydney/5/97 H3N2 and the homologous ferret antiserum as positive control. Those sera that presented titers $\geq$ 40 were considered positives. Eighty positive sera were detected in 8 (42%) farms, giving a global positivity of 16,32 %(80/483). If we consider the positive farms separetely, the % of positivity and the titer gemetric means were: 77.8 (160), 46,15(160), 31,8(160), 30,9(80), 23,1(80), 22,2(80), 18,5(60) and 12.2(190) each. With the obtained results it is possible to be inferred that influenza A (H3) virus would have circulated in the population of the studied pigs. It becomes essential to make deep studies including viral isolation to attempt the antigenic and genomic characterization of the recovered virus and see the relationship with the circulating strains in the human population and other species.

Abstract P604

Isolation and Genetic Characterization of Novel Human-Swine Reassortant H3N2 Influenza Viruses Circulated in Taiwan

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Prior to our intensive surveillance programme for influenza (flu) activities in Taiwanese pigs launched in July 1998, two kinds of H3N2 viruses had been reported: the earliest and wholly human H3N2 (A/Hong Kong/1/1968)-like flu virus (A/Swine/Taiwan/7251/1970) and an intermediate human-like swine H3N2 flu virus (A/Swine/Hong Kong/127/1982), possessing hemagglutinin (HA) and neuraminidase (NA) genes from human flu viruses and the other six genes coding for internal proteins derived from classical swine flu viruses. Sequence comparisons of H3N2 viruses isolated after 1998 presented in this report, however, indicated that at least three individual outbreaks caused by H3N2 flu viruses, which were identified in 2002, 2003 and 2004, respectively, revealed different constellation of genes from their genome compositions. Five genes coding for HA, NA, and 3 polymerases (PB2, PB1 and PA) were best matched with those of human H3N2 flu viruses circulated around the early 1980s in Hong Kong and Nanjing. The other 3 internal protein genes (nucleoprotein, matrix proteins and non-structural proteins) were highly similar to the corresponding genes from the contemporary North American swine H1N1 flu viral variants. The results suggested that one unique version of human-swine reassortant H3N2 flu viruses, which might emerge around the 1980s, had perpetuated in pigs and raised threat concerns on swine industries and public human health owing to the potential of inversely infecting people from pigs, at least in Taiwan.

Abstract P605

Genetic Analysis of the H5 Hemagglutinin Genes from North American Avian Influenza Viruses of Wild Bird Origin

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Some strains of the highly pathogenic avian influenza (HPAI) H5N1 viruses currently circulating in Asia have the unusual ability to cause morbidity and mortality in certain species of domestic and wild birds including ducks and other wild bird species. Currently little is known about the molecular basis of this increased pathogenicity in wild birds, however because of the role of the hemagglutinin (HA) protein in the pathogenesis of the virus in other species, evaluating the differences in HA gene sequence between pathogenic and non-pathogenic viruses of the same subtype may provide insight. Evaluation of low pathogenic avian influenza (LPAI) viruses isolated from wild birds in North America provides one such data set for initial evaluation. Here we present initial genetic analyses, including proteolytic cleavage site sequence analysis, and phylogenetic analysis of the H5 HA genes of 70 H5 viruses isolated from wild birds in North America between 1975 and 2006. Sequences of the duck-origin viruses from North America were also compared to the H5 HA from chicken-adapted avian influenza viruses from Mexico and the Asian H5 HPAI HA. Numerous sublineages of H5 viruses in wild bird-origin isolates were observed. All North American wild bird viruses had low pathogenic type cleavage sites and all but one were of North American lineage. In experimental inoculation studies in chickens with selected viruses, no clinical disease was observed.

Abstract P606

Genetic Identification of Influenza A Virus From Wild Birds in Barrow, Alaska

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Influenza A viruses have evolved into two different genetic lineages corresponding to the Eurasian and the North American waterfowl migration systems. However, the Beringia area links the Old and the New world, and is an intersection of flyways for migrating birds where Eurasian and American birds meet. The Beringia, therefore, is one of the most interesting sites to study the epidemiology of influenza viruses. We visited and collected samples from shorebirds and waterfowl at Barrow, Alaska during a scientific Beringia expedition organized by the Swedish Polar Research Secretariate. The samples were immediately stored at -80°C and transported to the laboratory in an unbroken freeze chain. Screening for positive samples was performed in two real-time PCR systems, a SYBR Green PCR and a Taq-man PCR with probe. Four (2%) of the 201 samples were positive for Influenza A virus RNA and typing was performed by direct sequencing, and subtypes H3N8, H6N1 and H8N4 were found. After typing, the virus was isolated by inoculation in the allantoic cavity of 10-days old embryonated chicken eggs. Isolates were used for sequencing the remaining 6 genes. Phylogenetic analysis with all known sequences in the database shows that one HA and one NS gene clustered with the Asian rather than the North American lineage. We found chimerical virus possessing both American and Asian genes, which implies genetic leakage over the Bering Strait. How this can be without disturbing the distinction between the two lineages more than occasionally might be explained by the relatively low prevalence of Influenza A in the Beringia compared to other northerly regions in North America and Europe. Another factor can be the species of bird carrying the virus, since not all the important AIV reservoirs breeding in the area actually cross the strait. However, the true impact of the different birds is hard to determine from the information in the database, because normal annotation of influenza often only refer to the host as "shorebird". Both charadriiformes and anseriformes may well be annotated as "shorebird" although they would tell us different things about the natural ecology and spread of influenza virus in birds.

Abstract P607

Highly Pathogenic Avian Influenza A/H5N1 Vaccine Development in Kazakhstan

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The avian influenza A/H5N1 epizootic situation forced the Research Institute for Biological Safety Problems, Kazakhstan, to develop vaccines against the disease. Inactivated vaccines based on heterogeneous strains are recommended as AI specific prophylactic means (DIVA strategy). That is why we chose strain A/crachka/South Africa/61(H5N3) for developing an inactivated HPAI A/H5N1 vaccine. Virus was inactivated by dimer of ethyleneimine. ISA-70 and aluminium hydroxide were used as adjuvants. Immunogenicity and protective efficiency of emulsified and sorbed vaccines was detected on chickens. Vaccinated birds were challenged with HPAI strain A/domestic goose/Pavlodar/1/05(H5N1). Cloacal and tracheal samples were daily taken from the infected birds. In 28 days after single vaccination with emulsified vaccines chickens demonstrated 100% immunity with 9.4-10.1 $\log_2$ in hemagglutination inhibition test (HIT). After challenge there were no sick and dead birds, virus was not excreted. Birds vaccinated with sorbed vaccines demonstrated 50-80% immunity with 5.7-7.1 $\log_2$ in HIT. Challenge caused 7% death rate in vaccinated birds, virus excretion was registered in 72 hours after inoculation. 5% of clinically healthy birds also excreted virus but the titer was three times lower than in the control group. Emulsified vaccines containing AI virus heterogenic strain A/crachka/South Africa/61 (H5N3) antigens showed the best immunological and protective characteristics among all tested inactivated vaccines that were developed. Emulsified vaccine showed high efficiency against highly pathogenic avian influenza virus strain H5N1.

Abstract P608

Effect of an Inactivated Avian Influenza Vaccine Prepared From an Apathogenic H5N1 Reassortant Virus Generated Between H5N2 And H7N1 Isolates From Migratory Ducks in Asia on Protection of Chickens Against Challenge With Highly Pathogenic Avian Influenza Virus Strains

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Objective: Stamping-out policy is the base for the control of highly pathogenic avian influenza (HPAI). Vaccination may be a part of optional control measures in case when the outbreak widely spreads (OIE). In the present study, we developed an inactivated avian influenza vaccine prepared from apathogenic avian influenza virus strains isolated from migratory ducks in Mongolia. The vaccine was adjuvanted with mineral oil to form water-in-oil emulsion. Potency of the test vaccine was injected in chickens intramuscarly and evaluated by protection tests of the birds against the challenge with HPAI virus strains. Material and Methods: Of the library of influenza virus strains (Kida and Sakoda, 2006), 12 H5 subtype viruses were investigated for their phylogenicity and antigenicity. The vaccine strain was a reassortant virus generated from A/duck/Mongolia/54/01 (H5N2) and A/duck/Mongolia/47/01 (H7N1). The resulting reassortant virus, A/duck/Hokkaido/Vac-1/04 (H5N1) was grown in chicken embryos. The yielded virus was inactivated with formalin and adjuvanted. A single dose of test vaccine was injected into chickens intramuscularly. Three weeks post vaccination (p.v.), either A/chicken/Yamaguchi/7/04 (H5N1) or A/whooper swan/Mongolia/3/05 (H5N1) were inoculated intranasally into the birds. Clinical signs of birds and virus recovery from the birds were examined. Antibody responses were investigated by Hemagglutination-inhibition (HI) tests. In addition, the duration of effective protection provided by the test vaccine in chickens was investigated. Results: Serum HI antibody response was detectable from the vaccinated chickens at 8 days p.v., and reached the peak of 1,024 HI at 6 weeks p.v., then gradually reduced to 256 HI at 28 weeks p.v. Although infectious viruses were recovered from the vaccinated chickens, the test vaccine protected chickens from each of the two HPAI viruses without showing any disease signs for 14 days. All chickens challenged with A/chicken/Yamaguchi/7/04 (H5N1) on 6 days p.v. died, and all the chickens challenged with A/chicken/Yamaguchi/7/04 (H5N1) on 8 days p.v. survived, indicating that duration of protection provided by vaccination was 8 days. Discussion: The present test vaccine was confirmed to prevent the chickens from manifestation of disease signs but not shedding of infectious virus, although high titers of serum antibody response were induced.

Abstract P609

Influenza A Virus in Birds Nesting in Antarctica

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Introduction: The avian influenza viruses are related with influenza viruses isolated in other species. Non pathogenic strains are ubiquitous in aquatic birds which represent the original host of viruses that produce outbreaks in different mammalian species. Virulent strains can emerge by mutation or reassortment of preexisting strains. Possibilities of introduction of infectious agents in the Antarctica animal wild life and its spread from Antarctica to other continents are recognized issues since the signature of the Antarctica Treaty. The migratory birds could be a potential vector for those agents. The aim of this work was to know if birds that nest every summer in Antarctica could be potential carriers of influenza A viruses. Materials and Methods: The sampled species were: Pygoscelis adeliae, Pygoscelis Antarctica, Pygoscelis papua, Macronectes giganteus, Catarctha spp, Chionis alba, Larus dominica and Oceanites oceanicus. The sampling area was: Bahia Esperanza (63o24'S, 56o59'W), Peninsula Antarctica; Peninsula Potter (62o15'S, 58o39'W), isla 25 de Mayo; Punta Armonia (63o18'S59o11'W), isla Nelson, Islas Shetland del Sur. Cloacal swabs (CS) and sera of 140 healthy birds were collected between December 2003 and May 2004. Influenza A genome was investigated by RT-PCR amplifying a 560 nt portion of the M1 gene. Influenza A antigens in cells and influenza A Nucleoprotein were studied in those RT-PCR positive CS by Immunofluorescence Assay (IFA) and Western Blott (WB). A phylogenetic analysis was done with the M1 RT-PCR amplified products. The intent of viral isolation in MDCK and H292 cell lines and hemagglutination inhibition subtype especific antibody detection are ongoing. Results: We detected viral genome in 15/140 samples. From the positive ones, 7 also resulted positive by IFA and/or WB. The phylogenetic analysis showed close relationship with avian M1 protein sequences. The detection of genome and protein of influenza A virus are the evidence of the presence of viral components, making these birds influenza A virus potential carriers. It is necessary

to extend this kind of studies in the Southern Cone to improve the knowledge of the habitats and interaction of these birds with other species during its migratory routes.

Abstract P610

Migratory Bird Surveillance Program in Ukraine, Egypt and Kenya, July 2005-January 2007

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Migratory aquatic birds are thought to be natural reservoirs of avian influenza (AI) viruses and play a role in distributing virus throughout their flyways. Monitoring for emergence of AI strains, especially highly pathogenic AI (HPAI) in bird populations, is essential for the prevention of serious socio-economic and public health consequences. NAMRU-3 performed surveillance for influenza in migratory birds through collaborations with Ukraine, Egypt and Kenya. Between July 2005 and January 2007, 15 collection sites were established; five in the Black Sea, Ukraine, six in the Nile Delta, Egypt, and four in the Rift Valley, Kenya. Birds were humanely caught by mist-netting and cloacal swabs screened by real-time RT-PCR for the influenza A matrix gene with characterization of positive specimens for H5 and N1 genes. In Ukraine, 118 (6.5%) of 1,822 birds were positive for influenza A with one mallard found positive for the H5 gene characterized as low pathogenic avian influenza (LPAI). In February 2006, 32 additional samples were collected during a multi-species bird die-off and 14 were HPAI H5N1 (43.8%). In Egypt, 325 (11.5 %) of 2,821 birds tested positive for A matrix gene. Two specimens collected from Common Teals in October (LPAI) and December 2005 (HPAI) were determined H5 positive. The December specimen was closely related to the H5N1 viruses from subsequent poultry/human cases in Egypt and from the Ukraine bird die-off as judged by sequencing. In Kenya, 24 (5.5 %) of 438 birds were determined influenza A positive with one characterized as LPAI (H5N3). Overall, 467 (9.2%) of 5,081 specimens were influenza A positive with three characterized as LPAI H5 and one as HPAI H5N1. Detection of HPAI in the Common Teal implicates migratory birds as possible vectors for virus introduction into new geographical areas. Active surveillance within migratory flyways may serve as an early warning system to detect viruses with potential to spread into domestic flocks. The lack of H5N1 introduction into Eastern Africa clearly illustrates that generalized, multi-species "migratory flyways" are not useful in predicting spread. These data support the need for ongoing surveillance to define other major reservoir species and their individual migratory patterns.

Abstract P611

Use of Tetanus Toxoid as a DIVA (Differentiating Infected From Vaccinated Animals) Strategy for Sero-Surveillance of Avian Influenza Virus Vaccination in Poultry

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Strategies for differentiating infected from vaccinated animals (DIVA) require improvement for increased surveillance of avian influenza (AI). We propose a novel DIVA approach for poultry using tetanus toxoid (TT) as an exogenous marker independent of serotype and relatedness of circulating and vaccine strains. Of 1,991 wild and domestic poultry (1,640 chickens and 351 ducks) tested from Australia, Hong Kong and China, 100% were seronegative for TT antibodies without vaccination. Tetanus toxoid vaccine was immunogenic in both chickens and ducks, with mineral oil being a superior adjuvant to alum. Co-delivery of both TT and inactivated AI (H6N2) vaccines in chickens elicited strong TT and influenza-specific antibody responses with high antibody titres to both antigens persisting to 39 weeks post-vaccination. In addition, vaccination of chickens with a combined TT and AI (H6N2) vaccine elicited strong antibody responses to both antigens with no significant difference in titre between groups given the combined vaccine and either TT or AI vaccine alone. We conclude that TT is a highly suitable exogenous marker for AI vaccination in chickens allowing simple and effective monitoring of AI vaccination status.

Abstract P612

Sero-Surveillance of Avian Influenza Virus Vaccination in Ducks Using Tetanus Toxoid as a DIVA Strategy

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The FAO and OIE have recommended that all vaccination for avian influenza to be practiced using a DIVA (differentiating infected and vaccinated animals) strategy so that spread of infection can be monitored in poultry. Sero-surveillance of avian influenza in countries, including Asia and Mexico, utilising vaccination control programs requires improved DIVA strategies as difficulties have arisen from circulating virus strains of various subtypes eliciting cross-reactive antibody responses to vaccine strain. Ducks are highly susceptible to avian influenza infection and can shed and disseminate high titres of virus, especially in the village environment. We propose a novel DIVA approach using tetanus toxoid (TT) as an exogenous marker, since birds are not susceptible to tetanus and are not routinely vaccinated. Our first objective was to examine the natural seroprevalence to TT in ducks. Serum samples were analysed for antibody levels to TT using a competitive ELISA. Of 351 wild and farmed ducks tested, 100% were seronegative for TT antibodies. Despite a slow antibody response, TT was immunogenic in twice-vaccinated ducks (week 0 and 4), with mineral oil being a superior adjuvant to alum. Co-delivery of both TT and inactivated avian influenza (H6N2) vaccines in ducks elicited strong TT and H6-specific antibody levels at 6 weeks post-vaccination. We conclude that TT is a suitable exogenous marker for avian influenza vaccination in ducks allowing sero-surveillance of avian influenza.

Abstract P613

Avian Influenza in Saudi Arabia: Geographical and Historical Perspectives

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Saudi Arabia is a country that is located in the southwestern corner of Asia. Most of the country's land is desert and is located directly on the tropic of cancer. Most of poultry farms are established in the central and western part of the country. Chickens are the main source of poultry meat in the country. Importing of live birds for breeding purposes undergo a strict examination before bringing these birds into Saudi Arabia. Migratory birds cross the country seasonally, but few of them make a direct contact with citizens. The majority of the migratory birds stay in protected areas. Saudi Arabia has 15 different protected areas across the country. Avian influenza is a viral disease that causes respiratory symptoms in birds and other animal and human. The Low Pathogenic Avian influenza (LPAI), H9N2, was reported in 1998. The H9N2 Virus continued to cause mild respiratory disease and caused slight reduction in eggs' production. In the early 2006 the Highly Pathogenic Avian Influenza (HPAI), H5N1, was isolated from smuggled falcons that were brought to a veterinary clinic. Rapid diagnostic tests were conducted, positive samples were confirmed by PCR in agriculture ministry laboratory. Samples were sent to a reference laboratory for confirmation of the results, and parallel results were obtained. All infected and contacted falcons were killed. H5N1 was not reported in any commercial farms in the country. In conclusion the H9N2 virus is still circulating in the commercial farms. The H5N1 has been eradicated and no isolation of this virus has been reported in chickens and in any other birds yet.

Abstract P614

Clinical Evaluation of Conventional and Reverse Genetic Vaccines for Chickens Against H5N1 HPAI Viruses

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Avian Influenza virus H5N1 in Indonesia has been infected many chickens, pigs, humans and cats. Since 2004, to control H5N1 infection in chickens in Indonesia inactivated oil emulsion vaccines have been used including vaccines driven from seeds of avian influenza field isolate virus subtype H5N1 and low pathogenic virus H5N2 and H5N9. Recently, a non-pathogenic H5N1 master seed originated from H5N1 strain A/chicken/Indonesia/Legok/2004 created by reverse genetic method was used for production of inactivated oil emulsion vaccines. Modified HA and wild-type NA genes from influenza A/chicken/Indonesia/Legok/2004 (H5N1) virus and remaining genes from A/PR/8/34 (H1N1) virus was licensed for chickens and commercially available in Indonesia. The aims of this study are to know the clinical performance and the virus

shedding of chickens after vaccination and challenge test. The chickens vaccinated by vaccines, which were contained H5N1 subtype antigen showed better performance than H5N2 and H5N9 subtypes. Reverse Genetic H5N1 vaccine performance was better than H5N1 conventional vaccine. All conventional vaccines still showed virus shedding more than six days after challenge with Indonesian H5N1 virus and reverse genetic vaccine showed the virus shedding only two days.

Abstract P615

Characterization of H3N2 Influenza Viruses Isolated From Pigs in Southern China

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Human-like H3N2 influenza viruses have repeatedly transmitted to domestic pigs in different regions of the world. However, it is still unknown whether any of those human influenza variants could become established in pigs. Historically, Victoria/3/1975-like H3N2 virus was introduced into pig populations in southern China and was maintained in this host even after their counterpart has disappeared in humans. The detection of different avian influenza subtypes viruses in pigs made it a candidate for generation of reassortant viruses with human infectious potential; however, whether pigs could act as a "mixing vessel" for a possible pandemic virus is still unknown. To investigate this question, H3N2 influenza virus isolated from domestic pigs from 1998 to 2003 were antigenically and genetically analyzed. Antigenic analysis revealed that two different groups of H3N2 influenza viruses were present in pigs in southern China in this period: a current human-like viruses, represented by Sydney/5/97, and Port Chalmers/1/73-like (PC-like) viruses. Phylogenetic analysis of representative strains confirmed the presence of those two H3N2 sublineages in pigs. In general, the PC-like viruses were genetically most closely related to those H3N2 reassortants found in European pigs from the mid-1980s onwards. Interestingly, one of those PC-like isolates contained a classical swine H1N1-like NP gene, suggesting that after introduction to pigs in southern China, the European swine H3N2 virus further reassorted with swine virus from this region. However, the current H3N2 human influenza viruses appeared to have been repeatedly introduced into pigs from the human population. Interestingly, one isolate (Swine/Hong Kong/NS1128/2003) clustered with those human viruses observed in early 1990s, suggesting that some human H3N2 variants could be maintained long-term in pigs in this region. Sero-epidemiological findings suggested that Ck/Beijing/1/94-like virus (H9N2) might circulate in pig in this region as 5% pig sera collected from 2001 to 2002 testing positive. However, only two sera were positive for H5N1 virus during the past six years, suggesting that it is still rare for H5N1 influenza virus to infect pigs. The present study provides information on the role of pigs in the interspecies transmission and genetic reassortment of influenza viruses in southern China.

Abstract P616

Evolutionary Dynamics of Avian Influenza A Virus in the Natural Reservoir

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Avian influenza A viruses (AIV) were thought to exist in a form of evolutionary stasis within their natural reservoir of wild waterfowl. However, a recent study demonstrated very high evolutionary rates, with epidemic-like population growth, for individual influenza subtypes in both multiple wild aquatic bird species and from disease outbreaks in poultry, suggesting the stasis theory may be incorrect. Yet the evolutionary dynamics of the influenza gene pool within a single wild waterfowl host remains unclear. We therefore tested influenza virus population behavior by estimating rates of nucleotide substitution of the internal genes from different subtypes of influenza viruses from mallard ducks in North America. This dataset represents a co-circulating influenza gene pool in the natural reservoir from a region with no long-term endemicity of influenza viruses in terrestrial poultry populations. Our results showed high substitution rates for each gene examined ($>10^{-3}$ substitutions/site/year), in line with the results of previous studies. However, preliminary results indicate that none of the internal genes show epidemic-like population growth. Using the same approach we investigated the evolutionary dynamics of the hemagglutinin sequences of viruses that are not associated with disease in poultry, humans or other animal populations. Under these criteria, only H4 subtype viruses, which have predominantly been isolated from wild aquatic waterfowl, have sufficient sequences available for analysis. Results indicate that populations of H4 subtype influenza viruses in mallard were under purifying selection pressure and that the viruses had a constant population size. This is in contrast to the high background mutation rate and indicates that antigenic drift does not account for changes in a viral population within its natural host. These results indicate that the high substitution rates seen in influenza viruses in mallard, which are consistent with other RNA viruses, likely represent the neutral rate of evolution of the influenza gene pool in its natural host.

Abstract P617

Library of Influenza Virus Strains and Genes for Vaccine and Diagnostic Use as the Preparedness for Pandemics and Highly Pathogenic Avian Influenza

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Recent outbreaks of highly pathogenic avian influenza (HPAI) have spread from Southeast Asia to the northwest, Africa, and Europian countries. H5N1 viruses isolated from dead water birds in Mongolia in May 2005 and 2006 are genetically closely related to those isolated from birds in Quinghai Lake in China, Iraq, Croatia, Nigeria, Korea, and Japan in 2005 to 2007. It is concerned that these HPAI viruses may perpetuate in the lakes where migratory water birds nest in summer. In the surveillance of avian influenza fulfilled in autumn 2004 to 2006 in Mongolia and Japan, no HPAI virus was isolated from migratory ducks that flew from Siberia, indicating that the virus has not yet perpetuated in their nesting lakes in Siberia. It is important to continue intensive surveillance of avian influenza in migratory water birds. These H5N1 HPAI viruses have caused severe disease with high mortality in a few humans particularly susceptible to infection with the strain in Asia, Middle East, and Africa. The second concern is that H5N1 virus alone is assumed to cause next pandemic influenza in humans. Since avian influenza influenza viruses of any subtype can contribute genes in the generation of reassortants in pig, none of the 16 HA and 9 NA subtypes can be ruled out as potential candidates for future pandemic strains. Surveillance of swine influenza bacame more important as well as that of avian influenza. Avian influenza virus isolates have been stored for vaccine strain candidates and diagnostic use. Influenza virus isolates of 51 combinations of HA and NA subtypes have been isolated from fecal samples of ducks. So far, 82 other combinations have been generated by the genetic reassortment procedure in chicken embryos. Thus, avian influenza viruses of 132 combinations of HA and NA subtypes have been stocked. Their pathogenicity, antigenicity, genetic information, and yield in chicken embryo have been analyzed, registered in the database, and opened to WHO and OIE member countries through internet.

Abstract P618

Surveillance for Avian Influenza in Wild Birds in New Zealand

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The spread of a number of strains of highly pathogenic avian influenza (AI) subtype H5N1 around the world has prompted Biosecurity New Zealand to expand surveillance programmes for AI to include the current AI status of resident waterfowl as well as migratory shore birds arriving in New Zealand (there is no migration of waterfowl to New Zealand). Since 2004, surveillance for AI has been carried out on targeted migratory birds, in particular the bar-tailed godwit, red (lesser) knot, and ruddy turnstone, on their arrival to New Zealand at Miranda in the North Island, in late September through to November. Because of the theoretical risk of migratory birds passing new virus to resident waterfowl, the sampling of migratory birds is followed by the collection of samples from waterfowl in coastal areas in the summer (December–February), where shore birds and waterfowl mix. At the end of 2006 a total of 827 cloacal swabs were collected from migratory birds. In the surveillance of waterfowl, 1754 cloacal swabs and 583 blood samples were collected from waterfowl—mainly mallard ducks. At the end of 2006, no influenza A virus had been isolated from migratory birds although AI viral RNA was confirmed in the samples using real time influenza A RT-PCR. Twenty-eight AI viruses (subtypes H1, H2, H4, H10, and H11) were isolated from resident waterfowl. Although the risk of highly pathogenic AI entering and becoming established in New Zealand via the movements of migratory birds is considered to be low, on-going monitoring of shore birds and waterfowl continues.

Abstract 619

Application of DNA Barcoding Techniques to Avian Influenza Virus Surveillance in Wild Bird Habitats

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From 1997 to May 2005, H5N1 highly pathogenic avian influenza viruses spread rapidly to from central Asia to Eastern

Europe. If H5N1 virus is endemic in wild migratory birds then spread to domestic backyard poultry is inevitable. In migratory birds, influenza viruses prefer to infect cells lining the intestinal tract and are shed in high concentrations in their feces. Therefore, most of samples for isolation of influenza virus from the migratory birds were taken by cloacal swab sampling from captured birds or environmental sampling of bird feces on the wild bird habitats. The cloacal swab sampling is useful in identifying species of captured birds correctly, but hard to catch birds individually. On the other hand, the environmental sampling of bird feces is convenient to sample, but it is difficult to identify the species of birds. Both methods seem to be limited in inspecting epidemiology of influenza completely. DNA barcoding techniques are tools of a bio-barcodes for identifying and discovering animal species using DNA sequences from a standardized genomic region. Several reports suggested that sequence diversity in a region of the mitochondrial gene, cytochrome c oxidase I(COI) can serve as a DNA barcode for the identification of bird species. DNA barcoding techniques are useful to identify species of wild birds without bird capture. From 2004 to February 2007, we have isolated 104 avian influenza virus isolates from environmental feces samples collected from seasonal migratory bird habitats in Korea. In this study, we optimized the COI barcode technique to bird feces and then successfully identified the bird species from the collected bird feces. As a result, major types of migratory birds which excreted avian influenza virus were mallard ducks, green-winged teal, white-fronted geese and bean geese, which belong to Anseriformes. The results indicated that information obtained from the DNA barcoding will provide important clues about the role of migratory birds with regards to avian influenza ecology.

Abstract P620

The OFFLU Network

WG Dundon, I Capua

OIE\FAO and National Reference Laboratory for Avian influenza and Newcastle Disease, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy

In April 2005, the World Organisation for Animal Health (OIE) and the Food and Agricultural Organization (FAO) created and endorsed a joint network of expertise on Avian influenza (AI) for the benefit of member countries. The objectives of OFFLU are to develop research on AI and to offer veterinary expertise to assist in the control of the disease. In addition OFFLU aims to collaborate with the WHO influenza network on issues relating to the animal - human interface. The Network consists of a Steering Committee, a Scientific Commission, a Team of Scientific Collaborators and a Secretariat. OFFLU has a philosophy of openness and sharing of information and materials and particularly encourages the exchange of viral strains and sequences between members. The network is also keen to involve collaborators from as wide a field as possible and invite suitably qualified institutions and individuals to apply to become members of the network as Collaborators, Field and Laboratory Experts. The OFFLU website (www.offlu.net) provides information on how to join the network in addition to information on the diagnosis of AI, exchange of viruses, training opportunities, missions for field experts and the procurement of OIE certified reference reagents. Presently the website has an average of 30 visitors a day. The secretariat of OFFLU will be held by the Istituto Zooprofilattico Sperimentale delle Venezie, Italy until 2008.

Abstract P621

Isolation of Swine H1N1 Influenza Viruses Possessing Classical Swine H1 and Avian-Like N1 Genes in Thailand

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Background: Swine, as well as poultry husbandry, is one of the most significant livestock industries in Thailand. Not only economical losses, SIVs may cause public health concerns because pigs are considered to have a potential as mixing vessels of avian and human influenza viruses. Highly pathogenic avian influenza H5N1 viruses in Thailand have caused severe economical damage on poultry industry as well as public health concerns since 2004. Since those viruses may have a possibility of infection to pigs, sufficient care should also be taken to prevent the emergence of new pandemics by genetic reassortment in pigs. Continuous surveillance of SIV would be effective not only for economics in swine industry but also for an early detection of SIVs with public health concern. SIVs of H1N1, H1N2 and H3N2 subtypes have been detected in Thailand by serological and virological surveillance conducted by National Institute of Animal Health, Thailand since 2000. Materials and Methods: H1N1 subtypes, designated A/Sw/Ratchaburi/NIAH1481/00, A/Sw/Ratchaburi/NIAH550/03, A/Sw/Chonburi/NIAH9469/04, A/Sw/Chonburi/NIAH589/05 and A/SW/Chachoengsao/NIAH587/05 were isolated from pigs with respiratory diseases. Sequencing and phylogenetic analysis of both HA and NA genes were carried out. Results and Discussion: All of the H1 genes examined belong to classical SIVs lineage. However, independent introductions to Thai pigs were shown as H1 genes of Thai

isolates have at least two distributions within classical SIVs lineage. Three isolates, A/Sw/Chonburi/NIAH9469/04, A/Sw/Chonburi/NIAH589/05 and A/SW/Chachoengsao/NIAH587/05 form one cluster and the other isolates belong to different branches from the former three isolates within classical SIVs lineage. In N1 phylogeny, all of the isolates examined formed a single cluster within avian-like H1N1 SIVs lineage, suggesting that N1 genes of Thai isolates share one common ancestor. Thus, our results demonstrated that the H1N1 SIVs used in this study have genetic combination of classical and avian-like H1N1 SIVs. Present study revealed the existence of SIVs possessing surface antigens of classical and avian-like swine H1N1 in Thai pig population. Further analysis is necessary to elucidate the detailed features of the SIVs in Thailand.

Abstract P622

Differences in Influenza A Virus Detection Between Oropharyngeal and Cloacal Swabbing in Wild Mallard Ducks

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It has been experimentally shown that ducks infected with highly-pathogenic H5N1 display high viral replication in the upper respiratory tract. This was unexpected, as the general dogma had been that the highest virus titers were found in the intestines of birds. Accordingly, a recommendation to include oropharyngeal swabbing in the surveillance for HPAI was issued. Surveillance projects for influenza in wild birds are now standardized regarding sampling and analysis techniques, and it is important to validate these methods in order to ascertain optimal virus detection. Laboratory findings on HPAI are difficult to confirm with natural samples from H5N1 infected birds. However, the vast majority of viruses detected in wild birds are LPAI, and the study of these viruses is important for the understanding of influenza A virus ecology and epidemiology. Therefore it is also important to ensure optimal sampling for LPAI. We compared the efficiency of virus detection between oropharyngeal- and cloacal swabs from wild Mallards (Anas plathyrynchos). A total of 534 Mallards were sampled in fall 2006. From each bird, both cloacal- and oropharyngeal samples were obtained, and analyzed by Real-time RT-PCR, with protocol as recommended by the EC. With fecal samples, 83 (15.5%) were positive, compared to 21 (3.9%) of the oropharyngeal. Nine of the ducks (1.7%) tested positive with both sample types. Ct-values for the fecal samples were generally much lower than those for the oropharyngeal samples (mean: 36,1 vs 39,0; $p<0,001$) indicating higher viral titers in fecal samples. Noteworthy, however, is the fact that 12 (2.2%) of the ducks tested positive only in the oropharyngeal samples. While several different reasons could explain this last result, it clearly shows that not all virus infections are detected with cloacal swabbing. This study shows that, using current recommended techniques for detection of AIV in wild mallards, most but not all viruses are detected by cloacal sampling. Hence, a combination of cloachal- and oropharyngeal sampling proved to be the best option to ensure optimal detection of both LPAI and HPAI viruses.

Abstract P623

Influenza Viruses in Wild Birds in Hong Kong

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Wild waterfowl are considered the natural reservoir of influenza A viruses and play a role in the genesis of pandemic influenza. The recent major regional outbreak of highly pathogenic avian influenza (HPAI) H5N1 in Asia highlights the potential role played by migratory waterfowl in these outbreaks and the importance of defining the influenza virus gene-pool in wild birds. A study of avian influenza viruses in feral birds from 2003 to 2007 at Mai Po Marshes and Lok Ma Chau in Hong Kong revealed a diversity of influenza virus subtypes in migratory waterfowl. During the 4-year surveillance period from Sep 03 to Feb 07, influenza viruses were isolated from 73 out of 16,724 fecal dropping samples collected from migratory birds in Mai Po Marshes and Lok Ma Chau fish ponds. These viruses, representing hemagglutinin subtypes of H1, H2, H4, H5, H6, H7, H8, H9, H10 and H11; neuramidinase subtypes of N1, N2, N3, N4, N6, N7, N8 and N9, were all from wild waterfowl. Seventy-two of these 73 positive isolates were collected during the winter period coinciding with the southern migration of waterfowl along the East Asian flyway. None of the water samples collected from the ponds where these birds congregate was found to be positive for the virus. No HPAI H5N1 viruses were isolated in this study of apparently healthy birds. However, as part of a surveillance of dead wild birds carried out by the Department of Agriculture during this same period, over 30 dead birds were tested to be positive for H5N1. Phylogenetic analyses of the HA gene of the LPAI H5 viruses isolated in the study were clustered with that of the other LPAI H5 viruses isolated from Hokkaido, Mongolia and Siberia and are not closely related to the HP H5N1. Six blood sample (out of 150) collected from wild ducks and one (out of 43) from shorebirds, revealed neutralisation of LPAI H5N3 in neutralisation assay. This result suggests that these birds could have been infected with H5 viruses.

Abstract P624

Genetic and Pathogenic Evolution of Influenza Viruses Isolated From Korean Chicken Farms

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Several Korean chicken farms were seriously affected in 2004 by outbreaks of influenza virus infection that caused typical clinical signs, such as significantly reduced egg production, depression, diarrhea, and relatively high mortality. To understand the potential threat posed by these viruses, we analyzed their evolution in Korean chicken farms during the period 2002 through 2004. Antigenic analysis revealed that the H9 viruses of Korea formed two antigenically distinct groups: those isolated from 1996 to mid-2003 and those isolated in late 2003 and 2004. Most of the 2004 isolates showed greater cross-reactivity with the second group than with the first group. Phylogenetic analysis of all eight genes of these 12 viruses revealed threegenotypes of H9N2 viruses and showed that reassortment had occurred. One isolate, Ck/Kor/164/04, belonged to the H9N8 subtype. Its HA and PB1genes were similar to those of the other isolated H9N2 viruses, but the remainder of its genes were closely related to those of an H3N8 virus (aquatic bird/Kor/W04/04) isolated from a wild aquatic bird in 2004. None of the viruses contained genes derived fromH5N1 viruses (Ck/Kor/ES/03 and Dk/Kor/ESD1/03) isolated during an outbreak in 2003. All 5 isolates of one genotype replicated well in the lungs of mice without prior adaptation, but those of the other two genotypes did not. The H9N2 isolate Ck/Kor/Q30/04 caused severe clinical signs and a mortality rate as high as 50% in inoculated chickens and replicated in internal organs, such as kidney and spleen. This study showed that at least 2 subtypes of avian influenza viruses, including the novel reassortant H9N8, were isolated in Korean chicken farms during the period 2000–2004. After evolution through antigenic drift and reassortment with influenza A viruses from aquatic birds, their pathogenicity in mice and chickens was altered. Our results show the continuous evolution in Korea of H9 avian influenza viruses that are potential expand their host to mammalina hosts.

Abstract P625

H5N1 Avian Influenza Outbreak in Wild Birds in Western China

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H5N1 influenza A viruses are widely distributed among poultry in Asia, but until recently only a limited number of wild birds were affected. During late April through June 2005, an outbreak of H5N1 virus infection occurred among wild birds at Qinghai Lake in China. Here, we describe the features of this outbreak. First identified in bar-headed geese, the disease soon spread to other avian species populating the lake. Sequence analysis of 15 viruses representing 6 avian species and collected at different times during the outbreak revealed 4 different H5N1 genotypes. Most of the isolates possessed lysine at position 627 in the PB2 protein, a residue known to be associated with virulence in mice and adaptation to humans. However, neither of the 2 index viruses possessed this residue. Importantly, H5N1 viruses isolated in Mongolia, Russia, Inner Mongolia were genetically closely related to one of the genotypes isolated during the Qinghai outbreak,and this genotype virus was also spread to some countries in Europe and Africa, suggesting the dominant nature of this genotype and underscoring the need for worldwide intensive surveillance to minimize its devastating consequences. In this report, We also present the data about 12 H5N1 HPAIVs isolated from dead migrate birds in China in 2006.

Abstract P626

Characterization of the Complete Genome of H5N1 Influenza Virus Isolates From the Poultry Outbreak in 2006 in India: Support for Involvement of a Single Strain

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India experienced a Highly Pathogenic Avian Influenza A (HPAI), H5N1 outbreak in poultry during January – April, 2006. The outbreak was contained swiftly and the country was declared HPAI free by August 2006. The outbreak was limited

to two districts in Maharashtra, Nandurbar and Jalgaon, and adjoining areas of Ucchal in Gujarat and Burhanpur in Madhya Pradesh. Two waves of the outbreak were reported. The first in Navapur and Uchhal from January end to mid-February, and the second in Jalgaon and Ichhapur from February end to mid-April. We characterized the full genome of two isolates from post-mortem samples of sick chicken obtained from two farms in Navapur, Nandurbar district. Both isolates were identical except for a single nucleotide difference in the M gene. Phylogenetic analysis placed all eight gene segments of each isolate in clade 2.2 (Qinghai like) of the Z genotype. An earlier report based on partial sequences of the HA gene from one isolate each from Navapur and Jalgaon concluded that the outbreaks were caused by two independent populations of viruses introduced at different times. The Jalgaon outbreak was hypothesized to be due to a strain closer to the circulating clade 2.2 viruses and introduced earlier than Navapur. However, the sequences of our Navapur isolates, closer to other clade 2.2 viruses and to the Jalgaon HA sequence reported, do not support this hypothesis. Also, the NA sequences that we have described belong to clade 2.2 compared to the NA sequences from Navapur isolates deposited in the GenBank by Pattnaik et al., which cluster in clade 1. The close identity of our isolates to the viruses carried by birds in the East Africa/West-Asia flyway strongly suggests that the viruses in India may have been introduced through the migratory route, causing the 2006 outbreak of HPAI in India. At present, surveillance is being carried out in poultry and water birds in the areas where the outbreaks occurred in 2006 and other representative poultry farms and water bodies in the country. So far, there has been no detection of H5N1 in the fecal droppings collected.

Abstract P627

Ecological Origins of the Novel H3 Avian Influenza Viruses in Korean Live-Bird Markets

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The role of live-bird markets in the ecology of influenza viruses is being emphasized but still little is known about the influenza virus gene pool of live-bird markets in South Korea. Previously we reported novel H3N2 subtype avian influenza viruses were circulating among duck and chicken at live-bird markets in 2004. To investigate the ecological origin of the novel H3 avian influenza viruses in Korean live poultry markets, we conducted genetic characterization of the H3 viruses isolated from live bird markets and wild birds of Korea during the period of late 2004 to 2006. Phylogenetic analysis revealed that at least 4 genotypes of H3N2 and two genotypes of H3N6 avian influenza viruses were co-circulating in live bird markets in Korea. The viruses were reassortants between the viruses of H9N2 circulating in the Korean chicken population and migratory birds circulating in wild birds from southeastern China. It is interesting to note that several gene segments (HA, NS, M, PB1, and PB2) of wild bird isolates were clustered with Dk/Korea/S6/03-like viruses isolated from live bird markets, suggesting that there may be two way transmission events between wild birds and domestic poultry. Furthermore, the results of experimental inoculation in animals demonstrated that these H3N2 and H3N6 viruses have the potential to replicate in mammalian host as well as avian hosts. Continuous monitoring of viruses in domestic and wild bird populations is essential to better understand interspecies transmission, such as events of recent human infections with H5N1, H9N2, H7N, and H7N7 avian influenza viruses. Future related studies and such monitoring will give a better view of the importance of avian hosts and live poultry markets in the ecology of influenza viruses.

Abstract P628

SCHOLARSHIP International Scholarship

Detection of H5 Avian Influenza Virus in Migratory Birds on the Northwest of México

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In Mexico, as around the world, programs for prevention and control of highly pathogenic H5N1 avian influenza virus has begun. Sonora, a border state located in the northwest of México, is a potential site for the entrance of Asian strain H5N1 virus. Therefore, a regional program of surveillance on migratory birds is being developed. The aims of this study were: a) To develop a detection system based on real time PCR for H5 and N1 genes of highly pathogenic Influenza virus, and b) the implementation of a surveillance program on migratory birds. Amplified PCR products from a reference virus strain were cloned into pCR2.1 vector and sequenced. To construct standard curves, vectors (pCRH5 and pCRN1) were linearized with BamHI and in vitro RNA transcript was generated, quantified and used as positive control. Twenty one hunter-killed ducks of several species commonly found in this region were sampled. RNA was extracted from traquea and cloaca and our results showed that analyzed samples were negative for H5 or N1 avian influenza virus. We consider that this study represents an initial step into the programs of prevention and control in the Northwestern of Mexico.

Abstract P629

Sequence Analysis of Avian Influenza Virus Isolated From a Live-bird Market in Taiwan, Dec. 2005

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Interface areas between avian species and humans including live-bird markets had been documented to have avian influenza virus (AIV) H5N1 isolated in Hong Kong. The widespread of H5N1 in many countries from Asia to Europe and Africa during 2003-2006 suggests a possible pandemic if the virus has not been contained early. To verify the H5N2 virus[A/duck/30-2/Tw05 (H5N2)] isolated in Taiwan during September–December of 2005, we conducted sequence analysis to compare with the H5N2 viruses from the past years in Taiwan and several neighboring countries. A 200 bp HA cDNA product covering the cleavage site of HA1 and HA2 was used to compare with the available sequences of H5 from the GeneBank of NCBI. We found that our 2005 H5N2 isolate had much higher similarity with the 2003 H5N2 virus obtained from the 2003-2004 outbreak in Taiwan (79.7%) than the other H5N1 and H5N2 viruses isolated from the neighboring countries (38% to 86% similarity). Phylogenetic analysis also found that our [A/duck/30-2/Tw05 (H5N2)] isolate grouped together with the 2003 Taiwan's H5N2 virus but it was entirely separate from the avian H5N1 viruses. More importantly, there were no multiple basic amino acids inserted at the cleavage site of HA. Therefore, this H5N2 isolated from ducks was low pathogenic avian influenza (LPAI) virus. Future efforts will involve to compare the full length sequencing of the eight segments of the [A/duck/30-2/Tw05 (H5N2)] virus with other AIVs, to investigate its possible sources related to epidemiological importance and its antiviral drug sensitivity. This work is in progress. Since novel influenza has become more and more important around the world, the continuous virological surveillance to monitor avian influenza viruses and compare each segment of the AIVs globally, especially H5 AIVs, will be very helpful to understand the micro- and macro-evolutions of AIVs and thus avoid possible pandemic with more international collaborations and efforts.

Workshop Topic #7

Clinical Vaccine Evaluation

Poster Abstracts

Abstract P701

Dose Sparing Strategy With Intradermal Influenza Vaccination in Cancer Patients

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Objectives: Influenza vaccine is considered to reduce influenza-related morbidity and mortality in patients with underlying chronic medical conditions. For fear of the vaccine shortage under the influenza pandemic, several antigen sparing strategies have been investigated. We intended to evaluate the immunogenicity of the intradermal influenza vaccination with one half the antigenic contents compared to the conventional intramuscular vaccination in solid cancer patients, and assessed adverse events after vaccination. Methods: We performed a randomized case-control study on September 2005; 59 patients were vaccinated with a conventional dose of influenza vaccine (hemagglutinin 15ug from each strain) via intramuscular route in the deltoid region and 54 patients received influenza vaccination with one half the conventional dose (hemagglutinin 7.5ug from each strain) via intradermal route in the forearm region. Two successive serum samples were collected from each volunteer: a prevaccination sample immediately prior to the administration of the vaccine and a postvaccination sample 4-6 weeks after the vaccination. Haemagglutination-inhibiting (HI) antibodies were measured by a standard microtiter assay. Results: There was no significant difference between the injection routes in the hemagglutinin inhibition (HI) response and the fold increase in the titer for A/H1N1, A/H3N2, and B 4-6 weeks after the vaccination; seroconversion factors increased by more than 2.5 folds. Seroresponse rates were more than 40% and seroprotection rates were above 70% against all three influenza strains irrespective of the vaccination routes. No serious events were observed, and local skin reactions were more frequent in the intradermal recipients than in the intramuscular recipients (32.7% vs. 9.1%). Conclusion: This study shows that intradermal injection of one half the dose of commercial influenza vaccine elicits immune responses comparable to those elicited by a full dose of intramuscular vaccine among cancer patients, and it can be tolerated without serious adverse reactions.

Abstract P702

Effect of Influenza and Pneumococcal Vaccines in Elderly Persons in Years of Low Influenza Activity

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The present prospective study was conducted 2002/2003, among all individuals 65 years and older, in Uppsala County, a region of 302,654 inhabitants situated close to Stockholm urban area. The objectives of this study was to assess the benefits of influenza vaccination in a year with low influenza activity. The additive preventive effect of influenza and pneumococcal vaccination regarding hospitalisation and duration of hospital treatment was evaluated. Data on hospitalisation and mortality during 1-year period were obtained from the administrative database of the Uppsala County Council. The total study population was 41,059, of which 28,152 (non-vaccinated cohort) did not accept the offer of vaccination. Influenza vaccination was performed in 12,907 (31%) elderly persons; and of these, 4,447 had also received the pneumococcal vaccine. Thirty-five percent of the vaccinated cohort were assessed as belonging to a medical risk category. A reduction of hospital admissions was observed for both influenza (35%) and for pneumococcal pneumonia (26%) for the elderly who also had received the pneumococcal vaccine. The duration of hospital treatment was significantly shorter for both influenza and pneumococcal pneumonia compared with the non-vaccinated cohort. An additive effectiveness was observed, for pneumonia overall and cardiac failure, among persons aged 65-74, when both vaccines were given, with a reduction of hospitalisation and length of in-hospital treatment. A reduction of death from all causes was observed among persons 85 yeas and older.

Abstract P703

Immunogenicity and Safety of Trivalent Inactivated Subunit Influenza Vaccine in Children

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The burden of influenza in children is substantial but under-recognized, and extends beyond the primary infection to complications such as acute otitis media and exacerbations

of asthma. Trivalent inactivated influenza vaccines have been shown to be effective and safe in children at high risk of the complications of influenza, including those with hemophilia, impaired immune response, cystic fibrosis, asthma and other chronic respiratory diseases, and in healthy children, including young children (< 5 years). In this abstract the clinical data for the trivalent inactivated surface antigen (subunit) influenza vaccine Influvac® are discussed. Influvac® was investigated in 13 studies in children at risk for influenza complications (n=910) and in healthy children (n=1,427) and in a field efficacy study (n=27,590), including young children. In young children with respiratory disease (n=52) Influvac® induced a high antibody response fulfilling the CHMP requirements for influenza vaccines; local (in 23%) and systemic (in 48%) reactions to Influvac® were minor and transient. In a field efficacy study, vaccination of kindergarten and schoolchildren reduced influenza-like infection rates by 39% and 29% respectively compared to non-vaccinated children; no serious adverse reactions were observed immediately after vaccination or at 3–5-day follow-up. Vaccination of children also reduced morbidity in household contacts and mortality in the elderly. Recently, a study performed in China which included 300 healthy children demonstrated that vaccination with Influvac® induced antibody titers in children which were comparable to adults and elderly for all three strains. In a subgroup of children from 3-5 yrs of age similar results were obtained confirming that Influvac® is highly immunogenic in young children. The incidence of local and systemic reactions was low in this study. In conclusion, these data demonstrate that trivalent inactivated subunit influenza vaccine Influvac® is safe, tolerable and immunogenic in children at risk and in healthy children.

Abstract P704

Intradermal Administration of a Virosomal Adjuvanted Influenza Vaccine: A Promising Antigen Sparing Strategy

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Background: Intradermal (i.d.) administration of a reduced dose of influenza vaccine would facilitate the introduction of universal influenza vaccination against endemic influenza and would help to meet the global need for increased amounts of antigen in case of an influenza pandemic. Objectives: To compare safety and immunogenicity of i.d. administration of a virosomal adjuvanted influenza vaccine (Inflexal® V) with intramuscular (i.m.) administration in adults. Methods: 80 healthy adults aged ≥18 - ≤ 60 years (mean 41.5 years) received single doses of either 0.1 mL of 2006/2007 influenza vaccine i.d. (Group A: n=23) or 0.5 mL i.m. (Group B: n=57) on Day 1. Safety was assessed for 3 weeks after vaccination. Serum anti-influenza antibodies were determined by haemagglutination inhibition assay on days 1 and 22 (± 2 days). Results: I.d. administration of a fifth of the conventional i.m. dose of Inflexal® V fulfilled the EMEA criteria set for assessment of influenza vaccines in adults. High geometric mean titer fold increases (ranging from 6.92-19.13) for all three influenza strains and seroconversion rates of 69.6% for both A strains were observed three weeks after vaccination, which were comparable to the response after i.m. vaccination. Both vaccine administrations were well tolerated; 8.7% and 17.5% of the injections were associated with solicited systemic adverse events in Groups A and B, respectively. Pain was reported less frequently following i.d. vaccination (21.7%) compared to i.m. administration (38.6%). Ecchymosis ≥5 mm occurred in 1.8% of subjects in Group B but was absent in Group A. All reported adverse reactions were generally considered to be mild to moderate and resolved within a few days. The tolerability of i.d. vaccination was rated as good or very good by all subjects and 100% of them would accept to be re-vaccinated with Inflexal® V compared to 98.2% of subjects in Group B. Conclusion: The virosomal adjuvanted influenza vaccine (Inflexal® V) was highly immunogenic and well tolerated when given intradermally at a reduced dose of 0.1 mL - thus providing a promising antigen sparing strategy for universal influenza vaccination with potential use as a carrier/adjuvant system in a pandemic situation.

Abstract P705

Influenza Vaccination in Children With Bronchial Asthma and Antibody Response to Neuraminidase

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Seasonal vaccinations against influenza are recommended due to clinical reasons for various groups of patients, including those suffering from asthma. This study was performed in a group of 36 children with bronchial asthma (mean age 8.3 years, median 9 years) to assess their antibody response to inactivated split influenza vaccine ('Fluarix', GSK). Vaccine was administered in the epidemic season 2005/06 and antineuraminidase antibody levels were measured by neuraminidase inhibition test before vaccination, 1 month after vaccination and 3 months after vaccination. The same

procedures were carried out in the control group of 36 healthy persons (mean age 10.4 years, median 9 years). Statistical analysis was performed using non-parametric U Mann-Whitney test and Wilcoxon test. In the asthmatic children pre-vaccination geometric mean antibody titers (GMTs) ranged from 8.4 to 9.6 and did not significantly differ from healthy persons with GMTs ranging from 9.8 to 12.1. After vaccination antibody levels were significantly higher than before vaccination and mean fold increases (MFIs) of antibody titers in patients with asthma ranged from 4.2 to 6.4 one month after vaccination and 3.1 to 3.9 three months after vaccination. In healthy children MFIs ranged from 4.2 to 5.0 and from 3.1 to 4.8, respectively. There were observed decreases of antibody levels measured 3 months after vaccination when compared with those measured 1 month after vaccination, but these differences were significant only for the response to neuraminidase N2 in the asthmatic children and neuraminidase N1 in healthy persons. Humoral response to neuraminidase component of influenza vaccine in children with asthma was similar to healthy persons from the control group. There were only two exceptions in the above observation: anti-N1 antibody levels measured 1 month after vaccination and anti-NB antibody levels measured 3 months after vaccination were significantly higher in healthy children than in those suffering from asthma. In the most studies regarding antibody response to influenza vaccination only antihemagglutinin antibodies are taken into consideration and the role of the response to neuraminidase is underestimated. The present study showed that influenza vaccine is immunologically effective in the induction of production of antineuraminidase antibodies in the asthmatic children.

Abstract P706

Prophylaxis Against Influenza in Children With Inflammatory Bowel Diseases

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Patients requiring regular medical follow-up or hospitalization because of immunosuppression, including immunosuppression caused by drugs, are one of the target groups for which seasonal influenza vaccination is recommended. Patients with inflammatory bowel diseases (IBD) belong to this group as they are treated with long-term immunosuppressive therapy. The aim of the study was to assess response to neuraminidase component of influenza vaccine in such 29 pediatric patients with IBD (mean age 12.9 years, median 14 years) in comparison with 36 healthy persons (mean age 10.4 years, median 9 years). Both groups received inactivated split influenza vaccine ('Fluarix', GSK) in the epidemic season 2005/06. Levels of antineuraminidase antibodies were measured before vaccination, 1 month after vaccination and 3 months after vaccination by neuraminidase inhibition test. Statistical analysis was performed using U Mann-Whitney test and Wilcoxon test. In patients with IBD pre-vaccination geometric mean antibody titers (GMTs) ranged from 8.7 to 9.5 and were similar to those in healthy persons where GMTs ranged from 9.8 to 12.1. After vaccination antibody levels were significantly higher than before vaccination. One month after vaccination mean fold increases (MFIs) of antibody titers ranged from 2.5 to 4.9 in children with IBD and from 4.2 to 5.0 in healthy persons. Three months after vaccination MFIs ranged from 2.7 to 4.2 and from 3.1 to 4.8, respectively. Humoral response for two of three influenza antigens included into the vaccine was better in healthy children than in those with IBD. Anti-N2 antibody levels 1 month after vaccination and 3 months after vaccination as well as the anti-NB antibody levels 1 month after vaccination were significantly higher in the control group than in the patients with IBD. Nevertheless, significant increases of antineuraminidase antibody titers after vaccination as well as MFI values similar to those observed in healthy persons showed that influenza vaccine is effective in children with IBD in the aspect of the production of antibodies against one of two influenza glycoproteins which contribute to a milder course of the disease by the inhibition of the release of the progeny virions from the infected cells.

Abstract P707

Antibody Response to Influenza Vaccine in Patients With Non-Hodgkin Lymphoma Vaccinated in Two Consecutive Epidemic Seasons

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The aim was to assess humoral response to hemagglutinin component of influenza vaccine in 26 patients with non-Hodgkin lymphoma (mean age 56.8 years, median 59 years) vaccinated in two consecutive epidemic seasons. Ten of 26 patients were previously subjected to immunosuppressive therapy (group A), while the remaining 16 patients (group B) were not treated with such therapy. In autumn 2003 and 2004 they received inactivated subunit vaccine ('Influvac', Solvay Pharma). Antihemagglutinin antibody levels were measured

by hemagglutination inhibition test before vaccination, 1 month after vaccination and 6 months after vaccination. Statistical analysis was made by using Wilcoxon test and U Mann-Whitney test. Pre-vaccination antibody levels in the season 2003/04 were similar to those in season 2004/05. One month after vaccination antibody titers significantly increased and mean fold increases (MFIs) of antibody levels ranged from 4.6 to 10.1 in the season 2003/04 (A: 5.7-11.3, B: 3.8-9.5) and from 5.1 to 7.0 in the season 2004/05 (A: 4.9-5.7, B: 5.2-8.0). Six months after vaccination MFIs ranged from 1.9 to 3.5 and from 2.1 to 2.5, respectively. The percentage of patients with protective antibody titers ≥40 (protection rates) ranged one month after vaccination from 69.2% to 96.2% in the season 2003/04 (A: 80%-100%, B: 62.5%-93.8%) and from 69.2% to 80.8% in the season 2004/05 (A: 70%-80%, B: 68.8%-81.3%). Six months after vaccination protection rates ranged from 34.6% to 38.5% in the season 2003/04 and amounted to 34.6% in the season 2004/05. The percentage of patients with at least a 4-fold increase of antibody titers (response rates) ranged one month after vaccination from 69.2% to 96.2% in the season 2003/04 (A: 70%-100%, B: 62.5%-93.8%) and from 69.2% to 76.9% in the season 2004/05 (A: 60%-80%, B: 75%-81.3%). After six months response rates ranged from 30.8% to 65.4% and from 34.6% to 46.3%, respectively. There were no significant differences in antibody responses between patients treated with immunosuppressive drugs and those not treated as well as in the response to vaccination between two seasons. The results confirmed immunological efficacy of influenza vaccine in patients with non-Hodgkin lymphoma showing the legitimacy of seasonal vaccination for this group.

Abstract P708

Vaccination Against Influenza and Antibody Response in Patients With Coronary Artery Disease–A Single Center, Prospective, Randomized, Double-Blind Placebo Controlled Study

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Single center, randomized, prospective, double blind, placebo controlled study was performed in a group of 658 patients with coronary artery disease (mean age 59.9±10.3 years). The randomization schedule was 1:1 (placebo : influenza vaccine). In autumn 2004, 325 patients received subunit influenza vaccine ('Influvac', Solvay Pharma.), and 333 patients received placebo. Blood samples were collected before administration of the vaccine or placebo and 8 to 10 weeks after this intervention. Antihemagglutinin (anti-HA) antibody levels were measured in sera by hemagglutination inhibition test performed with turkey 0.5% red blood cells and influenza strains recommended by WHO as the vaccine components for the season 2004/05. Serological tests were done only for those patients from whom paired sera were obtained, i.e. for 78 patients who received influenza vaccine and for 97 patients who received placebo. Statistical analysis was performed by using non-parametric Wilcoxon paired test and Mann-Whitney unpaired test. After administration of the vaccine geometric mean antibody titers were significantly higher than before vaccination and mean fold increases (MFIs) of antibody levels ranged from 4.9 to 5.7, while in the placebo group MFIs amounted to 1.2 for all antigens. The percentage of patients with protective anti-HA antibody titers, i.e. ≥40 (protection rate), ranged from 0% to 2.6% before administration of the vaccine and from 1% to 5.2% before administration of placebo. After influenza vaccination protection rates ranged from 56.4% to 60.3%, while in the placebo group the values of this parameter were from 6.2% to 8.2%. The percentage of patients with at least a fourfold increase of anti-HA antibody titers (response rate) ranged from 62.8% to 68% in patients who received the vaccine and from 5.2% to 7.2% in the placebo group. Antibody levels measured before administration of the vaccine and placebo did not differ significantly. After this intervention antibody titers were significantly higher in the vaccinated patients than in the placebo group. Vaccination against influenza provided patients with coronary artery disease with some degree of protection, at least for 60% of them and should be widely use in this group to minimize a risk of infection and serious post-influenza complications.

Abstract P709

Clinical Experience With an Inactivated Subunit Influenza Vaccine

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Influvac®, Solvay Pharmaceuticals' trivalent inactivated surface antigen (subunit) vaccine is available in more than 60 countries worldwide. During the last 25 years (1982-2006), more than 70 clinical studies were performed with this vaccine, including the annual update studies that are required for licensing procedure in Europe. Since 1991, after the introduction of the harmonized guidelines, the immunogenicity of the yearly updated influenza vaccine needs to be assessed annually according to serological criteria published by the Committee for Medicinal Products for Human Use (CHMP).

Most of the studies were done in healthy subjects but also in nursing home residents, subjects at risk for complications from influenza and healthy children. Post-vaccination (at three weeks) seroprotection rates, seroconversion rates, and mean fold increase were measured to determine whether the CHMP requirement for influenza vaccine immunogenicity was met. Reactogenicity and safety data were also compiled and evaluated. A previous analysis of clinical trial data from 1982-2002 demonstrated that the influenza vaccine Influvac® was immunogenic and safe (1). The 25 years' update of the analysis will be presented at the meeting. Reference: 1. Palache AM et al. Twenty years' experience with the safety and immunogenicity of an inactivated subunit influenza vaccine. J. Drug Assess. 2005; 8:1-15.

Abstract P710

Adverse Effects of Influenza Vaccine in Workers of a University Hospital 2004-2005

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Introduction: One of the reasons why influenza vaccine coverage continues to be low among healthcare workers, despite its proven benefits, is fear of adverse effects (AEs). Objective: Assess the frequency and characteristics of AEs of influenza vaccine occurring during the 2004-2005 vaccination campaign in workers from Hospital Universitario. Material And Methods: A cross-sectional, descriptive study. Study population consisted of hospital workers receiving influenza vaccine both in 2004 and 2005. The survey was self-administered during the 2005 campaign and was intended to ascertain whether any symptoms had occurred within 3 days of the 2004 vaccine. Variables included name and vaccine history. Date of birth, sex, and professional group of workers were taken from the hospital database. Results: The study population consisted of 1471 workers, 1071 females (72.8%) and 400 males (27.2%). Mean age was 44.25 years (SD: 11.76, median 46, range 19-70 years). At least one AE was reported by 40.6% (597). Local symptoms were reported by 34.7% of the surveyed staff: local pain (29.8%), redness (15%), and inflammation (13.9%). General symptoms including malaise (10.7%), headache (3.4%), and fever (1.8%) were reported by 12.9%. Seven percent reported both types of symptoms. Significant differences were found by sex - 28% and 45.3% for males and females respectively (OR=0.47, $p<0.05$) - and between healthcare and non-healthcare staff - 41.9% and 34.9% respectively (OR=1.3, $p<0.05$)-. More workers under 46 years of age reported AEs (41.9%, as compared to 34.9% in the group ≥ 46 years, OR 1.38 ($p<0.05$)). Four or more symptoms were reported by 2.6% of those surveyed. Among healthcare staff (physicians, nurses, auxiliary staff, etc.), nurses were the group that reported more AEs (46.9%). Conclusions: 40.6% of hospital workers vaccinated against influenza reported at least one AE after influenza vaccination on the previous year. Most AEs were local. Four or more symptoms were reported by 2.6%. A higher frequency was found in females, healthcare staff, or workers under 46 years of age. We think that vaccine coverage should be increased among healthcare staff by more aggressive information campaigns.

Abstract P711

Antibody Responses to Influenza Vaccination in Adults and Elderly in Taiwan, 2006

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The aim of this study was to assess antibody responses against influenza viruses between adults and elderly who were vaccinated with influenza vaccine (GlaxoSmithKline) at the end of 2006. Total 120 sera (including pre-vaccination and post-vaccination) were collected from 30 adult and 30 geriatric volunteers, respectively. For each individual, blood was collected before and after vaccination at an interval of three weeks. Antibody titers were assayed by hemagglutination inhibition (HAI) test against local circulating strains, A/Taiwan/0586/2006(H1N1), A/Taiwan/083/2006(H3N2) and B/Taiwan/0050/2006. The antibody titers of both groups were analyzed by geometry mean titers (GMTs), seroconversion rates, seroconversion factors and seroprotection rates, according to the criteria of guidelines of the European Committee for Proprietary Medicinal Products (CPMP). The results indicated that the GMTs of both groups were significantly increased after vaccination. The seroconversion factors indicated that the immune status of both groups fitted almost the criteria of guidelines of the European CPMP, except that against A/Taiwan/0586/2006(H1N1) in adults. Similarly, the seroconversion rates of elderly were greater than 30% while the rates of adults were greater than 40%, except that against B/Taiwan/0050/2006. The seroprotection rates of both groups ranged from 70 to 100% after vaccination. In conclusion, our results indicated that the influenza vaccination program could provide a protective immunity in adults and elderly in our country.

Abstract P712

Rapid Assessment of Influenza Vaccine Effectiveness During Three Consecutive Seasons: 2004-05 to 2006-07

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Background: Timely information on the effectiveness of influenza vaccine for preventing laboratory-confirmed infection has not been routinely available. Objective: Rapidly assess the effectiveness of trivalent inactivated influenza vaccine (TIV) in central Wisconsin. Design: Prospective cohort and case-control studies during the 2004-05, 2005-06 and 2006-07 seasons. Methods: Each season, population-based cohorts of 11,565 (2004-05) to 20,693 (2006-07) adults and children were identified; all cohort members met ACIP criteria for influenza vaccination in a given season. Consenting individuals with medically attended acute respiratory illness (MAARI) were tested for influenza by culture (2004-05) or by culture and RT-PCR (subsequent seasons). Separate cohort and case-control analyses were performed; the latter used both asymptomatic controls and influenza-negative MAARI controls. Main Outcome Measure: Vaccine effectiveness (1 – relative risk) for prevention of laboratory-confirmed influenza illness, adjusted for age, high-risk medical condition, and frequency of health care utilization. Interim mid-season analyses are shown for 2006-07 but final results will be presented. Results: During the 2004-05 season, 167 (20%) of 818 ill patients had culture-confirmed influenza; 69% of cases and 64% of controls were vaccinated. Adjusted vaccine effectiveness (VE) was –0.20 (95% CI, -0.74 to 0.18) in the cohort analysis and 0.03 (95% CI, -0.54 to 0.38) in the case-control analysis. Only 3 (5%) of 62 characterized viruses antigenically matched the 2004-05 vaccine strains. In 2005-06, 51 (14%) of 356 ill patients had laboratory-confirmed influenza infection. VE was 0.01 in the cohort analysis and 0.04 in the case-control analysis; 2 (5%) of 42 characterized viruses antigenically matched the 2005-06 vaccine strains. During 2006-07, initial estimates of VE were 0.29 (95% CI, -0.24 to 0.59) in the mid-season cohort analysis and 0.45 (95% CI, -0.34 to 0.77) in the mid-season case-control analysis after identification of 60 laboratory-confirmed cases. Conclusion: During the 2004-05 and 2005-06 influenza seasons, TIV was not effective in preventing medically attended influenza illness in central Wisconsin due to local circulation of drift variants of vaccine strains. Interim analyses for the 2006-07 season suggest higher vaccine effectiveness, and enrollment is ongoing. Rapid assessment of influenza vaccine effectiveness is feasible and can inform prevention strategies during influenza epidemics.

Abstract P713

Immunogenicity of Trivalent Inactivated Influenza Vaccine Among Children Less Than 4 Years of Age

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This study was conducted to investigate the immunogenicity of trivalent inactivated influenza vaccine. Study subjects were 259 children under 4 years of age who visited six pediatric clinics to receive influenza vaccine: age distributions were 64 in <1.0, 65 in 1.0-1.9, 64 in 2.0-2.9 and 66 in 3.0-3.9 year age groups. Two doses of vaccine were given subcutaneously four weeks apart. Dosages were 0.1 ml for <1.0 year and 0.2 ml for 1.0-3.9 years. To measure hemagglutination inhibition (HI) antibody titer, triplet sera were obtained before vaccination (S0), 4 weeks after first vaccination (S1) and 4 weeks after second vaccination (S2). The geometric mean of HI antibody titer, the seroconversion proportion (titer rise≥4-fold) and the seroprotection proportion (postvaccination titer ≥1:40) were calculated by age group. Analysis of variance was also employed to estimate the independent effect of age and prevaccination titer on the antibody rise. The geometric means of HI antibody titer were lower among the two younger age groups than among the two older age groups, regardless of vaccine strains or when blood samples were collected. The seroprotection proportion after 2 doses in the <1.0 year, 1.0-1.9 year, 2.0-2.9 year, and 3.0-3.9 year age groups were: 38%, 58%, 89% and 85% against A(H1); 52%, 54%, 81% and 73% against A(H3); and 23%, 49%, 67% and 71% against B, respectively. Regarding the analysis of variance, prevaccination titer consistently indicated strong effects on antibody rise, regardless of vaccine strains or combinations of paired sera. After two doses (S2/S0), significant effects of age on antibody induction were shown against A(H1) and B (P=0.000 and 0.002). Thus, the immunogenicity of trivalent inactivated influenza vaccine was strongly affected by prevaccination titer and age. Even two doses of vaccine did not induce the protective level of antibody in about 50 to 80 percent of those <1.0 years and 40 to 50 percent of those 1.0-1.9 years.

Abstract P714

MF59-Adjuvanted Influenza Vaccine (FLUAD®): Assessment of the Immunogenity Against Homologous and Heterologous Strains in Elderly Subjects

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Background: Influenza infection is associated with high morbidity and mortality in elderly (≥65 years of age) subjects. A high mortality rate is observed, with 95% of influenza-related deaths occurring in the elderly, primarily in those with an underlying chronic health condition. Although vaccination is the recommended method to prevent influenza, currently available influenza vaccines have limited efficacy in the elderly (17-53%) due to their waning immune system. Addition of the MF59 adjuvant to subunit influenza vaccine has been shown to enhance immunogenicity and offer superior clinical protection in the elderly compared with conventional non-adjuvanted vaccines. This retrospective study compared the immunogenicity of the MF59-adjuvanted subunit vaccine (sub/MF59) (FLUAD®, Novartis Vaccines) with a virosomal vaccine (Inflexal-V®, Swiss Serum and Vaccine Institute) against homologous and heterologous vaccine strains in elderly nursing home residents with chronic conditions. Methods: In a randomized, double-blind trial, elderly nursing home residents (≥65 years of age) in north-east Italy received either Sub/MF59 (n=72) or virosomal vaccine (n=39) during the winter season of 1998–1999; the majority of subjects (87.5% versus 79.5%, respectively) had ≥1 underlying chronic disease, including a heart or lung condition or diabetes. Blood samples were obtained pre-vaccination and at 4 weeks post-vaccination and geometric mean titers (GMT) of hemagglutinin-inhibiting (HI) antibodies were retrospectively measured against the homologous vaccine strains (A/H3N2/Sydney/5/97; A/H1N1/Beijing/262/95 and B/Beijing/184/93) and three heterologous strains recommended for the 2006-2007 vaccine: A/H3N2/Wisconsin/67/2005; A/H1N1/New Caledonia/20/99 and B/Malaysia/2506/2004. The post-vaccination mean-fold increase (MFI) in HI antibodies, the number of subjects with protective titers (≥1:40), and the number of subjects with a 4-fold increase in post-vaccination titers were calculated. Results: Baseline characteristics were similar for both vaccine groups. Higher GMTs were observed post-vaccination against all strains, for recipients of both Sub/MF59 and the virosomal vaccine. Against the homologous strains, vaccination with Sub/MF59 resulted in significantly higher post-vaccination GMTs than the virosomal vaccine (A/H3N2: 48.0 versus 38.6, P=0.02; A/H1N1: 145.3 versus 114.1, P=0.05; B: 83.1 versus 59.1, P=0.001, respectively). Furthermore, significantly (P=0.03) more subjects achieved a 4-fold increase in titers against the homologous B strain following Sub/MF59 vaccination compared with the virosomal vaccine (66.7% versus 46.2%, respectively). For the heterologous strains, higher GMTs were also reported in the Sub/MF59 group, achieving significance for the A/H3N2 (80.0 versus 49.5, respectively, P=0.02) and A/H1N1 strains (176.0 versus 93.8, respectively, P=0.01). In addition, significantly (P<0.02) more subjects achieved a 4-fold increase in titers following Sub/MF59 vaccination compared with the virosomal vaccine against all three heterologous strains (A/H3N2: 41.7% versus 22.9%, P=0.05; A/H1N1: 68.1% versus 43.6%, P=0.05; B: 25.0% versus 7.7%, P=0.02, respectively). Conclusion: FLUAD®, the only MF59 adjuvanted influenza vaccine on the market, induces a higher level of immunogenicity than a virosomal vaccine in elderly subjects with chronic underlying disease, even against strains that bear no close genetic homology to the vaccine strains. Thus, FLUAD® offers greater and broader protection against influenza in this population who are at high risk of influenza-related complications.

Abstract P715

Immunogenicity of Influenza Vaccination in Reduced-Intensity Conditioning Allogeneic Hematopoietic Stem Cell Transplant Recipients Compared to Healthy Controls: A Pilot Study

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Background: Influenza causes significant morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo HSCT). Objectives: The purpose of this pilot study was to compare immunogenicity of the 2002-2003 influenza vaccine in reduced-intensity conditioning (RIC) allo HSCT recipients to a healthy adult control group. Materials and Methods: Pre- and four-week postvaccination antibody titers against the 3 vaccine strains were measured by a vaccine-specific hemagglutination inhibition (HAI) assay. Results: Five patients and 14 controls were enrolled. The fold increase in hemagglutination inhibition vaccine-specific antibody titers was similar in both groups. Patients had lower rates of seroprotection prevaccination and seroconversion postvaccination. Conclusions: Influenza vaccination may be less immunogenic in RIC allo HSCT recipients compared to healthy controls. Larger studies are needed to confirm these findings, and to elucidate factors affecting vaccine responsiveness in these patients.

Abstract P716

Non-Inferiority of a New Influenza Subunit Vaccine Produced in Madin-Darby Canine Kidney Cells When Compared to an Egg-Based Conventional Influenza Vaccine

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Background: Egg-based influenza vaccine production relies on readily available chicken flocks and manual production systems. Cell culture-based systems are more attractive for large-scale production of influenza vaccines from an operational perspective, as they are likely to be more controllable. This study aimed to demonstrate that the new influenza subunit vaccine, produced in Madin-Darby canine kidney (MDCK) cells, shows a similar safety profile and non-inferior immunogenicity when compared with an egg-based conventional subunit influenza vaccine. Methods: This Phase III, randomized, observer-blind, multi-centre study was conducted in Poland in 2004-2005. Adult (18-60 years of age; n=1300) and elderly (>60 years of age; n=1354) subjects were randomized (1:1) to receive one dose of the MDCK cell-derived or the control vaccine, an egg-based conventional subunit influenza vaccine (Agrippal®, Novartis Vaccines). Solicited local and systemic reactions within 7 days, all adverse events (AE) up to Day 22, and all serious AE (SAE) and AE leading to withdrawal up to 6 months were evaluated. Immunogenicity was evaluated at 3 weeks post-vaccination according to the Committee for Medicinal Products for Human Use (CHMP) criteria and was assessed by hemagglutination inhibition (HI) using both egg-derived and cell-derived antigens. To demonstrate non-inferiority, the MDCK cell-derived vaccine was required to meet all three CHMP criteria in both age groups when compared with the control vaccine. Results: Safety: There were no differences in the safety profile of the two vaccines. Solicited local or systemic reactions were experienced by 40% and 41% of adult subjects, and by 34% and 32% of elderly subjects who respectively received the MDCK cell-derived vaccine and the control vaccine and were mostly mild or moderate in severity. Other AE reported by adult subjects (14% MDCK cell-derived vaccine, 15% control vaccine) and elderly subjects (15% MDCK cell-derived vaccine, 13% control vaccine) were mostly mild or moderate in severity. Immunogenicity: For each of the three influenza strains, there were no differences in the immune responses induced by the two vaccines in the adult and elderly groups. For adults, post-vaccination seroprotection rates (HI titer >40) for all three virus strains were 83-100% and 82-100% for MDCK cell-derived and control groups, respectively for adults; for the elderly, post-vaccination seroprotection rates were 76-99% and 74-99%, for MDCK cell-derived and control groups, respectively. All three CHMP criteria were met both for adult and elderly subjects in both vaccine groups when assessed by HI using both egg-derived and cell-derived antigens. Furthermore, the MDCK cell-derived vaccine was non-inferior to the control vaccine in both age groups and for all three CHMP criteria. Conclusions: In adult and elderly subjects, both MDCK cell-derived vaccine and egg-based vaccine were well tolerated and highly immunogenic, and met all the CHMP criteria for the assessment of influenza vaccines. Additionally, the immune responses induced by the MDCK cell-derived vaccine were not inferior to those induced by the egg-based influenza vaccine for all of the CHMP criteria.

Abstract P717

Safety, Tolerability And Immunogenicity Of A Madin-Darby Canine Kidney Cell-Based Influenza Vaccine Compared To Fluvirin®: A Phase II Study

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Background: Conventional influenza vaccine production uses hen eggs for virus propagation. Reliance on eggs has a number of limitations, especially in the event of a pandemic, thus Novartis Vaccines has developed a cell culture-based subunit influenza vaccine (flu cell culture [FCC] vaccine) which uses Madin-Darby canine kidney cells for virus propagation. FCC offers significantly reduced lead times and greater production flexibility over egg-based vaccine production. The objective of this study is to demonstrate that the FCC vaccine is as safe and well tolerated as, and immunologically non-inferior to, a US-licensed egg-based subunit influenza vaccine. Methods: A Phase II, randomized, active-controlled, observer-blind study was conducted in the US during the 2005-2006 influenza season. Adult subjects (n=613; 18-49 years of age) were randomized (1:1) to receive either the FCC vaccine or an egg-based, subunit influenza vaccine (Fluvirin®, Novartis Vaccines). Safety and tolerability were assessed by evaluation of solicited indicators of reactogenicity and unsolicited adverse events (AE) within 7 days after vaccination. All serious adverse events (SAE) and AEs leading to withdrawal from the study were recorded throughout the study (180 days). Immunogenicity was evaluated by the hemagglutination inhibition assay using egg-derived or cell-derived test antigens against A/H3N2, A/H1N1, and B influenza strains. Immunological non-inferiority

of the FCC vaccine was assessed using the ratio of post-vaccination (Day 22) geometric mean titers (GMT) between the two vaccine groups. Results: Safety and tolerability: The safety and tolerability profiles of the two vaccines were similar. Local reactions were experienced by 54% and 61% of subjects who received the FCC vaccine and the control vaccine, respectively. Systemic reactions were experienced by 58% of subjects in each group. Significantly more recipients of the control vaccine reported ecchymosis and chills compared with recipients of the FCC vaccine (9% versus 4%, P=0.017 and 9% versus 5%, P=0.044, respectively). Among all subjects, 16% of the FCC vaccine group and 25% of the control vaccine group reported at least one AE, and only 7% and 10% of these subjects, respectively, reported AEs that were considered to be possibly or probably vaccine-related. No vaccine-related SAEs were reported for either vaccine group. Immunogenicity: Higher post-vaccination GMTs were demonstrated for both vaccines compared with pre-vaccination GMT. The post-vaccination GMT ratio of the point estimates between the two vaccine groups ranged from 0.64-1.22 for the three strains, and the lower limit of the 95% confidence interval was >0.5, demonstrating non-inferiority of the FCC vaccine. Post-vaccination seroprotection rates (HI titer >40) in the FCC vaccine group were 85-97% for the three strains and 79-98% in the control vaccine group. Seroconversion (a 4-fold increase in HI titer) rates in the FCC group were 61-89% for the three strains and 63-91% in the control group. At Day 22, both vaccines met the CBER criteria (lower limit of the 95% CI for seroprotection rate ≥70% and for seroconversion rate ≥40%) for all three strains using both cell-derived and egg-derived antigens. Conclusions: In adult subjects, both the FCC and egg-based vaccines were safe, well tolerated and highly immunogenic. The immune responses induced by the FCC vaccine were non-inferior to those induced by the egg-based influenza vaccine. Additionally, both vaccines met the CBER criteria for demonstration of immunogenicity.

Abstract P718

Consistent Immunoresponse and Tolerability of Different Lots of a New Influenza Subunit Vaccine Produced in Madin-Darby Canine Kidney Cells

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Background: Cell culture-based systems represent new and promising technology for the large-scale manufacture of influenza vaccines, thus Novartis Vaccines has developed a new cell-based subunit influenza vaccine using Madin-Darby canine kidney (MDCK) cells. The immunogenicity and safety of the MDCK cell-derived vaccine has been assessed by extensive testing. With this study Novartis Vaccines intends to demonstrate that its cell-based production process is able to generate a consistently immunogenic and well-tolerated MDCK cell-based subunit influenza vaccine. Methods: A Phase III, randomized, observer-blind, multi-centre study was conducted in Lithuania in 2005-2006. Healthy adult subjects (18-60 years of age; n=1200) were randomly assigned to receive a shot of either one of three different lots of the new MDCK cell-derived vaccine or the control vaccine, an egg-based conventional subunit vaccine (Agrippal®, Novartis Vaccines). Solicited reactions within 7 days, all adverse events (AE) up to Day 22, and all serious AE (SAE) and AE necessitating a physician's visit and/or leading to withdrawal up to 6 months were evaluated. Immunogenicity was evaluated at 3 and 26 weeks post-vaccination according to the Committee for Medicinal Products for Human Use (CHMP criteria and was tested by hemagglutination inhibition (HI) using egg-derived antigens. Post-vaccination geometric mean titers (GMT) were used to demonstrate bioequivalence between lots. Results: Safety: There were no differences in the safety profile of the two vaccines and between the three lots of the MDCK cell-derived vaccine. Solicited local or systemic reactions were experienced, respectively, by 29% and 25% of subjects who received the MDCK cell-derived vaccine and by 25% and 23% of subjects who received the control vaccine; reactions were generally transient and graded as mild to moderate. Other reported AE (14% MDCK cell-derived vaccine, 12% control vaccine) were mostly mild or moderate in severity. Immunogenicity: There were no differences in the immune responses induced by the two vaccines at 3 or 26 weeks post-vaccination. At 3 weeks, the GMT ratio between the three MDCK cell-derived vaccine lots ranged from 0.69-1.30; for all ratios, the 95% confidence intervals were within the range 0.5-2.0, demonstrating bioequivalence between the three lots. At 26 weeks, antibody titers remained high in all groups for all strains tested. Seroprotection rates (HI >40) against A/H1N1, A/H3N2, and B were 81-86%, 68-76%, and 57-59%, respectively for the three MDCK cell-derived vaccine lots and 83%, 82%, and 56% for the control vaccine. Conclusions: In adults, both MDCK cell-derived vaccine and egg-based vaccine were well tolerated and highly immunogenic, and met all the CHMP criteria for assessment of influenza vaccines. Good antibody persistence at 6 months was demonstrated for both vaccines. The immunogenicity and safety profiles induced by the three different lots of MDCK cell-derived vaccine were very similar, confirming that the flu cell culture manufacturing technology translates into consistent clinical results.

Abstract P719

Impact of Clinical and Demographic Features in Influenza Vaccination Responses in Systemic Lupus Erythematosus

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Influenza is an important pathogen in the US, with approximately 20,000 deaths/year, and immunocompromised populations often suffer the greatest morbidity and mortality. In principle, vaccination prevents serious outbreaks; however, individuals needing the greatest protection may have impaired responses to vaccination. The goal of this study is to understand intrinsic and extrinsic defects in influenza vaccination responses in one representative immunocompromised population, patients with systemic lupus erythematosus (SLE). SLE is a prototypic systemic autoimmune disease which can affect nearly every organ system in the body but which is unified by the production of high concentrations of select autoantibodies. In the initial year of this study, we enrolled 30 SLE patients and 30 matched controls to evaluate anti-influenza antibody responses, influenza-specific T cell responses, autoantibody titers and disease activity after vaccination. In addition, detailed clinical information regarding the underlying disease process, as well as demographic and therapeutic information, was serially collected. Individuals received the current influenza vaccine and blood samples were collected at baseline, 2, 6, and 12 weeks post vaccination. Based upon post-vaccination antibody levels, SLE patients were grouped into "high" and "low" responders and several factors were found to correlate with the antibody response. Surprisingly, the high responder group was enriched in older subjects and African Americans. In addition, Hispanic and Native American individuals were all found to have low responses. SLE patients with high anti-influenza antibodies had an increased frequency of arthritis and anti-nRNP autoantibodies, while patients with low influenza antibody responses had an increase in hematological criteria and anti-Ro autoantibodies. Interestingly, Ro autoantibodies were induced in two SLE patients after vaccination and several additional patients had increased titers of this specific autoantibody. There were, however, no increases in disease flare following vaccination. Overall, the strongest association with a poor influenza vaccination response was the concurrent use of corticosteroids. In summary, specific demographic, clinical, and autoantibody features were associated with poor antibody responses to influenza vaccination in SLE patients. Ongoing research focuses on understanding the magnitude and pertinence of these findings to other immunocompromised populations, as well as more detailed analysis of B and T cell responses to vaccination.

Abstract P720

Detailed Assessment of T and B Cell Responses of Lupus Patients to Influenza Vaccination

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Immunocompromised individuals are at considerable risk of morbidity and mortality from influenza infection. This is due, at least in part, to inadequate vaccination responses. To more completely understand how immunocompromised populations respond to vaccination with the standard split vaccine, we analyzed both the antibody and T cell responses of 30 patients with systemic lupus erythematosus (SLE) and their matched controls. Anti-influenza antibodies against the H3N2 virus of the 2005-2006 vaccine, A/California/04, were assessed prior to and at 4-6 weeks after vaccination using ELISAs to native and denatured viruses. Titration curves were fitted to binding equations using Kaleidagraph software to obtain A_{max} (level of influenza-specific antibodies) and $K_{a(apparent)}$ (overall affinity of the antibodies). Although the SLE patients had significantly higher pre-vaccination antibody concentrations than controls, there were no significant differences between the two groups in post-vaccination levels of antibodies against either native or denatured viruses. No differences were noted between lupus patients and controls with respect to the fold increase in A_{max} or $K_{a(apparent)}$. The T cell response to the vaccine was assessed by IFNγ ELISPOT assays prior to and at 2 weeks after vaccination. CD4 T cell proliferation in response to the vaccine was also evaluated by CFSE dye dilution at both time points. As a group, the SLE patients had significantly fewer T cells producing IFNγ when cultured with the vaccine, both before and after immunization. This difference was more pronounced prior to immunization and was exaggerated in the patients with the lowest post-immunization antibody levels. In contrast, no significant differences between controls and patients in CD4 T cell proliferative responses were noted. Thus, although lupus patients' CD4 T cells can recognize vaccine components and undergo cell division, they are defective in their ability to produce IFNγ. Current prednisone usage was highly correlated with low antibody responses, but had no apparent impact on either the IFNγ ELISPOT or T cell proliferative assays. Thus, lupus patients as a group make reasonable responses to influenza vaccination as assessed

by in vitro assays. However, subsets of SLE patients have insufficient responses. Understanding why is the focus of ongoing investigation.

Abstract P721

Assessment of Immunological Response to Influenza Vaccination in Patients With Wegeners Granulomatosis

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The aim was to assess response to influenza vaccine in 35 patients with Wegeners granulomatosis (group A) who were in clinical and serological remission after immunosuppressive treatment. They were vaccinated with inactivated subunit vaccine in the season 2006/07. Antihemagglutinin (anti-HA) and antineuraminidase (anti-NA) antibody levels were measured before vaccination and after 1 month by hemagglutination inhibition test and neuraminidase inhibition test, respectively. Antibody levels were also determined in 28 patients with Wegeners granulomatosis who were treated immunosuppressively and were not vaccinated (group B) and in 35 healthy people who received vaccine (group C). After vaccination anti-HA and anti-NA antibody titers significantly increased in groups A and C when compared with pre-vaccination values. Mean fold increases (MFIs) of anti-HA levels ranged from 5.4 to 12.8 in group A and 6.7 to 24.1 in group C. In group B, MFIs ranged from 0.9 to 1.4 for anti-HA antibodies and 0.9 to 1.1 for anti-NA antibodies. Before vaccination protection rates (percentage of subjects with anti-HA antibody titers ≥40) ranged from 0% to 5.7% in group A and 2.9% to 14.3% in group C. After vaccination protection rates ranged from 51.4% to 74.3% and 65.7% to 94.3%, respectively. In group B, protection rates ranged from 0% to 21.4%. Response rates (percentage of subjects with at least a 4-fold increase of anti-HA antibody levels) ranged after vaccination from 60% to 74.3% in group A and 71.4% to 88.6% in group C. In group B, response rates were between 7.1% and 21.4%. The only significant difference in anti-HA antibody titers between group A and group C was found after vaccination for hemagglutinin HB where higher values were observed in group C. In response to neuraminidase, significantly higher post-vaccination anti-N1 titers and pre-vaccination anti-NB titers were found in group A than in group C. Significantly higher post-vaccination anti-NB titers were found in group C than in group A. In group B, anti-HA and anti-NA antibody levels were significantly lower than in group A and group C. The study showed that response to influenza vaccine in patients with Wegeners granulomatosis is comparable to those observed in healthy people.

Abstract P722

A Phase I-II, Randomized, Controlled, Dose-Ranging Study of the Safety, Reactogenicity, and Immunogenicity of Intramuscular Inactivated Influenza A/H5N1 Vaccine Given Alone or With Aluminum Hydroxide to Healthy Adults

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Background: Dosage-sparing strategies are being explored for the development of vaccines against potential pandemic influenza viruses. We evaluated whether aluminum hydroxide adjuvant (AlOH) could reduce the amount of antigen needed to immunize against influenza A/H5N1. Methods: 600 healthy 18-49 year old subjects were enrolled in a multi-center, double-blinded, dose-ranging clinical trial of intramuscular injection with subvirion inactivated H5N1 vaccine. Subjects were randomized into 8 groups to receive 2 doses 1 month apart of a candidate H5N1 vaccine (rgA/VN/1203/2004; sanofi pasteur) containing 3.75, 7.5, 15, or 45µg of H5N1 hemagglutinin without or with 600µg/dose AlOH (N=55-60/per group for the 3.75, 7.5 and 15µg dosage levels, and 112 &113 for the 45µg dosage groups). Serum samples for antibody assays were obtained at baseline, 1 month after each dose, and 6 months after dose 2. Results: No serious adverse events related to vaccination were reported. Injection site discomfort was reported by 32-77% of subjects after dose 1 and 25-66% after dose 2. Systemic reactions were reported by 16-26% after dose 1 and 9-22% after dose 2. Most reactions were mild, and peaked on day 0 or day 1 after vaccination. Geometric mean serum HAI antibody titers after 2 doses of vaccine without or with AlOH were 5.7 and 5.4; 5.3 and 7.7; 8.5 and 8.1; and 12 and 14.8 for the 3.75, 7.5, 15, and 45µg dosage levels, respectively. The proportions of subjects who developed ≥4-fold rise in serum HAI antibody titers after the 2nd dose of vaccine without or with AlOH were 4 and 2; 0 and 14; 13 and 14; and 25 and 33 for the 3.75, 7.5, 15, and 45µg dosage levels, respectively; similar proportions of subjects achieved titers of 40 or greater. Summary and Conclusions: The AlOH-adjuvanted vaccine was well tolerated in dosages up to 45µg. Dose-response relationships for antibody responses were observed for both formulations. A statistically significant effect of AlOH was observed only at the 7.5µg dosage level; however, the response to 7.5µg of non-adjuvanted vaccine was unexpectedly low. Overall, a clinically significant beneficial effect of this aluminum hydroxide-adjuvanted formulation was not observed. Acknowldegments: Staff and subjects at participating sites; R.Levandowski, S. Shorer, K Muth, J Hu-

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Abstract P723

Effectiveness of Trivalent Inactivated Influenza Vaccine to Prevent Hospitalization in Children 6-23 months of Age During Two Influenza Seasons in Canada

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Background: Routine influenza vaccination has been recommended and provided as a publicly funded program for children aged 6-23 months in Canada since 2004. The objective of this study was to estimate the trivalent, inactivated influenza vaccine (TIV) effectiveness (VE) against hospitalization due to influenza among children aged 6-23 months. Methods: This prospective case-control study was conducted through the Immunization Monitoring Program-Active (IMPACT) network and 2 additional community hospitals during the winters of 2005-2006 and 2006-2007. IMPACT is a network of 12 pediatric centers distributed across Canada that accounts for over 90% of the nation's tertiary care pediatric beds. Study participants were <24 months of age on November 1 of each season and ≥6 months on January 31 of the same season. Cases were children hospitalized for laboratory-confirmed influenza illness. Controls were children hospitalized for acute respiratory infection with negative laboratory test results for influenza. Results: During the 2005-2006 season, influenza activity in Canada was low and characterized by mixed H3N2 and B activity and dual vaccine mismatch for these components. Forty one influenza cases (28 influenza A, 13 B) and 78 controls participated in this study. Cases were more likely than controls to be male, born full-term, living with ≥3 adults or ≥3 other children and less likely to attend daycare. The crude overall VE was estimated at –0.2% [-142%; 59%]. VE estimates varied between 9% and 20% after adjustment for confounders however, none approached statistical significance. VE did not vary by influenza type and was not better in children aged ≥18 months than in younger children. For the second season (2006-2007), influenza season started late and recruitment of cases and controls is still on-going but VE estimate will be available by May 2007. Conclusion: The observed VE during the first season does not suggest great benefit from TIV to children age 6-23 months in preventing influenza-related hospitalization. The results should be interpreted cautiously in the context of a vaccine mismatch season and an unexpected small sample size because of low influenza activity. The second season will provide further data to estimate VE in young children.

Abstract P724

First-season Results From a Multi-site Case-control Study of the Effectiveness of Trivalent Inactivated Influenza Vaccine (TIV) in Preventing Laboratory-confirmed Influenza Hospitalizations Among Children Aged 6-23 Months

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Background: We began a multi-season assessment of TIV effectiveness during 2005-06 within the Emerging Infections Program, a network of state health departments that conducts surveillance for laboratory-confirmed influenza hospitalizations. Methods: Cases were children aged 6-23 months hospitalized with laboratory-confirmed influenza infection in six metropolitan areas: San Francisco; Denver; New Haven county; Atlanta; Oregon; and Nashville. Influenza was diagnosed by a positive result from ≥1 of the following tests: direct fluorescence antibody (DFA), viral culture, RT-PCR, or a commercial rapid diagnostic test. For each case, attempts were made to enroll four age- and residence-matched controls. Case and control families were interviewed and primary care providers contacted to determine vaccination status and collect information on possible confounders. Conditional logistic regression was used to estimate the effectiveness of 1 and 2 doses of TIV in preventing influenza-associated hospitalization; children were considered immunized 14 days after receipt of each dose of vaccine. Results: Forty-five (41%) of 111 eligible cases and 157 (28%) of 561 eligible controls were enrolled. Influenza was diagnosed by DFA in 46%, by rapid test in 35%, by viral culture in 11%, by RT-PCR in 2%, and by multiple tests in 4%. Influenza A was diagnosed in 80%, B in 18%, and type was unknown for one case. Sixty percent of cases and 47% of controls were male. The average age of cases and controls was 13 months. The most common underlying

condition in cases and controls was asthma/chronic lung disease, reported for 31% and 10%, respectively. Sixty-nine percent of cases and 55% of controls were not immunized, 24% of cases and 31% of controls had one immunization, and 7% of cases and 15% of controls had two immunizations After controlling for gender and asthma/lung disease, immunization with 1 dose of TIV was 40% effective (95% CI, -48% to 76%) and with 2 doses was 71% effective (95% CI, -35% to 94%) in preventing influenza-confirmed hospitalization. Conclusions: While the first-season data were not, as expected, sufficiently powered to provide definitive results, we have demonstrated the feasibility of multi-site assessments of the effectiveness of TIV in preventing hospitalization with laboratory-confirmed influenza.

Abstract P725

Effectiveness of the 2006-07 Influenza Vaccine: Data From US Military Basic Training Centers

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Background: Influenza vaccine effectiveness (VE) can vary each season, depending primarily on how well vaccine strains match circulating strains of influenza. Each season's VE is important public health information, but is difficult to measure in a manner that is consistent, cost-effective, and timely. Virtually all US military basic trainees receive seasonal influenza vaccination, and ongoing febrile respiratory illness (FRI) surveillance at basic training centers captures data and specimens that are leveraged to estimate influenza vaccine effectiveness on an annual basis. Methods: Data from ongoing FRI surveillance at six US military training centers were used to estimate VE against both laboratory-confirmed influenza (LCI) and nonspecific FRI (oral temperature of $\geq$ 100.5° F and either cough or sore throat). Vaccine protection was assumed to begin 14 days post-vaccination in the primary analysis of VE. Alternative analyses were conducted using 7 days to vaccination coverage or less than complete vaccination among trainees. Separate calculations for VE against H1 and H3 subtypes were performed. Results: Between November 2006 and February 2007, during periods when all trainees were vaccinated, 774 FRI cases were enrolled, including 56 LCI cases, among 355,022 person-weeks of observation. Twenty-five percent (190/774) of FRI cases and 79% (44/56) of LCI cases were from unvaccinated trainees. Vaccine effectiveness against FRI and LCI was -7% and 90%, respectively. VE estimates using shorter time to vaccine protection or less complete vaccination yielded VE of 89% and 85% against LCI, respectively. VE against H1 (92%) and H3 (89%) subtypes was similar. Conclusion: The 2006-07 vaccine was highly protective against laboratory-confirmed influenza in this population. This methodology allows uniform comparison of vaccine effectiveness season to season. Protection against nonspecific febrile respiratory illness was much lower, due to a preponderance of adenovirus and a tendency for adenoviral infection to occur after the second week of training. Surveillance strategies sensitive to changes in vaccine effectiveness are important as we heighten preparedness for pandemic influenza strains.

Abstract P726

Phase I and II Trials of a Prototype Adjuvanted Inactivated Split-virion Influenza A/Vietnam/1194/2004NIBRG (H5N1) Vaccine

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The global spread of H5N1 avian influenza virus and its potential to cause an influenza pandemic demand a prototype vaccine to contribute to protective measures. We conducted 2 trials in 800 healthy adults of a prototype H5N1 (A/Vietnam/1194/2004NIBRG) inactivated, split virion vaccine produced from standard embryonated egg culture: Phase I trial, (N=400, 18-45yr), Phase II (N=400, 18-64yr). In the Phase I trial, subjects were randomized to one of 4 treatment groups (N=100/group); 7.5µg or 15µg H5N1 antigen alone or with 0.5mg aluminium phosphate ($AlPO_4$) adjuvant. Vaccine was given IM in 2 doses on d0 and d21 and a booster dose at 6 months. In the Phase II study, subjects were randomised to receive the same $AlPO_4$ adjuvanted vaccine in doses of 30µg or 45µg H5N1 antigen (N=200/group) given IM on d0 and d21. Local and general solicited symptoms were monitored for 7d after vaccination, unsolicited adverse events (AEs) for 21d, serious AEs for 6-12mo. Immunogenicity was determined by hemagglutination inhibition (HI) antibody titre using horse red cells, and by microneutralisation (MN) assay. There were no vaccine-related serious AEs. Minor injection site reactions were more marked in groups receiving $AlPO_4$ adjuvanted vaccine but did not vary with dose of antigen. The most common systemic symptoms, headache, fatigue, myalgia sweating and nausea, occurred with similar frequency and severity in all groups in both trials and were as expected for influenza vaccine. In the Phase I trial,

MN (titre ≥1:20) after dose 2 was detected in 37%, and 51% (7.5µg -/+ $AlPO_4$), and 51% and 54% (15µg -/+ $AlPO_4$), and after dose 3 (N=268) in 45%, 70%, 65% and 71% of subjects respectively. In the Phase II trial, MN (≥1:20) was found post dose 2 in 73% of subjects in both 30µg and 45µg groups. HI titres ≥1:32 were detected in 59% of subjects in both groups, and the mean GMT fold increase (dose 2/1) was 6.8 (30µg) and 7.3 (45µg). This prototype H5N1 adjuvanted vaccine is adequately immunogenic at the 30µg H5N1 dose, and appears to be safe and generally well tolerated. Further trials in children and elderly subjects are underway.

Abstract P727

Immunogenicity of a Novel Influenza Vaccine Delivered by Intradermal Microinjection in Over 60-year-olds

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Immune responses to influenza wane with age, causing higher morbidity and mortality. Vaccination is our most potent weapon against influenza infection and its complications, yet there is a need to improve vaccine efficiency in this vulnerable population. Current influenza vaccines for the elderly are delivered IM. In contrast to muscle, skin is a key component of the immune system. Intradermal antigen delivery provides a direct and potentially more efficient access to the immune system. A microinjection system has been designed to provide simple and reliable ID vaccination, overcoming the technical difficulties historically limiting the use of this route. In a randomized controlled phase II trial (Australia and New Zealand, January 2006), we investigated the immunogenicity of trivalent inactivated split-virion influenza vaccine administered using this microinjection system. Two ID dosages (15 and 21µg of haemagluttinin/strain/0.1ml dose) were tested versus the licensed IM vaccine, Vaxigrip® (15µg HA/strain/0.5ml dose) in adults aged 60+. Vaccines were the 2005 southern hemisphere formulation. The objective was to demonstrate that one or both ID dosages could induce a superior immune response against ≥2 strains compared to IM. Day 21 haemagluttination inhibition titers were assessed using a standard assay. 1107 adults aged 60-85 years were enrolled (~370/group). Groups were comparable by age (mean age/group ranged between: 70-71), male/female ratio (0.8-0.9) and % vaccinated against influenza during the 2005 season (92.4-92.6). Both ID dosages induced superior immune responses to IM against all three strains (adjusted p-value <0.0001). Day 21 GMTs (95%CI) against H1N1, H3N2 and B strains were: 15µgID: 86.7 (76.7-98.1), 400 (354-452), 100 (89.6-112); 21µgID: 90.4 (81.3-100), 400 (357-449), 96.0 (84.6-107); 15µgIM: 56.9 (51.1-63.4), 235 (205-268), 67.9 (60.6-75.7). Seroprotection rates with 15µgIM were exceeded by 4.7-10.8 percentage points with 15µgID, and 5.8-13.8 points with 21µgID. Using a simple and reliable microinjection system, each of two dosages of a trivalent inactivated influenza vaccine administered ID elicited superior immune responses against all vaccine strains than an IM control vaccination. These increased serum antibody responses should provide improved protection against influenza.

Abstract P728

Serological Protection Induced by Influenza Inactivated Vaccine Against Drifted Variants: The Importance of Adjuvant MF-59

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The prevention of influenza is based on annual vaccination with an inactivated virus vaccine, the effectiveness of which largely depends on the match between the vaccine strains and the circulating viruses. To assess the effect of the oil-in-water adjuvant MF-59 on serological protection induced by inactivated vaccine against drifted variants, the humoral response was evaluated in four groups elderly subjects (n.=25 for each group) that received adjuvanted and non-adjuvanted commercial vaccines containing A/Panama/2007/99-like and A/Wyoming/3/03-like reassortants as the A/H3N2 component. At the time of vaccine administration, A/Panama/2207/99 was in the vaccine composition and widely circulated for 4 years, while A/Wyoming/3/03 was chosen for the first time. Blood samples were collected just before vaccination and 21±1 days later. The immunogenicity was evaluated by HI and neutralization tests, using A/Panama/2007/99-like, A/Wyoming/3/03-like, A/California/7/04-like and A/Wisconsin/67/05-like strains. HI and neutralization titers were transformed into binary logarithms and corrected for pre-vaccination state, as described by Beyer et al. and comparisons between groups receiving the adjuvanted or the non-adjuvanted vaccine were performed by analysis of variance with a post hoc Bonferroni test (titers) and Chi-square test (proportion). Vaccination with vaccine containing A/Panama/2007/99-like and A/Wyoming/3/03-like reassortants induced a significant increase of the HI antibody titers not only against the homologous strains. In subjects immunized with vaccine containing A/Panama/2007/99,

HI titers in adjuvanted and non-adjuvanted vaccine groups were overlapping, while individuals immunized with MF-59-adjuvanted vaccine containing A/Wyoming/3/03 showed higher titers against A/Panama/2007/99, A/Wyoming/3/03 and A/California/7/04 strains as compared to subjects immunized with the non-adjuvanted vaccine ($p<0.01$ for all strains). Seroprotection rate (proportion of subjects with protection HI titre>40) was higher in MF-59-adjuvanted group when sera were tested versus A/California/7/04-like (28% versus 80%, $p<0.01$) and A/Wisconsin/67/05-like (20 versus 64%, $p<0.01$) strains. Neutralization assay confirmed the higher and broader serological protection elicited by MF-59-adjuvanted vaccine. These findings clearly highlight that the adjuvant MF-59 enhances the humoral response when a vaccine containing a new strain is administered, inducing higher antibody titers against the homologous strain and broader serological protection against drifted variants.

Abstract P729

Cross-Neutralisation Antibodies Elicited by a Prototype H5N1 Pandemic Influenza Vaccine

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Avian Influenza A viruses of the H5N1 subtype are emerging as likely candidates for the next influenza pandemic. Highly pathogenic H5N1 has spread from original source in Southeast Asian poultry expanding the geographic range up to Europe and Africa. Genomic and serological analyses of H5N1 isolates from birds and humans revealed that two distinct clades with a non-overlapping geographic distribution have arisen from geographically separate regions within Asia. In several countries, clinical trials on pre-pandemic vaccines using clade 1 viruses have been concluded and it is now crucial to determine the cross protectiveness of these vaccines against the currently circulating virus strains which fall into different viral clades. The most recent human avian influenza infections are caused by clade 2 H5N1 viruses, whereas completed H5N1 vaccines have been based on clade 1 H5N1 viruses. Sera from an earlier phase I trial of a monovalent, inactivated subunit vaccine containing the HA of the A/Vietnam/1194/2004/NIBRG14(H5N1) reference strain with and without AlPO4 adjuvant were analysed in microneutralisation (MN) assays using the vaccine virus (clade 1) as well as reverse genetic viruses from various subclades of the H5N1 clade 2. Evidence for cross neutralisation of clade 2 subclade 2 reverse genetic (RG) virus after vaccination with clade 1 virus and limited cross neutralisation of a clade 2 subclade 1 virus were observed, with approx. 2 fold reduction in GMT between vaccine strains and clade 2 subclade 2 and a further 2-fold reduction with the clade 2 subclade 1 strain. Analysis of cross neutralisation data shows no significant dose effect and suggests that alumn adjuvantation does not induce a better humoral response in subjects receiving adjuvanted vaccine. Our serological analysis indicates that vaccination with a clade 1 virus could provide limited protection against viruses belonging to clade 2 and underlines the necessity for a close match between vaccine virus and circulating / pandemic virus to achieve maximal protection.

Abstract P730

A Nasally Administered Trivalent Inactivated Influenza Vaccine (TIV) is Well Tolerated, Stimulates Both Mucosal and Systemic Immunity, and Potentially Protects Against Influenza Illness

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Aim: A randomized controlled double-blind trial of a nasally administered proteosome trivalent influenza vaccine (FluINsure™) was conducted in the winter of 2003-04 to determine its protective efficacy against laboratory-confirmed influenza infection. Methods: 1349 healthy adults aged 18-64 years at 28 Canadian sites were assigned to vaccine (V) containing 15 µgs of haemagglutinin per strain on day (d) 0 and d14, or 30 µgs d0 and saline placebo (P) day 14, or P d0 and d14. The primary outcome measure was culture-confirmed influenza infection and fever and one or more of sore throat, runny nose or nasal congestion, muscle or joint ache, headache, fatigue, or chills (CCI). Secondary outcomes were influenza confirmed by RT-PCR or serology, or CCI

without fever, and serum hemagglutinin inhibition (HAI) reciprocal titers and salivary secretory IgA (sIgA) d0 and d28. Adverse events (AE) were assessed d0-7 and d28, and severe/serious AE throughout the study. Results: Immunization was well tolerated, with similar reactogenicity to P. Statistically significant increases in mean HAI titers and in mean salivary sIgA to all 3 antigens were observed at d28 for V vs. P for both vaccine dose groups. The incidence of culture-confirmed influenza was low (16/1349; 1.2%). In the intent-to-immunize population (n=1348) too few CCI occurred to perform analysis (n = 4). Fever occurred infrequently, even in the presence of positive cultures and disabling multi-symptom disease. In participants receiving all doses of either V regimen the incidence of CCI with or without fever was 0.77 % (7/904) compared to 2.03% (9/443) in P recipients (p = 0.045, Fisher's exact test; relative risk reduction 62 %). A drift variant A/H3N2 circulated in 2003-04 which matched the vaccine poorly. Conclusions: A nasally-administered proteosome TIV was well-tolerated in a large population and reduced the risk of symptomatic culture-confirmed influenza in healthy adults despite the 2003-04 circulation of a drift variant. Vaccine efficacy will need to be validated in a season with a higher attack rate.

Abstract P731

Evaluation of Inactivated Monovalent Rga/Vietnam/1203/04 X PR8 Subvirion Vaccine in Healthy Elderly Adults

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Background: Protection of older persons is an important component of pandemic planning. Therefore, we evaluated the rg A/Vietnam/1203/04 subvirion H5N1 vaccine (sanofi pasteur) in healthy subjects 65 and older. Methods: 259 subjects were randomized in a 2:2:1 ratio to receive two intramuscular doses of 45 mcg, 90 mcg, or placebo separated by 28 days. Results: The median age was 71.5 years (min 65.1, max 87.4) and 55% were female. Both 45 and 90 mcg doses were well tolerated with mild pain and tenderness at the site of injection the most frequently reported adverse experiences. There were no vaccine-related serious or severe adverse events. Serum antibody responses were assessed by microneutralization (MN) and hemagglutination-inhibition (HAI). In those receiving 45 mcg the geometric mean titer (GMT) of HAI antibody at day 56 (28 days after dose 2) was 16.7 (95% CI 12.8, 22.0), with 35% (95% CI 26%, 45%) achieving a titer of 1:40 or greater. In those receiving 90 mcg, the GMT at day 56 was 26.2 (19.5, 35.2) and 46% (36%, 56%) achieved 1:40 or greater. These results are very similar to those previously reported in a separate study in healthy adults under 65, where the HAI GMT at day 56 after two doses of 90 mcg was 27.7, with 44% achieving a titer of 1:40 or greater. Summary and Conclusions: The results suggest that in carefully selected older subjects, immune responses to the H5 antigen may not be appreciably diminished compared to younger subjects. Of note, the results at 45 mcg are also very similar to those reported (Brady et al) in a separate study evaluating subvirion vaccine at 45 mcg in the elderly, demonstrating the consistency of the assays used in these studies. Further studies to evaluate alternative vaccines and dose-sparing strategies should include elderly subjects.

Abstract P732

Estimating Influenza Vaccine Effectiveness Using a Sentinel Physician Network: Results From Two Seasons of Vaccine Mismatch (2005-2006) and Match (2006-07) in Canada

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Introduction: We report a case-control design using sentinel physician networks in British Columbia, Alberta and Quebec to estimate vaccine effectiveness (VE) against laboratory-confirmed, medically-attended influenza (LC-MAI). We provide results for the 2005-06 season of dual A and B vaccine mismatch and the 2006-07 season of dual vaccine match in Canada. Methods: Participants were patients ≥5years of age presenting with influenza-like illness (ILI) to a sentinel physician between November and April. Cases were participants in whom influenza was identified; controls tested negative for influenza A and B by PCR and culture. Isolates were characterized by gene-sequencing and hemagglutination-inhibition (HI) assays. Odds ratios (OR) for LC-MAI in vaccinated versus non-vaccinated persons were derived with adjustment for age and chronic conditions. VE was estimated as [1-OR(vaccinated/unvaccinated)]. Results: For the 2005-06 season, the sample included 442 patient visits: 10% were ≥65 years,15% had a chronic condition and 22% received the 2005-06 trivalent inactivated vaccine. 206 participants were positive for influenza; 107 (52%) had influenza A/H3N2 and 99 (48%) had influenza B/Victoria lineage. Based on gene sequencing followed by repeat HI assay, three-quarters of influenza A and

all B isolates were mismatched to the vaccine. Point estimates for VE against LC-MAI ranged between 50% and 70% for both types of influenza in 2005-06. For the 2006-07 season, the influenza peak was delayed until February/March. By March 10, 738 patient visits were included in the VE sample; 226 (31%) have been positive for influenza with even split between H1N1 and H3N2 strains, both matched to vaccine. The influenza season is ongoing; full 2006-07 VE estimates with adjustment will be presented at the conference in June. Conclusion: Dual A and B mismatch resulted in diminished VE estimates but substantial cross-protection against LC-MAI during the 2005-06 season. Estimates for the 2006-07 season of dual vaccine match will be of interest to compare. This case control design using test-negative controls is an efficient way to estimate VE annually and may have pandemic applications. Current efforts are focused on increasing the precision of real-time VE estimates through the participation of more sentinel networks across Canada.

Abstract P733

Superior Immunogenicity Following MF59-Adjuvanted Influenza Vaccination (FLUAD®) in At Risk Adults (18-60 Years Of Age)–A Randomized, Observer-Blind Study

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Background: Influenza spreads rapidly during seasonal epidemics, affecting 5-15% of the adult population worldwide and imposing a considerable health and economic burden. Although vaccine effectiveness is 70-90% in healthy adults, in those with underlying chronic disease this is reduced due to their weakened immune response. Addition of the MF59 adjuvant to subunit influenza vaccine can enhance the immune response in vulnerable people, such as the elderly; however, few studies have assessed the potential of adjuvanted influenza vaccines in younger, high-risk groups. This study compared the immunogenicity and safety of an MF59-adjuvanted subunit influenza vaccine, (Sub/MF59) (FLUAD®, Novartis Vaccines) in comparison with a conventional subunit influenza vaccine (Subunit) (Agrippal®, Novartis Vaccines), in adults (18-60 years of age) with underlying chronic disease who are at increased risk of influenza-related complications. Methods: In a randomized, observer-blind, single-center clinical trial, at-risk adults (18-60 years of age) in north-east Italy received either Sub/MF59 (n=180) or Subunit (n=179) vaccine during the 2006-2007 influenza season. Subjects were affected by one or more underlying chronic disease, such as moderate-to-severe hypertension (n=265), chronic obstructive pulmonary disorder or moderate-to-severe asthma (n=85), moderate-to-severe hepatic or renal insufficiency (n=26), moderate-to-severe congestive heart failure (n=22) and arteriosclerosis or diabetes mellitus (n=18). Blood samples were obtained pre-vaccination (Day 1) and post-vaccination (Day 22) and geometric mean titers (GMT) of hemaglutinnin-inhibiting (HI) antibodies were measured. Immunogenicity was evaluated according to the Committee for Medicinal Products for Human Use (CHMP) criteria. In the preliminary safety analysis, solicited local and systemic reactions within 7 days and all adverse events (AE) and serious AEs (SAE) up to Day 22 were evaluated. The 6 month safety follow-up for SAEs is on-going. Results: Immunogenicity: Post-vaccination, a significant increase in GMTs was observed for both vaccine groups, compared with pre-vaccination GMTs for all three strains tested; however, significantly ($P<0.001$) higher GMTs were observed in the Sub/MF59 vaccine group compared with the Subunit group for all strains. Post-vaccination GMTs against A/H1N1, A/H3N2 and B were 102, 232, and 56, respectively for the Sub/MF59 vaccine and 60, 133, and 38 for the Subunit vaccine. Significantly ($P<0.01$) higher seroprotection rates were recorded in the Sub/MF59 group for all three strains, compared to those of the conventional subunit vaccination group. Furthemore, the geometric mean ratios (Day 22/Day 1) observed for the Sub/MF59 group were significantly higher for the A/H1N1 ($P<0.001$), A/H3N2 ($P=0.01$) and B ($P<0.001$) strains. For all strains more subjects achieved seroconversion, or at least a 4-fold increase in HI titer, in the Sub/MF59 group than in the Subunit group, reaching statistical significance for A/H1N1 and B strains ($P<0.001$). The Sub/MF59 vaccine met all three CHMP criteria required for healthy adults, for all three strains, whereas the Subunit group met all three criteria for A/H3N2 and A/H1N1 and two of the three criteria for the B strain. Safety: Both vaccines were well tolerated, with only one SAE reported for the Subunit vaccine which was not considered to be vaccine related. During 7 days post-vaccination, injection-site pain, malaise, and myalgia were significantly ($P<0.05$) more frequent in the Sub/MF59 vaccine group than the Subunit group (45% versus 20%; 22% versus 13%; 31% versus 9%, respectively). All local and systemic reactions were generally mild or moderate in severity, and of short duration. Conclusions: The results of this study confirm the superior immunogenicity of the MF59-adjuvanted influenza vaccine, compared with a conventional subunit influenza vaccine, for high-risk adults. In addition, the MF59-adjuvanted vaccine was well tolerated, making it suitable for use in this vulnerable population who are at increased risk of influenza-related complications.

Abstract P734

MF59-Adjuvanted Influenza Vaccine is Well Tolerated and Effective At Low Doses, Offering a Suitable Vaccine for Pre-Pandemic Vaccination Programs

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Background: Pathogenic avian influenza A viruses (ie, A/H5N1) have the potential to cause a major global pandemic with substantial morbidity and mortality worldwide. In the event of such a pandemic, large supplies of an effective vaccine will be urgently required, as the World Health Organization has predicted that a pandemic would spread globally within approximately 3 months. Surge vaccine production however, will lag behind the spread of the disease, with sufficient vaccine only available 1-3 months after the pandemic has circled the globe. This lag time may mean that distribution of a pandemic vaccine would be an exercise in 'vaccinating the survivors'. In view of this, pre-pandemic vaccination has been proposed to prime the population and provide an initial level of protection against pandemic influenza. For this vaccination strategy, pre-pandemic vaccines with the potential to offer cross-protection against a range of heterovariant influenza virus strains are needed, whether as a multivalent formulation; a vaccine containing adjuvants or other immunomodulators to broaden the immune response; or as the long-sought universal antigen. Furthermore, vaccines that use the minimum antigen content are required, rather than the current dose (15 µg) used in seasonal influenza vaccines, so that more people may be vaccinated. Addition of the MF59 adjuvant to seasonal influenza vaccine (MF59-adjuvanted vaccine [FLUAD®], Novartis Vaccines) has been shown to enhance the immune response in adults and the elderly, and offer well-tolerated protection, with >27 million doses distributed worldwide. Thus, the use of MF59 with a pre-pandemic vaccine (A/H5N1) offers the potential to achieve well-tolerated seroprotection at a lower dose, resulting in dose-sparing and the possibility to reduce the number of doses needed in a vaccination schedule. The objective of this study was to assess the safety and immunogenicity of an MF59-adjuvanted A/H5N1 influenza vaccine at two doses, and demonstrate non-inferiority of a low dose of the vaccine. Methods: A multicenter, observer-blind study was conducted in Italy in 2006. Healthy adults (18-60 years of age; n=313) and healthy elderly (>60 years of age; n=173) subjects were randomized (1:1) to receive an MF59-adjuvanted influenza vaccine (A/Vietnam/1194/2004-like H5N1) containing either 7.5 or 15 µg of hemagglutinin antigen. Each vaccinee received two intramuscular doses administered 3 weeks apart. Local and systemic reactions occurring up to 7 days post-vaccination and all adverse events (AE) occurring up to 3 weeks after each vaccination were recorded. Blood samples were taken pre-vaccination (Day 1) and on Day 22 and Day 43. Serum antibody titers against H5 were measured in all samples by hemagglutination inhibition (HI), single radial hemolysis (SRH) and microneutralization (MN) assays. Results: The incidence of local and systemic reactions and AE were similar in each vaccination group. Mild pain at the injection site was the most common AE for both vaccine groups. No serious AEs were reported during the 3 weeks after each vaccination. The immune response to two doses of 7.5 µg A/Vietnam/1194/2004-like H5N1 avian influenza vaccine was non-inferior to that of two doses of the higher dose (15 µg A/H5N1). Both vaccines met all three of the Committee for Medicinal Products for Human Use (CHMP) criteria when assessed by SRH for both adult and elderly subjects. Conclusions: A two-dose regimen of MF59-adjuvanted influenza A (H5N1) vaccine was well tolerated and immunogenic at both doses (7.5 and 15 µg) in adults and the elderly. The low-dose vaccine was non-inferior to the high-dose vaccine, resulting in the opportunity for dose-sparing. The MF59-adjuvanted influenza vaccine is, therefore, suitable for use in pre-pandemic priming strategies.

Abstract P735

Seroprevalence of Antibodies Against Low Pathogenic Avian Influenza (H5N2) Virus in Children at an Elementary School Near a Migratory Bird Habitat in Taiwan in 2006

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Highly pathogenic avian influenza (HPAI) H5N1 viruses have caused many human infections and deaths in epidemic areas. Among those HPAI H5N1 cases, most were younger children who may contract the H5N1 virus through contact with diseased or dead poultry. In addition, children are most susceptible group and may shed the H5N1 virus longer. In this study, we enrolled schoolchildren from grade 3 to 5 at

one elementary school in a high risk area A, where had been reported low pathogenic avian influenza (LPAI) H5N2 virus in 2001 and also a place near one migratory bird habitat, to investigate the possible infection of the LAPI H5N2 virus among school children. We collected 250 serum samples and questionnaires with parents' inform consents in April, 2006. All sera were tested by modified haemagglutinin inhibition (HI) test (Meijer et al.,2006) using horse erythrocytes and 1 HA units to detect antibodies against low pathogenic avian influenza virus (H5N2). Sero-positive was defined as "HI sero-titer higher than 1:40". Of 250 samples collected, 178 (71.2%) had H5N2 antibody titer < 1:10, 54 (21.6%) had 1:10, 14 (5.6%) had 1:20. Only 4 out of 250 sera had their serotiter above 1:40, and the seropositive rate was 1.6%. In those 4 children, 3 females and 1 male, no one reported avian contacts during the past six months. Of the 13 who reported contact with chickens, 9 had H5N2 antibody titer < 1:10, 3 had 1:10, and 1 had 1:20. Four children with duck contact and 1 with goose contact were all showed serotiter < 1:10. Only one schoolchild who traveled to Vietnam with her Vietnam mother also demonstrated her serotiter < 1:10. This is the first community-based seroepidemiological studyon possible infection against LPAI H5N2 in schoolchildren. The data showed that those schoolchildren were not a high risk group to the LPAI H5N2 in Taiwan since the seropositive rate against the virus was low and none of them had direct contacts with poultry or wild birds, according to the answers filled by parents. Therefore, different laboratory tests will be employed to confirm and exposure environment will be further examined.

Abstract P736

Safety, Tolerability and Immunogenicity of Two Influenza Subunit Vaccines, Combined and Not With MF-59 Adjuvant Emulsion, Administered to HIV-1 Seropositive and Seronegative Adults

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Introduction: Few studies, to our knowledge, have been published regarding the effects of MF-59 adjuvanted vaccines when administered to adults and to subjects affected with co-morbid conditions, even if the need to improve immune response to vaccination, particularly in potential low responders, is highly desirable. This could explains that MF-59 adjuvanted formulation is currently recommended for the use only for elderly subjects. Materials and Methods: An open, randomized, comparative clinical trial was performed during the 2005-2006 season. Enrolled subjects, divided in two groups by their HIV-1 seropositive status, received intramuscularly one dose of the WHO recommended formulation of two influenza vaccines, adjuvanted (Fluad) and not (Agrippal). Adverse events and side effects have been monitored using a clinical diary. Blood samples were collected at the time of vaccination visit (T0) and then 30 (T30) and 90 (T90) days after, in order to determine the CD4+ T lymphocyte count as well as viraemia (HIV-1-RNA) in HIV-1 infected individuals and to assess immunogenicity for all subjects (HI test). Results: A total of 256 subjects, 216 males (84,3%), with a mean age of 35 years (SD= 8,05) were recruited during the study period. Safety and tolerability of both the vaccines was excellent: particularly, in HIV-1 infected subjects, no significant changes in viremia and in CD4+ cell-count were detected and side effects solicited by the adjuvanted vaccine were nearly superimposable to that observed among subjects immunized with the not adjuvanted formulation, with the exception of fever ($p<0,01$) and shivering ($p<0,05$) that were significantly higher. A good immune response was detected for both the vaccines, reaching all the requested EMEA criteria. A general better immunogenicity profile was registered for Fluad compared with Agrippal: this assessment was true for all influenza subtypes, reaching a statistical significance for types A/H1 ($p=0,005$) and B ($p=0,023$) in the group of HIV-1 seronegative subjects and for type A/H3 ($p=0,003$) in HIV-1 seropositives. Conclusions: These results suggest the need to conduct further studies investigating MF-59 influenza vaccines in adults, in the view to up-date recommendations for their use, particularly for those belonging to the high risk categories, such as HIV-1 infected people.

Abstract P737

Evaluation of a Third Dose of Subvirion H5N1 Influenza Vaccine (rgA/ Vietnam/1203/04 x PR8) in Healthy Adults

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Background: Previous studies have suggested that administration of booster doses of unadjuvanted subvirion H5N1 vaccines can enhance the serum antibody response.

Methods: In a previous trial, we evaluated immune responses in healthy adults 18-64 yrs old who received two doses of unadjuvanted H5N1 influenza vaccine (sanofi pasteur), separated by ~1 month. Participants had been randomized to receive either placebo, 7.5, 15, 45, or 90 mcg/dose in a 1:2:2:2 allocation sheme. In the present study, participants received a third (booster) dose of the same vaccine dose they received in the original study, 6 months after the second dose. Microneutralization (MN) and hemagglutination-inhibition (HAI) antibody titers were measured before, 28 days, and 6 months after the third dose in 84-100 participants/group. Results: The booster doses was generally well tolerated and there were no serious vaccine-associated adverse events. Although MN and HAI GMTs declined substantially after receipt of the second dose of the primary two-dose vaccine series, we observed a significant and dose-related increase in GMTs after the booster dose. After the booster, 78% (67,86 95% CI), 67% (55,77), 43%, (32,54) and 31% (21,42) of recipients of the 90, 45, 15, and 7.5 mcg dose groups had a MN GMT ≥1:40, respectively. By HAI, 64% (52,74; 90 mcg) and 44% (32,55; 45 mcg) achieved GMTs of ≥1:40. MN titers of ≥1:20 were seen in 92%, 86%, 61%, 58% for those that received 90, 45, 15, and 7.5 mcg/dose, respectively. Over the range of vaccine dose concentrations, there was a 1.8 to 2.3-fold increase in the MN GMT 28 days after the booster dose compared to 28 days after the second dose (P<0.001). By HAI, there was a 1.1-1.3-fold increase, by dose. Six months after the booster dose, MN and HAI GMTs had declined, but were still significantly greater than those seen before the booster, by MN for all doses and by HAI, for the 90 mcg dose group only (P<0.01). Approximately 12% all participants were non-responders to the second dose, but responded to the third dose (defined as a 4-fold rise in antibody level). Female sex and younger age (18-39 years old vs 40-64 years old) were associated with ~30% higher MN GMTs after the booster (P<0.04), but this effect was not seen by HAI. Conclusions: Six months after a two dose schedule of subvirion H5N1 vaccine, serum antibody levels decline substantially. Administration of a third dose results in a dose-dependent and significant increase in immune response to levels greater than those seen after the second dose, with evidence of persistence 6 months thereafter. These data suggest that a ?prime-boost? vaccine approach may contribute to pandemic preparedness against avian influenza.

Abstract P738

Serum and Plasma Protein Biomarkers of Influenza Vaccine Response in the Elderly and Young Adults

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Influenza virus infections account for more than 40,000 deaths annually in the U.S. with 90% or more of these occurring in the elderly population. Vaccination for influenza is recommended for the elderly, yet it is only 30-40% effective in this population. To address this, longitudinal serum and plasma samples from multiple influenza vaccine cohorts were collected for analysis by different proteomic techniques. Advances in mass spectrometry instrumentation have allowed for more comprehensive characterizations of complex proteomes. We hypothesized that comparative proteomic analysis of longitudinal blood samples from responsive and non-responsive elderly and young vaccinees could identify biomarkers predictive of response at earlier time points than currently used. Two separate sera and plasma cohorts from elderly (>65 yr) and young adult (21-40 yr) vaccinees were utilized based on antibody titers from hemagglutination inhibition assay. Plasma and serum samples were collected on days 0, 4, 7, 14 and 28 following trivalent split influenza virus vaccination. Initially, four sub-groups were selected (n=10/group) comprising of young and elderly responders and non-responders. Plasma proteins were incubated with weak cation beads and processed robotically. Eluted proteins were mixed with CHCA matrix (1:1) and spotted on an AnchorChip plate for analysis on a Bruker Daltonics ClinProt UltraFlex MALDI-TOF mass spectrometer. Over 100 low-mass differentially expressed protein peaks (p<0.05) were identified between the young and elderly vaccine responders across the time points. Among all the vaccine responders and non-responders, 81 differentially expressed protein peaks (p<0.05) were identified. These protein identities are being determined using MALDI-TOF/TOF and comparative iTRAQ analyses. Functionally, these protein identities are being correlated with other immunologic parameters such as T cell responses to vaccination. Use of the longitudinal samples allows the levels of different markers to be followed over time and head to head sample day comparisons across each response group. The initial results suggest that this comparative proteomic approach will be useful for identifying differential biomarkers of influenza vaccine response in the elderly versus young adults which can be readily validated in the larger longitudinal serum/plasma cohorts.

Abstract P739

A Phase I-II, Randomized, Controlled, Dose-Ranging Study of the Safety, Reactogenicity, and Immunogenicity of Intramuscular Inactivated Influenza A/H5N1 Vaccine Given Alone or with Aluminum Hydroxide to Healthy Elderly Adults

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Background: We evaluated the ability of aluminum hydroxide, a commonly used adjuvant, to enhance the immune response to influenza A/H5N1 in healthy elderly adults. Methods: 600 ambulatory, medically stable, elderly subjects ≥ 65 years were enrolled in this multi-center, randomized, double-blinded, dose-ranging clinical trial of intramuscular injection with a subvirion inactivated influenza rgA/Vietnam/1203/2004 (H5N1) vaccine. Subjects were randomized into 8 groups to receive 2 doses one month apart of influenza A/H5N1 vaccine containing 3.75, 7.5, 15, or 45 µg of H5N1 hemagglutinin (HA) with or without 600 µg/dose of aluminum hydroxide (AlOH) adjuvant. Serum samples for antibody assays were obtained at baseline, one month after each dose, and 6 months after the second dose. Results: No serious adverse events related to vaccination were reported. Injection site discomfort was reported by 39% of subjects after dose 1 and 29% after dose 2. Systemic reactions were reported by 23% of subjects after dose 1 and 14% after dose 2. Most of these reactions were mild and peaked on day 0 or day 1 after immunization. Geometric mean serum hemagglutination inhibiton (HAI) antibody titers one month after 2 doses of vaccine without or with AlOH were 6.6 and 8.9; 11.0 and 9.8; 13.0 and 10.3; and 19.2 and 19.6 for the 3.75, 7.5, 15, and 45 µg dosage levels, respectively. The proportions of subjects who developed ≥ 4-fold rises in serum HAI antibody titers one month after the second dose of vaccine without or with AlOH were 6 and 8; 15 and 12; 21 and 14; and 34 and 33 for the 3.75, 7.5, 15, and 45 µg dosage levels, respectively; similar proportions of subjects achieved titers of 40 or greater. Summary and Conclusions: Vaccine with or without AlOH was well tolerated at dosages up to 45 µg. Antibody responses increased with increasing dosages for both the vaccine alone and vaccine with AlOH groups. Even at the highest dosage, only 36%-37% of subjects achieved HAI titers ≥ 40. A beneficial effect of aluminum hydroxide was not observed. Acknowledgments: Staff and subjects at participating sites; R. Levandowski, S. Shorer, K Muth, J Hu-Primmer at DMID: EMMES and PPD; and members of the Safety Monitoring Committee: M Schilling, J Meier, M Keefer, A Falsey, C Tacket, W Bullock, P Darbari-Kaul, and G Taffet. Supported by NIAID contracts: N01-AI-25459, N01-AI-25465, N01-AI-25460, N01-AI-25461, and N01-AI-45250.

Abstract P740

Quantitative Review of Antibody Response to Influenza Vaccination

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Background: Influenza vaccines are licensed annually based on limited clinical immunogenicity data without efficacy data. We reviewed studies published between 1968-2006 assessing antibody response to influenza immunization to explore variation by virus (sub) type, population age, vaccine formulation, and dose and dosage regimens. Methods: Publications were identified in PUBMED using keyword search terms “influenza” “vaccine” “immunogenicity” and by bibliographic reviews. Selection criteria included a PUBMED citation, English language, and information on the proportion of immunized subjects achieving seroconversion (% with ≥ fourfold rise in titer) or seroprotection (% with titer ≥1:40). We used multiple logistic regression to evaluate how antibody response rates varied with antigen, age, year, pre-vaccination antibody titers, vaccine type, vaccine valency, dosage, presence of adjuvants, and number of doses. Results: Of the 193 studies included, 46% measured vaccine response in healthy adults, 23% in children (<18 years) and 28% in the elderly (≥60 years). Nearly 69% of studies evaluated split vaccine formulations whereas fewer assessed response to whole (27%) or live (24%) vaccine. Immune responses to influenza vaccination were highly variable between seroconversion and seroprotection rates, and across strain (sub) types, across age groups, number of doses, vaccine types and other factors. Preliminary analysis indicates the baseline antibody level of the population has important consequences for the measured immune response post vaccination. Populations composed of individuals with a range of baseline antibody levels were more likely to achieve higher rates of seroprotection and lower rates of seroconversion than populations seronegative at baseline (eg. for H3N2: odds of seroprotection 1.64 (95%CI 1.23-2.19); odds of seroconversion 0.22 (95%CI 0.19-0.26)). Overall, the elderly have poorer immune responses to influenza vaccination than adults (odds of H3N2 seroconversion 0.59 (95%CI 0.55-0.64)) and adjuvants increase seroconversion and seroprotection

rates. The effect of vaccine valency is unclear. Conclusions: Immunogenicity studies indicate that multiple variables are associated with standard measures of protection. Policy makers should consider the variability in vaccine response among different target populations when developing wider recommendations for influenza vaccine formulation and administration. Further research is needed to explore the heterogeneity of antibody response to vaccination and its association with clinical protection.

Workshop Topic #8

Genetic and Antigenic Evolution

Poster Abstracts

Abstract P801

Genetic Analysis of Influenza B (Victoria lineage) Viruses in Taiwan From 2003 to 2006: Multiple Lineages Cocirculating and Reassortment of the Internal Genes

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Introduction: The genome of the influenza B virus consists of eight RNA segments which encoded three polymerase proteins (PB2, PB1, and PA), hemagglutinin (HA), nucleoprotein (NP), neuraminidase (NA), Matrix (M1) and ion channel (M2), and nonstructural proteins (NS1 and NS2). The influenza B viruses, like other members of family Orthomyxoviridae has the potential to undergo reassortment between characterized by exchange of genome segments between two different strains. Thus, the severity of an influenza epidemic season may be influenced not only by variability in the surface glycoproteins but also by difference in the internal proteins of circulating influenza viruses. The two lineages are represented by the reference strains B/Victoria/2/87 (B/Vic) and B/Yamagata/16/88 (B/Yam). B/Vic-like outbreaks were observed in the 2004-05 and 2006-07 influenza season in Taiwan and were associated with several severe and death cases in adolescents and adults. All eight gene segments and amino acid comparisons of 42 influenza B epidemic strains isolated during 2003 to 2006 in Taiwan were done to provide a complete profile of protein variability, as well as the evolutionary patterns of these viruses. Materials and Methods: Throat swabs from sentinel physician networks and other sources, mainly hospitals were collected. Specimens were tested for influenza by virus culture and polymerase chain reaction amplification. Sequence analyses were performed using of Phylip software version 3.57c and were aligned with ClustalW software. Phylogenetic trees were generated by neighbor-joining method using MEGA3 program (Life Sciences, Tempe, AZ), and were carried out for entire genome of influenza B isolates obtained from January 2003 to December 2006 in Taiwan. Result: Taiwan B/Vic-like viruses isolated in 2003 and 2004 were phylogenetically grouped mainly around B/Brisbane/32/2002 or B/Ohio/1/2005 clade, however viruses isolated in 2005 and 2006 were around B/Malaysia/2506/2004 clade. Phylogenetic data shows the B/Vic lineage has little drift with viruses appearing similar to B/Shangdong/7/97 but more extensive drift has been seen in recently. All of the B/Vic viruses analyzed at the molecular level were reassortants that inherited the B/Vic-like HA with a B/Yam-like NA during 2003-2006. Moreover, all isolates of B/Vic-like having B/Yam-like MP, NP, NS, and PA genes. In addition, the phylogenetic trees of these genes are clearly divided into two branch clusters and revealed similar patterns of genetic divergence of the HA, but belonging to two different clades consisted of isolates from B/Yam HA lineage (clade Yi and Yii) respectively. For instance, the MP and PA genes revealed their divergence close to the clade Yi represented by B/Jiangsu/10/03, the NS gene were closed to clade Yii represented by B/Colorado/4/04. However, the PB1 and PB2 genes were evolving in a manner similar to that of HA genes remain belonging to B/Vic lineage. The genetic analysis results indicating multiple cocirculating lineages and genetic reassortment of the internal genes between the two lineages of influenza B viruses were observed in Taiwan during 2003 to 2006. Discussion: A significant amount of the impact of influenza is due to the influenza B viruses during 2003 to 2006 in Taiwan. While influenza B infections are usually associated with a lower mortality than influenza A infections, occasional deaths can occur. Comparison of the evolutionary profiles of all 8 gene segments of influenza B viruses reveled that these genes of recent isolates from 2003 to 2006 consistently divided into two major lineages which evolving independently. In contrast to that of influenza A viruses, observed reassortment among variable cocirculating internal genes of influenza B viruses resulted in more pronounced protein variability. The results of this study provide evidence that genetic exchange among cocirculating B/Yam-lineage and B/Vic-lineage viruses involving gene segments coding for the internal proteins occurs naturally in the human population and that mechanism of genetic reassortment may be important in virus evolution and pathogenicity. Changes in the internal proteins as well as antigenic variability in the surface proteins should be considered when analyzing and predicting newly emerging influenza viruses. In addition, our results indicate that antigenically and genetically distinct influenza B viruses co-circulated in Taiwan during 2003 to 2006 and caused seasonal outbreaks. The genetic reassortment occurred among the internal genes of these cocirculating viruses contributing to the variability of these viruses and giving rise to new variants with distinct genome constellations.

Abstract P802

Antigenic and Molecular Characterization of a Reassortant H3N2 Swine Virus Isolated From a Child in Canada

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Influenza A is endemic in a broad range of species, with avian and swine strains having the greatest potential for transmission to humans. Here we report a case of swine

H3N2 influenza virus (SIV) in a Canadian infant with both viral isolation and serologic evidence of infection. The influenza A isolate designated A/Canada/1158/06 was recovered from a 7-month-old hospitalized child who lived on a communal farm in Canada. Molecular characterization of the isolate revealed that it was most closely related to A/Swine/Ontario/33853/2005 (H3N2) virus, which shares the same human/classical swine /avian triple reassortant genotype as the H3N2 viruses that emerged in pigs in the United States in 1998. Sequence analysis showed that nucleic acid identity between A/Canada/1158/06 and A/Swine/Ontario/33853/2005 ranges from 98.4 % (HA) to 100% (M1), and that amino acid (aa) identities range from 97.9% (HA) to 100% (M1). A deletion of 4 aa at position 156 to 159 was observed in the HA1 region of the A/Canada/1158/06 HA protein. Phylogenetic analysis showed that the HA and NA gene of A/Canada/1158/06 clustered with Canadian isolates from 2005. Initial HI testing showed that the isolate was not inhibited by antisera against recent human influenza A strains (A/Wisconsin/77/05 and A/New Caledonia/20/99), but was inhibited by antiserum to A/Swine / Texas/4199-2/98 (H3N2) virus with an HI titer of 128, indicating that the A/Canada/1158/06 virus was antigenically related to swine influenza virus. The child recovered uneventfully. The index case had no direct contact with the farm animals and his household contacts did not work directly with animals but his father occasionally spent time in the barns and his uncle, who lived next door, worked in the swine barns. A serosurvey was done on sera collected from the index case, the parents, 5 siblings and 46 other occupants living in a total of 17 households. Seropositivity to A/Canada/1158/06 was demonstrated in the index case, 4 of 7 household members, and 4 of 49 non-household members, with only two of the seropositives having a history of an influenza-like illness. These results show that unrecognized human infection with SIV is occurring in Canada. The potential of SIV strains to adapt or reassort resulting in ready human-to-human transmission remains to be established, but surveillance of swine workers would be an important step in early identification of such a strain.

Abstract P803

The Influenza Virus Resource at NCBI Facilitates Genetic Evolution Studies of Influenza Viruses

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The Influenza Virus Resource (http://www.ncbi.nlm.nih.gov/genomes/FLU/) was created at NCBI to cope with the rapid increase of influenza virus sequences. It provides a curated database that contains nucleotide and protein sequences of influenza viruses in EMBL/DDBJ/GenBank databases. Sequences in the database can be searched and sorted in an easy interface by one or more of the following fields: subtype, genome segment, sequence size, host, country of origin, year and GenBank accession number. Complete genome sets of influenza viruses can also be retrieved to study reassortment among different virus isolates. Nucleotide and protein sequences from the database can be downloaded for further analysis. This database has open access to all users, and has been used as the backbone of several other influenza virus sequence databases. The resource also provides sequence analysis tools integrated with the database, such as multiple sequence alignment and clustering of protein sequences based on different metrics. This allows users to quickly modify a dataset to optimize the analysis. An influenza virus genome annotation tool is included in the resource to validate and predict protein sequences encoded by Influenza A viruses and Influenza B viruses. In addition, the resource has links to flu sequences, protein structures, Trace Archive, publications and general information about flu viruses. This resource not only enables scientists to study genetic evolution of influenza viruses, but also plays an important role in disease surveillance, vaccine selection and development in diagnostic techniques.

Abstract P804

Phylogenetic Analysis of Influenza Viruses Circulating in South Africa During 2006-2007

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The purpose of this study was to monitor the genetic drift in South African influenza viruses circulating in 2006 by

sequencing the genes coding for the surface glycoproteins of representative strains. Several viruses isolated early in 2007 were also analysed. In 2006, the predominant subtype in South Africa was influenza A H3N2, followed by influenza B, while H1N1 activity was sporadic. This is in contrast to 2005 where H1N1 accounted for 55, 6% of the isolates made. Sequence analysis of the HAI region of representative H3N2 isolates from the Gauteng, Kwa-Zulu Natal and Western Cape provinces revealed significant drift from the A/California/7/04 vaccine southern hemisphere vaccine strain. Some drift in antigenic sites A and B was also observed compared to the A/Wisconsin/67/05 vaccine strain for the 2006/7 season. Influenza B viruses from both the B/Victoria/2/87 and B/Yamagata/16/88 B lineages were isolated with the majority being B/Malaysia/2506/04-like. Phylogenetic analysis of the HA1 subunit of representative South African 2006 influenza B viruses showed that the 2006 B/Victoria/2/87-like isolates were closely homologous to the B/Malaysia/2506/04 vaccine strain. In the B/Shanghai-like viruses, substitutions were seen at four common residues and sporadic changes were seen at several additional other residues. A single H1N2 reassortant virus was identified in the six influenza A H1 viruses isolated in 2006 by RT-PCR typing of the neuraminidase genes. This is the first time an H1N2 reassortant has been detected in South Africa since it circulated in this country in 2002 and 2003.

Abstract P805

Phylogenetic Analysis of Hemagglutinin and Neuraminidase Genes of A/Domestic Goose/Pavlodar/1/05 (H5N1) Avian Influenza Virus Strain Isolated in Kazakhstan

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Avian influenza (AI) virus H5N1 was first detected in 2003 in the South-Eastern Asia. Migrated birds introduced it into Europe, Africa and Near East. By present the virus killed about 150 humans and over 200 millions of birds. Hereby we present the results of the phylogenetic analysis and molecular-genetic characterization of the AI virus, A/domestic goose/Pavlodar/1/05 strain, isolated in Pavlodarskaya oblast, Republic of Kazakhstan. Methods: AI virus RNA was extracted with QIAamp Viral Mini Kit (Qiagen). Pfu-polymerase (Fermentas) was used to produce PCR products (cDNA fragments). Nucleotide sequences were determined using dye-terminator cycle sequencing ready reaction kit (Applied Biosystems). Results: Phylogenetic analysis of the highly pathogenic AI virus, A/domestic goose/Pavlodar/ 1/05 strain, (H5N1) and data of the global database has shown that in neuraminidase the studied strain is highly homologous to the Russian strain A/turkey/Suzdalka/Nov-01/05 (H5N1) as well as to the Mongolian strains. Like in case of neuraminidase phylogenetic analysis of hemagglutinin demonstrated high identity of hemagglutinin to that in the strains isolated in Russian, China and Mongolia in 2005. However high homology with the strains isolated in Qinghai Province (China) was demonstrated. This fact evidences first of all that these strains are ancestors of the Kazakhstan strain since the outbreaks of highly pathogenic avian influenza have been recorded in Qinghai Province earlier than in Kazakhstan. Pathogenicity of the AI virus much depends on the structure of the proteolytic cleavage site of a hemagglutinin molecule. Strain A/domestic goose/Pavlodar/ 1/05 (H5N1) pos-sesses the same amino acid sequence of the site of proteolytic cutting of hemagglutinin P337QGERRRKKRGLF349 that is characteristic of a highly pathogenic AI virus as the strains isolated in Russia possess. To determine genotype of A/domestic goose/Pavlodar/ 1/05 strain neuraminidase se-quences were compared. In the result it was determined that A/domestic goose/Pavlodar/ 1/05 (H5N1) strain has 20 amino acid deletion between the 49th to 68th thus demonstrating its belonging to type Z. So, it is possible to assert that the virus was introduced to Pavlodarskaya oblast of Ka-zakhstan exactly from Novosibirskaya oblast of Russian and high homology in hemagglutinin gene evidences its "Chinese" origin.

Abstract P806

Complete Genetic Analysis of Influenza A Vaccine and Vaccine Candidate Reassortant Viruses From 2004-2007

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Currently approximately 300 million doses of vaccine are produced annually worldwide. In order to better understand and improve upon vaccine production it is of interest to know the complete genetic composition of the genes of high yield (hy) reassortants used for vaccine production. High yield reassortants for vaccine production must have the hemagglutinin (HA) and neuraminidase (NA) of the currently circulating wildtype virus with one or more genes that confer an increased capacity to grow in ovo. The augmented growth capacity of hy reassortants in ovo is the result of

genes donated from the highly egg adapted strain A/Puerto Rico/8/34 (A/PR/8/34). Thus far all hy vaccine reassortants analyzed have the M gene from the A/PR/8/34 parent strain. Reassortants are the result of two levels of selection, negative selection by antibodies against PR/8/34 HA and NA and positive selection of the internal genes by the egg host. In this study the complete genetic composition of a panel of influenza A vaccine and vaccine candidate reassortant viruses was analyzed. The panel included the 2004 (NYMC X-147), 2005 (NYMC X-157), and 2006 (NYMC 161B) H3N2 component of the influenza A vaccine and also the 2007 vaccine candidate reassortant for the H1N1 component. Restriction fragment length polymorphism was used to analyze gene composition. All hy reassortants derived their M genes from A/PR/8/34; in contrast two polymerase genes, PB1 and PA, were found to be of wild type origin in certain hy reassortants. The NP and NS genes also were of wild type origin in some reassortants. Therefore this analysis shows that the 6/2 gene composition (six genes from A/PR/8/34 with HA and NA from the circulating strain) is not a requirement for the development of hy reassortants suitable for vaccine production.

Abstract P807

Identifying Potential Immunodominant Amino Acid Positions in Influenza Hemagglutinin Protein and Predicting Antigenic Variants of Influenza A/H3N2 Viruses

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Background: Current inactivated vaccines provide protection when vaccine antigens and circulating viruses share a high degree of similarity in hemagglutinin protein. Five antigenic sites in the influenza H3 hemagglutinin protein have been proposed and 131 amino acid positions have been identified in the five antigenic sites. In addition, 20, 18 and 32 amino acid positions in the hemagglutinin protein have been identified as mouse monoclonal antibody-binding sites, positively selected codons, and the significant diversity codons, respectively. A previous study had documented that a model based on the 131 positions in the five antigenic sites has the best performance (agreement=83%) to predict antigenic variants of influenza A/H3N2 viruses (Lee MS et al. EID 2004;10:1385-90). Aims: In this study, statistical models were employed to identify potential immunodominant positions in the hemagglutinin protein and predict antigenic variants of influenza A/H3N2 viruses. Methods: Amino acid sequences of hemagglutinin protein of 45 A/H3N2 viruses isolated during 1971-2002 and 181 pairwise antigenic distances determined by hemagglutination-inhibition antibody cross-reactivity among the 45 viruses were available for analysis as a training dataset. In addition, 57 pairwise antigenic distances from 12 A/H3N2 viruses isolated during 1999-2004 were used as a validation dataset. Multivariate regression models were employed to identify potential immunodominant positions defined as amino acid substitutions that are positively associated with antigenic distance. Results: Fifteen amino acid positions were identified as potential immunodominant positions in the training dataset. Prediction models based on the potential immunodominant positions have good performance on predicting antigenic variants in the training (agreement=92%) and validation (agreement=93.0%) datasets. Conclusions: A prediction model combining serology, bioinformatics and statistics was developed to predict antigenic variants of influenza A/H3N2 viruses. The model could be readily integrated to the global influenza surveillance system and applied to predict vaccine-induced cross-reactive antibody responses in humans, which may further accelerate the selection of vaccine strains. (Finiancial support from Taiwan Pandemic Influenza Vaccine R&D Program, DOH95-DC-1401)

Abstract P808

A Quantitative Genotype Algorithm Reflecting H5N1 Avian Influenza Niches

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Computational genotyping analyses are critical for characterizing molecular evolutionary footprints, thus providing important information for designing the strategies of influenza prevention and control. Most of the current methods that are available are based on multiple sequence alignment and phylogenetic tree construction, which are time consuming and limited by the number of taxa. Arbitrarily defining genotypes further complicates the interpretation of genotyping results. In this study, we describe a quantitative influenza genotyping algorithm based on the theory of quasispecies. First, the complete composition vector (CCV) was utilized to calculate the pairwise evolutionary distance between genotypes. Next, Hierarchical Bayesian Modeling using the Gibbs Sampling algorithm was applied to identify the segment genotype threshold, which is used to identify influenza segment genotype through a modularity calculation. The viral genotype was defined by combining eight segment

genotypes based on the genetic reassortment feature of influenza A viruses. We applied this method for H5N1 avian influenza viruses and identified 107 genotypes among 283 viruses with a complete genome set. The diversity of viral genotypes, and their correlation with geographic locations suggests that these viruses form local niches after being introduced to a new ecological environment through poultry trade or bird migration. This novel method allows us to define genotypes in a robust, quantitative, as well as hierarchical manner.

Abstract P809

Genetically Destined Potentials for N-Linked Glycosylation Associated With Antigenic Changes of Influenza Virus Hemagglutinin

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The addition of carbohydrates to influenza virus hemagglutinin (HA) is believed to facilitate viral escape from neutralization by antibodies. It is well known that the number of carbohydrates in HA, especially in the globular head region, changes during circulation of the viruses in the human population. Retrospective sequence analysis confirmed a gradual increase in the number of N-linked glycosylation sequons (Asn-Xaa-Ser/Thr, where Xaa is any amino acid except Pro) in the amino acid sequences of human H3N2 viruses isolated from 1968 to 2006. We then analyzed the potential candidate codons that were not sequons, but able to become sequons with 1-3 nucleotide mutations (i.e., a set of three codons that required single, double, or triple nucleotide substitutions to produce sequons). All of the sites that acquired N-glycosylation sequons during the past 37 years were observed in these candidate codons in HA of the prototype strain, A/Hong Kong/1/68. These results suggest that the genetically destined potential for N-glycosylation in H3 HA could have been one of the key factors prerequisite for this virus to continuously circulate in the human population. Finally, we estimated the potentials of the acquisition of new N-linked glycosylation sites in 16 HA avian influenza virus subtypes, all of which might possibly be introduced into the human population in the future. We found that there was a difference in the capacities to acquire glycosylation sites among these subtypes. Notably, recently circulating H5N1 viruses may have comparable or rather greater possibilities to undergo mutations associated with glycosylation of HA than H3N2 viruses.

Abstract P810

Identification of Epitopes in Hemagglutinin and Nucleoprotein of Influenza B Viruses

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Influenza B is composed of two antigenically and genetically distinct sublineages. For determination of the neutralizing epitopes in structure of hemagglutinin of influenza B virus five monoclonal antibodies (MAbs) directed to the viruses of both contemporary lineages with neutralizing activity were developed and used to select the escape mutants of the virus. The first group (GI) of MAbs (4H7, D9 and G3) reacted in hemagglutination inhibition test (HI) and microneutralization test (MN) with the strains of Yamagata lineage. The second group (GII) of MAbs (B/4H1 and 5B7) reacted in HI and MN with the strains of Victorian lineage exclusively. Analysis of cross reactivity of three EM derived with MAbs of GI revealed two different epitopes for MAbs 4H7 and D9 and partially overlapping epitope for MAbs 4H7 and G3. The HA1 gene of EM for MAbs 4H7 and D9 were sequenced and predicted amino acid sequences were compared. Two amino acid substitutions (V75I and D150T) in 4H7 EM and single amino acid substitution (R149I) in D9 EM were identified. Two epitopes for MAbs B/4H1 and 5B7 (GII) were non overlapping. Additionally, new panel of eight MAbs directed according to western blot analysis to influenza B virus nucleoprotein (NP) was used for investigation of this protein structure. Four non overlapping epitopes in NP of influenza B virus common both for B/Yamagata and B/Victoria lineage were detected in competitive ELISA. Besides, one epitope specific for viruses of Victorian lineage and one other epitope common for Victorian lineage viruses and for strains of early (1954-1979) years of isolation was discovered.

Abstract P811

The Properties of Human Influenza Viruses Isolated in Russia in 2004-2006

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Influenza viruses A(H1N1), A(H3N2) and B co-circulating in different combinations caused epidemics every year in Russia. The epidemic strains were like or variants of modern reference

strains: A/New Caledonia/20/99 (H1N1) (16); A/Fujian/411/02 (13), A/California/07/04 (178) (H3N2) consistently; and B/Shanghai/361/02 (84) and B/Malaysia/2506/04 (56) representatives of two lineages B/Jamagata/16/88-like and B/Victoria/2/87-like since 2004 till 2006. So antigenic properties of epidemic strains, circulated in Russia, were similar to modern reference strains circulated in other countries in the world. The increases of antibody titres to circulated strains have been shown in 200 paired sera of ill patients in HI–test. Some of them were investigated in Western blot. The appearance of antibodies to virus proteins: hemagglutinin (HA), nucleoprotein (NP) and matrix M1 protein was detected. The population immunity varied during the year: the maximum of antibodies to circulated strains in donor sera were detected just after epidemic seasons. All epidemic strains were isolated in MDCK. It directs that the tropism to mammalian cells instead of embryonated eggs continued. Three viruses were isolated (2 – A(H1N1), 1 – B) from 86 throat swabs in eggs in period 2004-2006. During two seasons the 6 viruses of A(H1N1), 1 – of A(H3N2), 7 – of B from 37 strains isolated in MDCK were transmitted to eggs. The average differences between GMT hemagglutinin titers of 238 epidemic strains isolated in 2002-2006 with erythrocytes of human and chicken were the maximum for influenza viruses A(H1N1), (H3N2)- 6.3, 4.1 and minimum for influenza B viruses 1.4. The differences between chromatographic profiles of M1 protein tryptic hydrolyzates of influenza A(H1N1), A(H3N2) and B viruses of two lineages have been found. Influenza B viruses of both evolutionary lineages were more sensitive to proteolytic treatment by enzyme bromelain than influenza A(H1N1), A(H3N2) viruses. The tryptic hydrolizate chromatographic profiles obtained for conservative internal protein matrix (M1) for A(H1N1), A(H3N2) and B were different. The studies were performed in close cooperation with WHO Influenza centers in USA and UK and 16 viral laboratories, situated in Russian regions. This work was supported in part by ISTC grant #3070.

Abstract P812

Determination of the Molecular Basis for Antigenic Drift in the Hemagglutinin Genes of H3N2 Influenza A Viruses, 1992-1994

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The gradual accumulation of amino acid changes in the influenza A hemagglutinin (HA), or antigenic drift, allows the virus to evade inhibition by antibodies from prior infection, and is an underlying mechanism of annual influenza epidemics. One example of antigenic drift in the H3N2 serotype of influenza viruses occurred by replacement of the previously circulating A/Beijing32/92-like viruses by A/Johannesburg/33/94-like viruses. Alignments of the HA1 amino acid sequences of contemporary viruses revealed 4 conserved amino acid changes that were likely antigenic drift determinants during the evolution of the A/Johannesburg/33/94-like antigenic group. The contributions of the amino acid changes were determined by generating transfectant viruses, using plasmid-based reverse-genetics, bearing either the wild-type or mutant HA genes of the reference viruses and comparing the antigenic characteristics of the transfectant viruses with the parental reference strains in hemagglutination inhibition assays using post-infection ferret antisera. Combinations of multiple amino acids in as few as two antibody-combining sites are necessary to recreate the antigenic drift observed during the 1992-1994 epidemics. Several amino acid changes result in changes in sensitivity to γ-inhibitors of red blood cell agglutination, indicating adjustments in receptor specificity occurred during this period. Finally, the changes necessary to cause an antigenic change were not reciprocal between the reference strains, suggesting virus-driven differences in epitope-recognition patterns during the humoral immune response. Revelation of molecular mechanisms in antigenic drift will prove to be a crucial tool in the improvement of influenza vaccine strain selection and understanding the nature of changes necessary for a pathogen to successfully evolve and continue to cause epidemics.

Abstract P813

SCHOLARSHIP International Scholarship

Identification of Precursors of Indonesia and Vietnam Avian Influenza A (H5N1) Viruses From Southern China

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The transmission of highly pathogenic avian influenza (HPAI) H5N1 virus to Southeast Asian countries triggered the first outbreak wave of this virus in late 2003. Subsequently H5N1 influenza virus has become endemic in poultry in this region, which has lead to the sustained transmission of those viruses and repeated outbreaks in poultry and human infection cases. This situation has raised global concern of a coming influenza

pandemic sometime in the near future. Although surveillance work in market poultry had been strengthened following this initial outbreak in Southeast Asian countries, the lack of influenza surveillance prior to the outbreaks made it difficult to identify the precursor and transmission pathways of those H5N1 viruses. To determine the possible source of those H5N1 viruses responsible for this first transmission wave we recently conducted further sequencing of samples collected in live-poultry markets from Guangdong, Hunan and Yunnan Provinces in southern China from 2001 to 2003. Phylogenetic analysis of the HA gene of 50 H5N1 isolates from this period indicated that eight viruses exclusively from Yunnan fell as the direct progenitor to viruses isolated from Vietnam. A further two viruses isolated from Hunan were the direct precursor to those viruses from Indonesia. In general, phylogenetic analysis revealed similar relationships for the NA gene and each of the 6 internal genes, indicating that these viruses also belonged to the same H5N1 genotype Z that is predominant throughout Southeast Asia. These results clearly show a transmission of H5N1 viruses from Yunnan to Vietnam and from Hunan to Indonesia. Trade of poultry may be the major route of virus transmission between Yunnan and Vietnam, while the transmission route from Hunan to Indonesia remains unclear and could be either via migratory birds or poultry movement.

Abstract P814

Dating the Emergence of Influenza A (H5N1) Virus

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Since the first detection of highly pathogenic avian influenza (H5N1) virus in geese in Guangdong, China, H5N1 viruses have transmitted to poultry throughout southern China. In late 2003 the first transmission wave was detected, which originated in southern China and spread to multiple Southeast Asian countries. In May 2005, a second transmission wave westwards to Europe and Africa was initiated following an H5N1 disease outbreak in migratory birds at Qinghai Lake, China. There is also evidence for a third transmission wave being initiated since mid-2005. Those viruses are now endemic in poultry populations in affected regions and cause repeated outbreaks in poultry and subsequent human infection cases with high mortality, creating pandemic concern. Genetic data from systematic surveillance of H5N1 for the past seven years in apparently healthy market poultry, along with sequence data from outbreaks throughout the region, provide us with a unique opportunity to estimate the most recent common ancestor (MRCA) and therefore postulate the dates of introduction of H5N1 variants into affected countries. In this study, we estimated the time of emergence of those three transmission waves, based on their hemagglutinin genes. We then compared these MRCA estimates with the date of detection of either human or poultry disease and postulated an establishment time for the virus in the absence of control measures. These analyses indicated that the time intervals between the first detection of H5N1 disease and the MRCA for the wave 1 outbreaks, in both Vietnam and Indonesia, were 5 months. While for the Qinghai outbreak the MRCA was estimated at three months prior to their first detection among migratory birds. Remarkably, the mean time of the MRCA for the third transmission wave was the same day as the first virus (Dk/FJ/1734/05) from this lineage was isolated. The early detection of H5N1 viruses in the second and third transmission waves, as compared to the first wave outbreaks in Vietnam and Indonesia, highlights the importance of systematic influenza surveillance in apparently healthy market poultry.

Abstract P815

Recent Features of Evolution of Influenza A (H1N1) Viruses in Russia

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Antigenic and genetic analyses of present-day epidemic viruses showed that the influenza A (H1N1) isolates of 2004-2005 and 2005-2006 epidemic season were close related to A/New Caledonia/20/99 antigenic variant. However in the last season the divergent changes of this subtype became evident. The antigenic analysis demonstrated that Russian stains divided into three distinct groups. According to HI (hemagglutination inhibition) test the viruses from the first group interacted with antiserum to reference-stain A/New Caledonia/20/99 up to 1-1/2 homological titer. Viruses of the second group were neutralized with antibodies of the same serum with 2-4 times lower HI titers and the third ones with 4-8 time lower HI titer. The results of genetic and phylogenetic analyses corroborated antigenic data. Three genetically distinct clusters of influenza A (H1N1) viruses were shown. The first two clusters comprise of gradually evoluting during the last 7 years viruses. They accumulated 4-5 aminoacid changers in HA molecule. The third cluster distinguished from the two first groups (in 8 residues) as well as from reference-stain A/New Caledonia/20/99 (in 5 residues). The registered aminoacid substitutions were unique for this viral cluster. The third group

was formed only with the stains isolated in 2006. According to the criteria of Kendal and Cox who believe that a new antigenic variant can be divided if there are 4 and more substitutions accumulated in more than 2 antigenic sites, we also can speak of a new reference stain. The third group was formed only with the stains isolated in 2006. During the 2006-2007 epidemic season in Russia isolated influenza A (H1N1) stains also differed from A/New Caledonia/20/99 and interacted with antiserum to this reference-stain up to 1/16-1/32 homological titer, so we can say that antiserum did not work at all. As it is reported by WHO in 2007 influenza A (H1N1) viruses were associated with outbreaks in several countries. Many isolates were antigenically similar to the current reference virus A/New Caledonia/20/99, but an increasing proportion of recent viruses were more closely related to A/Solomon Islands/3/06 which is recommended to be used in vaccines in the next epidemic season. So A/Solomon Islands/3/06 is a new reference stain. We would like to stress that our findings were made independently and simultaneously with new WHO recommendations and they strongly boost WHO proposals for a new reference strain.

Abstract P816

Influenza Viral Research Data Management and Analysis Resource: A New Collaboration of the Influenza Sequence Database and BioHealthBase

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In the past 14 months, the number of records of influenza genomic sequences in the public domain has increased from ~22,000 to ~ 47,000, more than the increase in number during the previous six years. In conjunction with this increased attention to the evolution of the influenza virus has been an increased study of influenza viral infections in humans and in animal models, bringing insights into the complex host-pathogen interaction. Clearly, there is a great need for resources that support efficient searching, selecting and analyzing data from this vast pool in support of effort to develop improved vaccine strategies and therapeutics. In January 2007, the National Institute of Allergy and Infectious Disease (NIAID) (USA) launched a collaboration between two existing influenza databases: the Influenza Sequence Database (ISD: www.flu.lanl.gov) at Los Alamos National Laboratory (USA) and the NIAID-funded BioHealthBase (BHB: www.biohealthbase.org), a UT Southwestern-Northrop Grumman-Vecna-AMAR International (USA) consortium project. The ISD, established in June 1998, has focused on viral genomic/proteomic data and has developed high-quality curation methods as well as tools for molecular epidemiological studies of the influenza virus. The BHB, first released in January 2006, has focused on development of tools to support the study of host-pathogen interactions during influenza infection. The goal of the ISD-BHB collaboration is to provide a single, comprehensive data management and analysis resource to integrate these related activities. In this presentation, we will describe our alignment-based curation of sequence data, and other automated and manual efforts to provide a high-quality resource. We will also describe the current and projected data integration and tool development work in the ISD-BHB collaboration, including: •multiple sequence alignment and phylogenetic analysis; •visualization of immunological epitopes curated from the scientific literature by the Immune Epitope Database; •visualization of immunological epitopes predicted by NetCTL; •influenza infection life cycle pathways developed in collaboration with the Reactome database project; •sequence conservation analysis; •3-D protein feature visualization. In conjunction with this presentation, we will offer hands-on tutorials with the BHB, on laptops, in a dedicated room at the convention center (times and place to be announced).

Abstract P817

SCHOLARSHIP International Scholarship

The Molecular Characteristics of Heamagglutinin of Human Influenza A/H3N2 in China From 1995 to 2005

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To investigate the molecular Characteristics of Hemagglutinin of human influenza A/H3N2 from 1995 to 2005. We performed phylogenetic analysis 550 HA1 sequences of H3N2 virus isolated from China. The bisulfide bond sites in HA1 of most strains were conserved. Our data indicated that potential glycosylation sites on the head of HA were gradually increased from1995-2005. Some receptor binding sites?RBS?and contiguous sites have changed faster than the strains before 1995. The most regular variance sites are in the proposed antigen sites or near them. Others mutant sites were also shown in 25,50,57,121,122,124,186,202,276 of HA. The tree of

human H3N2 HA1 sequence is of long trunk with short side branches. The HA sequences of the different areas are of more consistency during epidemic time, and of more polymorphic during the intervals of H3N2 epidemic. Our data suggested the circulating H3N2 strain in China were not often consistent with the vaccine strains WHO recommended according the variant animo acid of HA1 sequences. (Corresponding author: Shu Yuelong, E-mail: yshu@vip.sina.com tel: 8610-63577499).

Abstract P818

The Thousand and One Antigenical and Virological Faces of Influenza B Virus: Ten Years of Surveillance in Italy

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Influenza B viruses evolutionary pattern is characterized by a single HA and NA type and by a low rate of antigenic changes. Since the 1987 two distinct lineages have circulated causing diseases in humans, represented by reference strains B/Victoria/2/87 and B/Yamagata/16/88. Isolates collected in the last 10 years in Italy were antigenically and molecularly characterized to define the epidemiological picture. One-hundred and ninety-nine B strains were isolated between 1996 and 2006, 25 were chosen as representative of circulating viruses and characterized by HI and neutralization (NT) assays and HA and NA sequencing. Surveillance of influenza showed circulation of strains belonging to Yamagata lineage, until 2000/2001 season, when viruses belonging to Victoria-lineage re-appeared on Italian scene and co-circulated with Yamagata viruses, causing the epidemic peak (69% of isolates). The co-circulation of viruses belonging to two different lineages allowed a genetic reassortment, promoting the circulation of HA-Victoria/NA-Yamagata recombinants, that was observed during the 2002/03 and 2003/04 seasons. During the last seasons (2004/05 and 2005/06), HA-Victoria/NA-Yamagata recombinants and HA and NA-Yamagata viruses were isolated, forming a significant amount of circulating strains (19-75%). The HI data showed that the two lineages had a clearly different antigenic pattern, but HI assay was not able to distinguish the two divergent clusters into Victoria (B/HongKong/330/01 and B/Shangdong/7/97) and Yamagata (B/Harbin/7/94 and B/Sichuan/379/99) lineage, that were highlighted by HA phylogenetic analysis. The microneutralization assay clearly distinguishes viruses belonging to different lineages and sub-lineages. Moreover, this assay showed (i) cross-reactivity of recombinants with anti-Victoria and anti-Yamagata antisera and (ii) that some viruses had an unexpected low titre when tested with the homologous antibody. The molecular analysis can explain this low affinity with an amino acid substitution over the antigenic site A, situated in the loop of the HA molecule determining changes in the antibody affinity. The continued co-circulation of 2 influenza B lineages makes selection for the annual vaccine difficult, although the partial cross-reactivity, observed in recently circulating recombinants, reduces the need of the presence of both lineages in the vaccine composition.

Abstract P819

Sequence Analysis of the Ns1 Gene of H7N3 Viruses Isolated in Pakistan Reveals a 13aa Truncation and Three Viral Subpopulations Circulating Between 1995 and 2005

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A recent study of the non-structural 1 (NS1) protein of H7N1 Italian avian influenza (AI) isolates revealed a progressive truncation of an immunogenic part of the carboxy terminal of the protein after extensive circulation in poultry. In order to establish whether this occurrence was also present following extensive circulation in poultry of other viruses, we sequenced the ns1 gene of 19 H7N3 AI viruses isolated from chickens in Pakistan between 1995 to 2005. The complete gene was amplified by RT-PCR, sequenced and analysed phylogenetically. All of the 19 ns1 genes belonged to the allele A subtype. Seventeen encode a protein that has a truncation of 13 aa at its carboxy terminal while only two genes, from isolates A/Chicken/Pakistan/NARC-1282/04 and A/Chicken/Pakistan/NARC-100/04, encode a full length protein of 230aa. The isolates exhibiting the truncated carboxy terminal cluster together and appear to be closest to A/ruddy turnstone/DE/1794/00(H12N4), even though the latter has a full length protein. In contrast, the amino acid sequence of one of the isolates with the full length NS1 protein (A/Chicken/Pakistan/NARC-1282/04) showed 99.6% homology with the NS1 protein from A/turkey/Italy/214845/02(H7N3) while the other A/Chicken/ Pakistan/NARC-100/04 showed the highest homology (94.8%) with an H2N2 subtype virus A/mallard/MN/26/98 isolated in the USA indicating that these two Pakistan isolates, both obtained in 2004, are probably reassortants. This suggests that within the H7N3 Pakistan virus populations there are at least three viral subpopulations

that have circulated in a 10 year period. The sequence data from the 19 Pakistani isolates is in keeping with previous work suggesting that the truncation of the NS1 protein may be associated with the extensive circulation of AI in domestic poultry populations, in addition to providing evidence of at least two reassortment events during the epidemic. Although the cause of the truncation and its implications are yet to be established, it appears that among sequences present in public databases, only a small proportion of viruses isolated from wild birds (0.8%) have a truncated NS1, while 19.7% of viruses isolated from poultry display a truncation. The availability of additional sequence and epidemiological data from wild and domestic bird isolates will be instrumental in understanding this and other aspects of the pathobiology of influenza A viruses. Funds for this work were provided by EU project FLUAID (SSPE-CT-2005-022417).

Abstract P820

Heterogenic Selective Pressure Acting on Influenza B Victoria- and Yamagata-like Hemagglutinins

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As a consequence of immune pressure influenza virus hemagglutinin presents some amino acids under positive selection. Several works report the existence of influenza A hemagglutinin codons under positive selective pressure (PSP). Using a maximum likelihood method, this work pretended to demonstrate the presence of PSP and evaluate his effect on Victoria- and Yamagata-like influenza B viruses, estimating the parameter $w= d_N/d_S$ (acceptance rate of non-synonymous substitutions) to describe the strength of selective pressure and identifying codons that may be positively selected. For this purpose, two groups of HA1 sequences (140 from Yamagata and 60 from Victoria lineage) were submitted to codeml program from PAML 3.15 (Yang, 1997). The maximum likelihood estimates were obtained for the models M0, M1, M2, M7 and M8. M3 and M8 (which support the existence of sites under PSP) were found to be the best-fit models for both lineages, when we compared them to M2 and M7 (only consider negative and/or neutral selection) using likelihood ratio tests. This way, the hypothesis of non existence of sites under PSP was rejected ($p<0.001$), conferring statistical significance to our results and demonstrating this way the presence of positive selection acting on hemagglutinin of Yamagata- and Victoria-like influenza B viruses. In HA1 of Yamagata lineage, a 99% of codons are under negative selective pressure with w=0.19 (M8 model) and being 1% of the codons under PSP (w=5.48). For Victoria lineage the percentage of codons under PSP was near the same as for the Yamagata-like viruses (1.1%) but with a higher acceptance rate of non-synonymous substitutions, w=7.42. Different sites were estimated to be under PSP on Yamagata and Victoria hemagglutinins, however with a posterior probability higher than 0.95 (Bayes Empirical Bayes) were found 197 and 199 in both lineages and 75 in Yamagata and 129 in Victoria lineage. The detected amino acids are located at antigenic sites or near them in the H3 of influenza A viruses.

Abstract P821

Phylogenetic Analysis of the HA1 Coding Region of Influenza B Strains (from 1994 to 2006)

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Influenza B viruses present different epidemiological and evolutionary patterns when compared to influenza type A viruses. We have conducted sequence and phylogenetic analysis based on the HA1 domain-coding region in order to understand the evolution of influenza B viruses isolated in Portugal since the 1994/1995 winter season. Nucleotide sequences of HA1 region were obtained from isolated influenza B viruses after RT-nested PCR amplification. Complete HA1 sequences were aligned by the Clustal method, and their phylogeny was constructed by the Neighbour-Joining algorithm. The best-fit nucleotide substitution model was found using maximum likelihood estimates (Yang, 1997). The model of evolution that better fitted our set of sequences was the Hasegawa-Kishino-Yano 85 plus Gamma (HKY85+G). This model assumed a transition/transversion rate ratio = 7.6 and rate heterogeneity among sites (a=0.27). Synonymous and non-synonymous substitutions per site were estimated between our sequences and B/Victoria/2/87 or B/Yamagata/16/88, using the method of Yang and Nielsen (2000), and then plotted against the time of divergence since these reference strains. The substitution rates estimated were $2.17x10^{-3}$(95% CI: 2.05-$2.28x10^{-3}$) non-synonymous substitutions/non-synonymous site/year (Yamagata-like viruses) and $1.09x10^{-3}$(95% CI: 1.08-$1.11x10^{-3}$) non-synonymous substitutions/non-synonymous site/year (viruses from Victoria lineage). In order to understand which domains of influenza B hemagglutinin are influenced by positive selection, nucleotide sequences were analysed by a maximum likelihood method using the program codeml (Yang, 1997). Likelihood Ratio Tests

between models 1 and 2 and models 7 and 8 rejected the null hypothesis that selective pressure along the sequence is either neutral or deleterious, demonstrating the existence of positive selective pressure acting on hemagglutinin of type B viruses.

Abstract P822

Influenza AH3 Virus: A Ten-year Genetic Analysis

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Genetic analysis of circulating influenza strains is essential for influenza surveillance, not only by contributing to a better understanding of molecular epidemiology and evolutionary relations of influenza viruses in circulation, but also predicting virus evolution for influenza vaccine optimisation. This study presents a genetic analysis based on HA1-coding region of influenza A(H3) viruses, isolated in Portugal over the last ten influenza winter seasons. Nucleotide sequences of HA1 region were obtained from isolated influenza A(H3) viruses after RT-nested PCR amplification. Complete HA1 nucleotide sequences were aligned by the Clustal method, and their phylogeny was constructed by the Neighbour-Joining algorithm. Using maximum likelihood (Yang, 1997), we found Hasegawa-Kishino-Yano 85 plus Gamma (HKY85+G) evolution model to best fit our H3 sequence data, assuming rate variation among sites (a=0.7) and a transition/transversion ratio k=3.99. Influenza A(H3) viruses, isolated between 1996/97 and 2001/02 seasons belonged to the same lineage of the correspondent vaccine strains. The first A/Fujian-like viruses were detected in Portugal at 2003/04, presenting a strong antigenic drift relative to the vaccine strain A/Panama/2007/99. Strains detected in that season had origin on two distinct phylogenetic groups, one similar to A/Kumamoto/102/2002 and another closer to A/Wyoming/3/2003. At 2004/2005, the isolated viruses were A/California/7/2004-like, that would became the vaccine strain for the next 2005/06 winter. At the latest, was isolated only one A/Wisconsin/67/2005-like strain. Hemagglutinin of circulating strains presented several amino acid substitutions relative to vaccine strains, the majority of them located at antigenic sites.

Abstract P823

Evolution of Influenza A Virus Genotypes Through Host Switching and Reassortment of Lineages from Distinct Hosts or Geographic Locations

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The evolution of the Influenza A virus genome is complex due inpart to the unique evolutionary rates and constraints of each of the eight gene segments. The ability of reassortment and recombination to occur between viral genomes compounds the study of its evolution. A genotype nomenclature is necessary to study the dynamic nature of Influenza A evolution. In order to develop a genotype nomenclature, we studied all available gene segments and categorized them into letter-based lineages using phylogenetic clustering and nucleotide difference. A genotype is given as the string of lineages in gene order, with the hemagglutinin, neuraminidase and non-structural gene retaining their allele number, i.e. [PB2 ,PB1,PA,HA,NP,NA,NS,M]. Using this nomenclature FluGenome a web-based tool, www.flugenome.org, was created to genotype Influenza A viruses. Lineages were assigned for known gene segments and genotypes for full genomes using FluGenome. Each gene sequence also includes information on the host, geographic location and year. While each gene is different, several trends across the genome were found. In all gene segments many lineages are constrained by geographic location, loosely segregated by continent. The movement of a lineage or genotype into a new geographic area can be natural, seen in the reassortment of genes between gulls in North America and Europe, or man-made as in trade or smuggling. Recent swine trading between North America and South Korea introduced several new lineages into the swine and now avian populations in Asia. In a number genes, known lineages are currently found in a single host, but lineages or whole genotypes have the potential to cross into new hosts. The most recent example of host-switching is the introduction a full equine genotype into the North America canine population. Host-switching may also be responsible for past and future epidemics. Introduction of a gene segment with novel lineage either to a particular host or geographic region could result in a shift in pathogenicity. A uniform genotype nomenclature will be useful in studying the epidemiology and evolution of Influenza A viruses.

Abstract P824

Phenotypic and Genetic Peculiarities of Influenza B/USSR/60/69 Master Donor Strain Based Reassortants

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B/USSR/60/69 (B/60) influenza virus is master donor strain (MDS-B) for Russian live influenza B reassortant vaccine. Vaccine 6/2 reassortants, with 2 genes expressing HA and NA antigens from epidemically actual virus, inherite temperature-sensitivity (ts), cold-adaptation (ca) and attenuation (att) for humans with 6 internal genes of MDS-B. B/60 master strain was derived from the wild type virus after 60 passages in eggs at 25°C. Our investigation has revealed that the MDS-B stock is a mixed population of ca variants of B/60 virus displayed different level of temperature sensitivity. Majority of ca clones isolated in eggs from master donor stock possess ts phenotype at 37°C, but some clones are non-ts. Previously it was shown, that mutations in PB2 and PA genes are responsible for ts phenotype manifestation of influenza B master donor strain. Comparative sequence analysis of PB2 and PA gene segments identified changes unique to ts and non-ts MDS-B clones. Multiple reassortants were generated between ca/ts MDS-B clone and wild type influenza B non-ca viruses. Their reproductive capacity (RCT_{25}) in eggs was evaluated as difference between virus infection titers ($lgEID_{50}$/ml) at optimal (32°C) and low (25°C) temperatures. All the reassortants containingPB1 and PA genes ofMDS-B expressed ca phenotype. RCT_{25} of this group of reassortants and MDS-B was 1.5-2.0 lg $EID_{50.}$ The reassortants containing M gene of MDS-B had better growth capacity at 25°C (3.0-3.5 lg EID_{50}) compare to wild type virus, but it was lower then that of MDS-B. Investigations contribute to multicomponent characteristic of master donor strain and stability of live attenuated reassortant vaccines.

Abstract P825

Emergence and Convergence of H5N1 Clade 2.2 Hemagglutinin M230I in Egypt

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Avian influenza virus H5N1 Clade 2.2 was first detected at Qinghai Lake in May 2005 in long range migratory birds. Clade 2.2 has been detected in wild birds, domestic poultry, and patients in Egypt. Polymorphism tracing was used to track and monitor emerging polymorphisms, including HA M230I, encoded by two previously detected codons, ATA and ATT. Between October 2006 and March 2007, NAMRU-3, a World Health Organization Regional Influenza Reference Laboratory in Cairo, Egypt, sequenced 13 poultry and 9 human samples which were H5N1 RT-PCR positive. All H5N1 hemagglutinin sequences were Clade 2.2 containing an HA cleavage site of GERRRKKR, and a number of regional polymorphisms detected in human and poultry samples collected in 2006 between March and June. This season, eight of the thirteen poultry hemagglutinin sequences had M230I. Six were encoded by ATT and two was encoded by ATA. Similarly, four of the nine human sequences had M230I. Three were encoded by ATA and one was encoded by ATT. The four M230I positive infections were fatal. Although M230I has been detected in H5N1 in Asia previously, the detection M230I in the twelve samples collected in the 2006/2007 season represents the first report of M230I in Clade 2.2. Two of the human samples were from a cluster of three patients from the same extended family, who died in December, 2006. In addition to M230I, which is adjacent to the receptor binding domain, the two samples also had V223I located within the receptor binding domain, as well as NA N294S, an alteration linked to oseltamivir resistance. Although these emerging polymorphisms also have not been reported in human Clade 2.2, all polymorphisms have been reported previously in H5N1 in Asia. Sequencing of H5N1 in Egypt has defined a number of newly emerging regional specific polymorphisms on a Clade 2.2 genetic background. These polymorphisms can be used to identify parental strains, which contribute polymorphisms which are placed onto new genetic backgrounds via recombination. The monitoring of these polymorphisms can be used to develop new vaccine targets prior to the emergence of the novel combinations of polymorphisms.

Abstract P826

H5N1 Clade 2.2 Polymorphism Tracing Identifies Influenza Recombination and Potential Vaccine Targets

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Avian influenza virus H5N1 Clade 2.2 was first detected at Qinghai Lake in May 2005 in long range migratory birds. Subsequently, Clade 2.2 has been detected in wild birds, domestic poultry, and patients in 47 countries in Asia, Europe, and Africa Polymorphism tracing was used to track and monitor emerging polymorphisms. Newly emerging polymorphisms in H5N1 clade 2.2 sequences in public sequence databases at Genbank and Los Alamos were analyzed and compared to each other and sequences from other H5N1 sub-clades or serotypes. The newly emerging polymorphisms define regional markers and linking markers between countries that correlate with known migratory bird flyways. The newly emerging polymorphism could be traced to both low and high path avian influenza isolates. Sequences in Egypt and Nigeria from the current season contained regional markers from last season, with new markers appended onto the established genetic background defined by isolates from the 2005/2006 season. HA from the first reported human H5N1 isolate from Nigeria, A/Nigeria/6e/07, had two western Africa region markers (identified in isolates from Nigeria, Ivory Coast, Burkina Faso, and Sudan) combined with one marker form northern Germany/ Switzerland, and three others from Egypt/ Djibouti. One of these three was also present in recent H5N1 isolates from chickens in Hunan and geese from Shantou in China. Although the isolates from China were not Clade 2.2, the isolates also contained additional polymorphisms in other recent Clade 2.2 isolates from Egypt. Isolates from the familial cluster in Gharbiya contained polymorphisms in HA, NA, and PB2 that matched the geese from Shantou. Two additional isolates from adjacent regions in Beni Suef and Fayoum governorates had a three BP deletion in HA which was also present in the chicken isolates from Hunan. Newly emerging Clade 2.2 polymorphisms in Africa define migratory pathways and parental strains of H5N1, which create novel combinations of polymorphisms via recombination. Use of these acquisition patterns to predict novel vaccine targets will be discussed.

Abstract P827

Genetic Analysis of Two Influenza A (H1) Swine Viruses Isolated Recently From Humans in Thailand and the Philippines

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Influenza A viruses are known to infect a wide variety of species, including humans, swine and birds. Swine are thought to play an important role in interspecies transmission, having receptors for both human and avian viruses and thus the potential to create novel reassortant viruses of interspecies origin. While the infection of humans with swine influenza is a relatively uncommon occurrence, there have been several previous cases, most famously in the US with the swine influenza outbreak in 1976. In this present study we report on two human influenza virus isolates submitted to the Melbourne WHO Collaborating Centre for Influenza from Philippines and Thailand which appeared to have originated from swine. Influenza viruses A/Philippines/341/2004 (H1N2) and A/Thailand/271/2005 (H1N1) were isolated from two males with mild influenza. Genetic and serologic analysis at the Centre revealed that both viruses were distinct from other influenza A (H1N1) and A(H1N2) viruses that have recently circulated in the human population. Genetic analysis of the haemagglutinin genes found these viruses to have the highest degree of similarity to classical swine H1 viruses currently circulating in Asia and North America. The neuraminidase gene and the internal genes were found to be more closely related to viruses circulating in European swine, which appear to have undergone multiple reassorting events. The nucleoprotein gene from both viruses and the matrix gene from the Thai virus all had their highest homology match with genes from A(H1) viruses isolated from turkeys. While transmission of swine influenza to humans appears to be a relatively rare event and there was no evidence of significant spread in this study, swine have been proposed as an intermediate host in the generation of potentially novel influenza viruses. These viruses may have the capacity to cause future human epidemics resulting in high morbidity and mortality.

Abstract P828

Genetic Analysis of Influenza-A Viruses Isolated During an Outbreak of Encephalitis in Children From West Bengal, India, 2003

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An outbreak of encephalitis with case fatality ratio of 13.7% in children below 10 years of age was reported during May-June, 2003 in Murshidabad district of West Bengal state, eastern India. The cases were found negative for encephalitis causing viruses such as Japanese Encephalitis, West Nile, Nipah and Chandipura.Detection of Influenza-A virus RNA in 50% of the patients and isolation of Influenza-A H3N2 virus led to the possible association of the virus with the outbreak. To understand the relationship of mutations in the Hemagglutinin (HA) and Neuraminidase (NA) genes with the encephalogenic potential of Indian strains, Influenza H3N2 viruses isolated from 2 encephalitis and 2 mild upper respiratory infection (URI) cases from Murshidabad and 4 mild URI cases from Pune, western India obtained during same period were studied. HA and NA genes were amplified using reverse-transcriptase polymerase chain reaction from viral isolates from and sequenced. Tyr-137-Phe located in the receptor-binding pocket of the virus, reported for Japanese isolates from encephaopathy cases was not present in the Indian isolates (Tyr-137-Ser). At position 291, substitution of Asp with Glu noted in Japanese isolates was not present in the Indian isolates. Deduced amino acid substitutions in HA and NA proteins were related to the time of virus isolation and not to the type of clinical presentation. Based on phylogenetic analysis clustering of viral sequences was shown to be a function of time of virus isolation and not type of clinical disease in the patient.

Abstract P829

Genetic Variation in Influenza A/H3N2 Virus Isolates From India

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Monitoring genetic variations in circulating influenza viruses is an important global activity, which contributes toward determination of trivalent vaccine. Under Multi-site monitoring project National Institute of Virology, Pune, the referral center carries out genetic characterization and compilation of national data. Objective: To identify variations in hemagglutinin genes from influenza A (H3N2) viruses isolated in different parts of India during 2004-2006. Method: 48 human influenza A (H3N2) isolates (Delhi-28, Chennai-10, Kolkata-1 and Pune-9) collected between November 2004 and July 2006 from influenza regional centers in different states of India were included in the study. Isolates were sequenced for Hemagglutinin HA1 gene (1200bp) and compared with 2003-2006 Northern Hemisphere vaccine recommended strains by constructing phylogenetic trees using MEGA. Result: Phylogenetic analysis of the HA1 proteins showed two distinct lineages i.e. A/Panama/ 2007/99 (2003-2004) and A/Fujian/411/02 (2004-2005) which were the vaccine recommended strains. Fujian-like lineage appears to be the ancestor for 2 subclusters comprising of the A/Wisconsin/2005-like and A/California /2004-like sequences. 12 Isolates from Delhi collected during December 04 to February 05 clustered with A/Panama/ 2007/99 with 2% amino acid divergence (PAD) within the group. 4 isolates collected during January 05 and March 05 and two isolates in January 06 from Delhi, 3 Isolates collected during October 05, March and July 06 from Pune, and 1 isolate collected in October 06 clustered together with Wisconsin 05 and had a 0.8 PAD within the group. 5 isolates collected between September 04 to April 05 from Chennai, 6 isolates collected between March 05 to January 06 from Delhi and 5 isolates collected during July 05 to March 06 from Pune clustered with A/California /2004 with 1.1 PAD value within the group. Conclusion: Genetic characterization of the 48 A (H3N2) isolates showed that these isolates were similar to the globally prevalent strains and genetically closer to the recommended vaccine component. Results obtained by the phylogeny were in concordance with the antigenic characterization of CDC. Continued genetic monitoring of the circulating strains is necessary for deciding vaccine component.

Abstract P830

Molecular Variations of Hemogglutinin HA1 of Influenza H1N1 Analysis In China

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Objective: To study molecular variation of hemogglutinin HA1 of influenza H1N1 viruses, about 400 strains isolated from influenza like illness patients in 1956-2005 in China were tested. Methods: RNAs of the 400 strains of influenza A subtype H1N1 were extracted. The HA1 fragments of hemogglutinin gene were amplified by RT-PCR, sequenced, analyzed through bioinformatics softwares. Results: 1020 bps of HA1 fragment from about 400 strains influenza A subtype H1 were amplified and analyzed. We found that 4 sites variation derived amino acids of the HA1 were interesting and these variation returned back to 1918 from 1977(e.g.G–R–G) and 3 of them in ABD antibody-biding region (ABR). 11 amino acid sites had changed from 1956 and all related to ABR and receptor sites. One amino acid of A antibody-biding regions were deleted since 2000. Conclusion: The variations of influenza A subtype H1N1 from 1956-2005 in China show a largely antibody-biding regions and closely related to antigenicity of HA. Otherwise, the 4 sites of amino acid variation of HA1 since 1977 and returned back in 1918 would suggest may be associated with H1N1 recurred in 1977.

Abstract P831

Canadian Swine H1N1 and H1N2 Evolution Via Recombination

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Several constellations of influenza genes have emerged in swine in recent years. Classical swine H1N1 was present in North America from the first influenza isolates in 1931 through 1998. In 1998 triple reassortants were identified which had human H3, N2, and PB1 genes, swine NP, MP, and NS genes, and avian PA and PB2 genes Subsequent isolates replaced the human H3 with swine H1or the N2 with N1. Recent H1N1 and H1N2 swine isolates in Canada contained a constellation of five or seven swine genes and one or three human genes. All had a human PB1 gene. Analysis of the sequences from the 2003/2004 swine isolates identified recombination with earlier swine sequences. Most striking was the recombination in swine PB2 and PA genes. Most had regions of identity with two 1977 H1N1 swine isolates, A/swine/Tennessee/26/77(H1N1) and A/swine/Tennessee/24/77(H1N1). One had regions of identity in PA with a 1931 H1N1 swine isolate, A/swine/Iowa/1976/31(H1N1). Additional regions of identity in PB2 in A/swine/North Carolina/35922/98(H3N2) and A/swine/Korea/CY02/02(H1N2) were found. The regions of identity were extensive, covering as much as 80% of the gene segments. Nested regions of recombination were also seen.. The regions of identity, which were maintained with absolute fidelity for over 25 years, raise questions about the role of random mutations in the seasonal variation in swine and human influenza. Although the PB1 sequence was human, the 2003/2004 swine sequences were most closely related to human sequences from the mid 90's. This slower rate of evolutionary change in swine provides an alternate gene pool for human influenza evolution. The conservation of these changes and the tracing of the changes to earlier isolates provides a novel approach for the analysis of the 1918 pandemic strain, which shares polymorphisms with human and swine isolates of the early 1930's. The relationship between the 1918 pandemic strain and human and swine isolates is maintained across all eight gene segments. The role of recombination in the evolution of human and swine influenza will be discussed.

Abstract P832

1918 Pandemic Evolution Via Recombination Between Human and Swine H1N1

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Although complete sequences of all eight gene segments of the 1918 pandemic strain of H1N1 have been published, the origin and evolution of the strain remain unclear. 10 polymorphisms in the four internal genes distinguished the 1918 strain from avian isolates, but pandemic polymorphisms match both human and swine H1N1 sequences. Similarly, phylogenetic analysis indicates the pandemic strain is most closely related to human and swine H1N1 from the early 1930's. Recent H1N1 and H1N2 sequences from swine in Canada demonstrate absolute fidelity with extended regions of PA and PB2 from 1977 swine isolates from 1977, as well as regions of PA identity with a swine isolate from 1931. These identities strongly suggested that the swine evolution was via recombination and support use of polymorphism tracing to determine parental strains. Although human and swine sequences from isolates collected prior to the 1918 pandemic are not available, full sequences have been

generated on human and swine H1N1 isolates from the 1930's. Representative human, A/WSN/33, or swine, A/swine/Iowa/15/30 or A/swine/Iowa/1976/31, contain over 90% of the polymorphisms in six of the eight gene segments of A/Brevig Mission/1/1918 or A/South Carolina/1/18. The matches in the remaining two genes, NA and NP, are 84% and 86%, respectively. In six of the eight genes, the percentage of human polymorphisms are higher than the swine polymorphisms, PB2(51/40), PB1(55/40), PA(52/39), HA(53/49), MP(52/40), NS(55/41) while the swine polymorphisms are greater in the remaining two gene segments, NP(42/47) and NA(34/51). Moreover, although the matching regions alternate, the regions are defined by clustered polymorphisms. These acquisition patterns provide additional support for generation of the pandemic strain via recombination between human and swine H1N1. The differences between the 1918 pandemic strain containing polymorphisms that are found in two mammalian sources, swine and human, and a pandemic strain such as avian H5N1 which has acquired the ability to efficiently transmit in a mammalian host, will be discussed.

Abstract P833

FluGenome: A Web Tool for Genotyping Influenza A Virus

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Influenza A viruses are hosted by numerous avian and mammalian species, which have shaped their evolution into distinct lineages worldwide. The viral genome consists of eight RNA segments that are frequently exchanged by genetic reassortment. Currently only two RNA segments are used to define a genome (HA and NA), however, a complete genotype nomenclature utilizing all eight segments is needed in order to describe genetic reassortment and understand its role in host range, virulence and transmissibility of influenza viruses. To meet this need, we have developed a nomenclature to name influenza A genotypes and implemented a web server, FluGenome (http://www.flugenome.org/), for detecting lineages of given gene segment sequences and identifying genotypes of given genome sequences. FluGenome provides functions for the user to interrogate the database in different modalities and get detailed reports on lineages and genotypes. These features make FluGenome unique in its ability to automatically detect genotype differences attributable to reassortment events in influenza A virus evolution.

Abstract P834

A Statistical Method for Genotyping Influenza A Viruses

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Recent outbreaks of highly pathogenic avian influenza A virus infections in poultry and humans have caused tremendous economic losses and threaten with becoming pandemic. To identify the genetic source and to understand the transmission pathways of influenza viruses, we recently proposed a nomenclature for influenza A viral genotypes and applied the concept in the development of a genotyping tool called FluGenome. This tool uses the BLAST algorithm for the detection analysis of genotypes. Here, we present a more accurate statistical method for genotyping influenza A viruses based on genomic sequences. The implementation includes two steps: computing the complete compositional vectors (CCV) for each sequence and estimating the Chi-Square distance for testing significance of genotyping analyses. Preliminary studies on real data sets and simulation indicates that the new method is more reliable than BLAST scores in the assignment of lineages and genotypes.

Workshop Topic #9

Antivirals and Resistance

Poster Abstracts

Abstract P901

Quest for New Anti-Viral Drugs Against Influenza A Virus

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Currently, four antivirals have proven efficiency in treatment and prophylactic of influenza A infections: two M2 inhibitors (amantadine and rimantadine) and two neuraminidase inhibitors (zanamivir and oseltamivir). The M2 protein is conserved among influenza A viruses what make it a 'universal' target for anti-influenza agents. The M2 protein forms a proton selective transmembrane ion channel, which is activated at acidic pH and is the specific target of the anti-influenza drugs amantadine and rimantadine. The M2 channel plays a role in the uncoating of influenza virions in endosomes. In addition, during infection by the highly pathogenic influenza A virus subtypes H7 and H5, the M2 protein functions during its transport through the exocytic pathway. There the M2 protein ion channel activity causes equilibration of pH of the lumen of the trans Golgi network with the cytoplasm. The M2 proteins of different influenza viruses vary in their ability to alter trans-Golgi pH. When amantadine/rimantadine are used in treatment, antiviral resistance develops rapidly, limiting their use. We have tested 26 new anti-viral agents (new analogues of amantadine) by virus neutralization test on MDCK cells. Influenza viruses of several strains and different subtypes (H1, H3, and H7) were used in the test. 17 tested agents showed no effect on replication of influenza viruses. 9 anti-viral drugs inhibited the synthesis of virus proteins (nucleoprotein) and virus replication. This research was supported by Slovak Research and Development Agency (APVV-51-004105).

Abstract P902

Investigation of the Effects of Verapamil on Influenza A Virus Replication: Impairment of PKCα-transmitted Raf/MEK/ERK Induction, RDRP- activity and Viral Protein Production

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Influenza Virus (IV) attacks mainly the upper respiratory tract. IV rapidly spreads around the world in seasonal epidemics and imposes a considerable economic burden in the form of hospital and other health care costs and lost productivity. IV activate the Raf/MEK/ERK-(MAPK) cascade late in their replication cycle. This is essential for efficient nuclear RNP-export and therefore for production of infectious IV. To characterize cellular factors involved in MAPK-activation in the context of the viral infection we recently analyzed the role of PKCα. The results so far indicate, that activation of the Ca2+ activated PKCα is involved in the IV induced MAPK-signaling and that specific inhibition of this function using a Ca2+-channel blocker (Verapamil) at non toxic concentration, negatively affects IV propagation (Marjuki et al., 2006). We have now further analyzed the action of Verapamil for possible additional negative effects on IV replication. Therefore we have investigated viral protein production in IV infected human lung epithelial cells and found that PB1, NP, M1 and NS1 production is significantly reduced in IV infected and Verapamil treated A549 cells. Cell survival and cellular protein production does not seem to be affected ruling out a general effect of Verapamil on translation. Since PB1 is a functional important subunit of the viral polymerase the activity of the polymerase might be affected. Therefore we analyzed the polymerase activity in Verapamil treated cells for the production of different viral RNA species (vRNA, cRNA and mRNA) using primer extension analysis of a reporter transcript expressed by either a plasmid based replication system or in virus infected cells. Furthermore we analyzed the expression of the reporter gene in plasmid transfected cells only and in transfected and virus infected cells with and without Verapamil treatment. Our current results show that viral mRNA production in the plasmid based replication system is not affected, while it seems to be reduced in virus infected cells. This indicates that Verapamil might alter viral transcription activity in virus infected cells. Taken together, PKCα plays an important role in transmitting the influenza virus induced signal to the MAPK-cascade. As PKCα-inhibition can be achieved at non-toxic Verapamil concentration, leading to strong reduction of virus titers, inhibition of this cellular activity might be a potential anti-viral approach.

Abstract P903

Long Term Expression of Small Interfering RNA Targeting M2 Gene Induces Effective Inhibition of Influenza A Virus Replication

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RNA interference (RNAi) is a form of posttranscriptional gene silencing mediated by short double-stranded RNA, known as small interfering RNA (siRNA). Recent reports suggest that siRNAs hold great promise for treating influenza A virus infection. However, to find high efficient antiviral target with long term protection effect is still a major issue. In our study, a lentiviral vector with H1-shRNA expression cassette and enhanced green fluorescence protein as surrogate marker is adopted to deliver siRNAs into mammalian cells. Upon the lentiviral integration property, we have successfully built up persistent cell lines to express siRNAs. Based on the established stable cell lines, the inhibition efficiency and long term effect of the rationally designed siRNAs specific for conserved genome of influenza A virus have been well studied. The results showed that siRNA targeting influenza M2 gene (siRNA-M2) conferred more effective inhibition of virus replication compared with previously reported siRNA targeting NP gene (siRNA-NP). More than 90% of influenza H1N1 virus replication has been inhibited by siRNA-M2 compared with controls using Plaque Reduction Assay. Comparatively, siRNA-NP can only offer about 60% inhibition effect. High suppressive effect of siRNA-M2 was also observed on highly pathogenic avian influenza H5N1 virus. Additionally, real time RT-PCR of mRNA extracted from influenza A virus infected stable cell lines showed siRNA-M2 abolished the accumulation of M2 mRNA. That indicated mRNA was one of likely targets of RNAi. Furthermore, a long term inhibition effect and the emergence of siRNA resistant mutants in influenza quasispecies are investigated. The current data demonstrated that there was no mutation on RNAi targeted sequence after 25 passages of H5N1 virus culturing on the stable cell line expressing siRNA-M2. In conclusion, our study suggested M2 gene can be an optimal RNAi target for antiviral therapy. Those findings provide rational information for the development of siRNAs as prophylaxis and therapy for influenza infection in humans.

Abstract P904

A High Through-put Assay to Identify Novel Anti-virals for Influenza A Virus

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Currently there are only four FDA-approved drugs available for the treatment and prevention of influenza: the M2 ion channel inhibitors (adamantanes), amantadine and rimantadine, and the neuraminidase inhibitors, oseltamivir and zanamivir. Influenza A viruses resistant to both of these classes have been described, and widespread resistance has precluded the use of adamantanes for the 2006-2007 influenza season. The spread of highly pathogenic avian influenza across several continents underlines the urgent need for novel anti-virals. Here we report the development of a cell-based high through-put screen for the identification of potential anti-influenza compounds. Human lung epithelial cells (A549) were transfected with an influenza virus mini-genome which encodes firefly luciferase in the negative sense, flanked by the cRNA promoter of the A/WSN/33 NP segment. Transcription of the reporter construct generates an RNA segment that mimics viral RNA (vRNA). After infection with A/WSN/33 virus (H1N1), the viral polymerase initiates the production of luciferase. Potential inhibitors can therefore be identified through suppression of luciferase signals upon incubation with a candidate compound. The antiviral assay was optimized in a 96-well format and its validity confirmed by exhibiting z' factors of ~ 0.74. Adaptation to a 384-well format allowed screening of standardized compound libraries. To date, two distinct groups of inhibitors have been identified: protein kinase C inhibitors, and several compounds which regulate intracellular sodium concentration. In summary, we have developed a robust assay which is able to detect inhibitors of influenza virus entry, fusion/uncoating, and replication/transcription. These preliminary data provide new directions for antiviral development.

Abstract P905

Effect of Oral Gavage Treatment With ZnAL42 and Other Metallo-ion formulations on Influenza A H5N1 and H1N1 Virus Infections in Mice

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Avian influenza H5N1 infections can cause severe, lethal human infections. Whether influenza A virus treatments effectively ameliorate avian influenza H5N1 human infections is uncertain. The research objective was to evaluate the efficacy of novel zinc and other metallo-ion formulations in two influenza A mouse models. Mice infected with influenza A/Duck/MN/1525/81 (H5N1) virus were treated orally 48 h before virus exposure and then twice daily for 13 days with ZnAL42. The optimal dosing regimen for ZnAL42 was achieved at 17.28 mg/kg 48 h prior to virus exposure, twice daily for seven days. The survival rate was 80% compared to 10% in the untreated control group and a 100% survival rate for ribavirin (75 mg/kg/day, twice a day for five days, beginning four hours before virus exposure). ZnAL42 treatment significantly lessened the decline in arterial oxygen saturation ($P<0.001$). This regimen was also well tolerated by the mice. Manganese and selenium formulations were not inhibitory to virus replication when given therapeutically. Mice were also infected with influenza A/NWS/33 (H1N1) virus and were treated 48 h before virus exposure with three dosages of ZnAL42 (8.64, 1.46, or 0.24 mg/kg/day). Treatment was by oral gavage twice daily for 13 days. The highest dose of ZnAL42 was significantly inhibitory to the virus infection as seen by prevention of deaths and lessening of decline in arterial oxygen saturation. The data suggest that the prophylactic use of ZnAL42 is effective against avian influenza H5N1 or H1N1 virus infection in mice and should be further explored as an option for treating human influenza virus infections. This work was supported, in part, by contract NO1-AI-15345 from the Virology Branch, NIAID, National Institutes of Health.

Abstract P906

Efficacy of the High-Mannose Binding Protein Cyanovivin-N Against Influenza A (H1N1) Virus Infections in Mice

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Cyanovirin-N (CVN), a 101 amino acid protein derived from Nostoc elipsosporum, neutralizes virus infectivity by binding to specific high-mannose oligosaccharides (oligomannose-8 and oligomannose-9) on the influenza virus hemagglutinin HA1 subunit. The compound is effective against numerous strains of influenza A and B viruses in cell culture, but viruses passaged in mice to increase virulence lose sensitivity to CVN due to mutations that eliminate glycosylation sites on the HA1 molecule. Recently we developed a hybrid virus that was adapted to kill mice: influenza A/WSN/33 (H1N1) containing the HA1 of A/New Caledonia/20/99 (H1N1) but possessing an Asp225Gly mutation. The virus retained its sensitivity to CVN when evaluated in MDCK cell culture, thus was suitable for animal experiments. Intranasal treatment with CVN (0.5 mg/kg/day) of mice infected with the virus when treatments started 4 hours prior to virus exposure and continuing twice a day for 3 more days led to 80% survival and to a 1,000-fold reduction in lung virus titer on day 3 of the infection. In contrast, ribavirin at 75 mg/kg/day caused only a 30-fold reduction in lung virus titer. Both compounds caused significant improvement in arterial oxygen saturation of mice relative to placebos measured on days 3-11. Lung consolidation scores and lung weights were significantly reduced by CVN and ribavirin treatment on day 6, although virus titers were near control levels. A follow-up experiment was conducted with treatments starting 4 hours before, 6 hours after, and 12 hours after infection. Significant survival benefit with CVN treatment was seen with treatments starting at -4 and +6 hours, but not at +12 hours. Reductions in lung consolidation and lung virus titers accompanied the increases in survival. Efficacy of the compound in mice was dose-responsive in the range of 0.1 to 2 mg/kg/day. CVN has the potential for prophylaxis and early initiation of treatment of influenza virus infections. [Supported by Contracts NO1-AI-15436 and NO1-AI-30048 from the Virology Branch, NIAID, NIH]

Abstract P907

Antiviral Susceptibility of Influenza Viruses From the 2005-2006 Winter Season in The Netherlands

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Introduction: During large-scale use of influenza antivirals, in e.g. outbreaks or a pandemic, emergence of resistance can be anticipated and therefore surveillance of antiviral resistance should be operational. In this study, the antiviral susceptibility of Dutch influenza viruses from the 2005-2006 winter season was assessed. Methods: The susceptibility of 39 A(H3N2) and 48 B influenza viruses for the neuraminidase inhibitors (NI) oseltamivir and zanamivir was determined using a fluorescence based neuraminidase inhibition assay (expressed as 50% inhibitory concentration: IC_{50}). Adamantane resistance of the A(H3N2) viruses was determined using M2 gene sequencing. Results: Six outliers for NI susceptibility were identified. Of the A(H3N2) viruses, one (2.6%) had an elevated IC_{50} for oseltamivir (1.1 nM). Of the B viruses, two (4.2%) had a reduced IC_{50} for zanamivir (1.5 and 1.7 nM), one (2.1%) a reduced IC_{50} for zanamivir (2.9 nM) and an elevated IC_{50} for oseltamivir (23.6 nM), and two (4.2%) an elevated IC_{50} for both NIs (114 and 46.4 nM oseltamivir and 102 and 50.4 nM zanamivir, respectively). Using the remaining data, baseline NI susceptibilities were determined. Baseline IC_{50}s were for the A(H3N2) viruses 0.24 ± 0.06 nM (mean ± SD) oseltamivir and 0.83 ± 0.18 nM zanamivir, and for the B viruses 13 ± 2.7 nM oseltamivir and 6.9 ± 1.3 nM zanamivir. In Europe, recent mean IC_{50} values for influenza A(H3N2) viruses were 0.6 nM oseltamivir and 0.7 nM zanamivir and for influenza B viruses 42 nM oseltamivir and 7 nM zanamivir. Of the A(H3N2) viruses, 74% had the adamantane resistant S31N mutation in the M2 protein. This is in line with recent adamantane resistance data from many countries. Conclusions: Baseline zanamivir susceptibility of Dutch influenza viruses is similar to that for the whole of Europe. For oseltamivir, the baseline susceptibility of Dutch influenza viruses is about threefold higher compared to influenza viruses from the rest of Europe. Outliers and a subset of baseline influenza viruses are currently being sequenced to find the origin of altered NI susceptibility. The adamantane resistance data indicate that the M2 inhibitors are no option any longer for influenza A(H3N2) treatment.

Abstract P908

Use of Rapid Diagnostic Tests to Initiate Influenza Antiviral Treatment in an Institutional Setting

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Introduction: In this study, the use of rapid influenza diagnostic tests for timely initiation of control measures, i.e. antiviral treatment, in an institutional setting was assessed. Methods: In the period December 2005 – May 2006, respiratory specimens were collected from patients with influenza-like illness (ILI) in a hospital (hospitalized with ILI or developed during hospitalization), in an asthma centre, and in a nursing home. When antiviral therapy was given, a second specimen was collected at the end of the antiviral treatment. Rapid testing using two different tests took place within two hours after specimen collection. Virus culture, PCR and antiviral susceptibility analysis was done retrospectively. Results: Of 28 patients subjected to laboratory diagnosis, seven were positive in the rapid tests, virus culture, and PCR, two in virus culture and PCR, and four in PCR only. Twelve were influenza A(H3N2) and one was influenza B. With PCR as gold standard, the sensitivity and specificity of the rapid tests were 54% and 100% respectively. In the hospital, of the 17 ILI patients two were positive in all tests and three in PCR only. In the asthma centre, 3/3 ILI patients were positive in all tests. In the nursing home, 5/8 residents with ILI were positive in PCR of which four were positive in virus isolation and only two in the rapid tests. Except one outlier for oseltamivir, susceptibility for oseltamivir and zanamivir of all nine primary virus isolates was within the range for influenza viruses from the 2005/2006 winter season. Only one of four second specimens obtained from four patients treated with oseltamivir was positive in PCR but negative in virus isolation. The neuraminidases of this virus and the outlier virus are being sequenced. Conclusions: As only half of the ILI patients eligible for antiviral therapy were confirmed infected with influenza virus by the rapid tests, use of these tests for initiation of influenza antiviral therapy of an individual patient was not sensitive enough. Antiviral susceptibility data indicated that all viruses were susceptible and therapy could have been useful to limit potential spread on a ward and to reduce symptoms and worse outcome.

Abstract P909

Detection of Oseltamivir Resistance Mutation N294S in Humans With Influenza A H5N1

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Introduction: From Mar 2006 to Feb 2007 Egypt had 22 human cases of Influenza A H5N1 with 13 deaths. All patients were treated with oseltamivir using WHO guidelines. Subjects and Methods: In Dec 2006 NAMRU-3 received 3 human specimens from the Ministry of Health that were reported positive for influenza H5N1. The cases included 2 females (age 35 and 16) and one male (age 26) from an extended family living in the Nile Delta. All patients participated in the slaughter of household ducks on Dec 13 and developed influenza like illness between Dec 15 and 19. On Dec 21, a throat swab was collected from each patient before beginning oseltamivir treatment. The patients were transferred to Abbassia Chest hospital in Cairo on Dec 23 for respiratory deterioration, and additional throat swabs were collected. All 3 patients died from pneumonia complicated by adult respiratory distress syndrome. The specimens were tested at NAMRU-3 using RT-PCR. H5N1 positives were further analyzed by genomic sequencing. Results: H5N1 was confirmed in 2 patients (16F and 26M), while the third remains under study. The NA gene sequence from specimens collected before and after antiviral therapy revealed a mutation (N294S) known to confer resistance to oseltamivir. The sequences were confirmed by the Influenza Division at the Center for Disease Control. Phenotypic analysis, using the chemiluminescent NA inhibition assay, revealed a 12-15 fold reduction in oseltamivir susceptibility, but sensitivity to zanaimivir. Conclusions: N294S is reported frequently in patients with seasonal influenza treated with oseltamivir, but has been reported in only one patient with H5N1. This patient was treated with a prophylactic dose of oseltamivir followed by a full course of therapy. N294S has not previously been described in humans before beginning oseltamivir treatment. Although N294S has been detected in H5N1 infected birds in Asia, this is the first demonstration of N294S in Clade 2.2. This is also the first report of a H5N1 virus containing N294S being transmitted directly from an avian species to a human as judged by the presence of the mutation in 2 patients before the administration of antiviral therapy.

Abstract P910

Screening and Evaluation of HA Antagonists or Neuraminidase Inhibitors Using Influenza HA-Pseudotyped Retroviral Vectors

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Influenza HA-pseudotyped retroviral vectors expressing a luciferase reporter were produced by co-transfecting 293T cells with plasmids for the expression of MuLV gag-pol, fire fly luciferase, and HA (from VN1194). For the preparation of high titer pseudotyped viral vectors for the transduction of A549 or MDCK cells, neuraminidase was needed, presumably to facilitate the release of pseudotyped viral vector from 293T cells. This requirement could be trans-complemented with bacterial neuraminidase or cotransfection using plasmids expressing flu NA activities. Thus, the titer of the pseudotyped viral vector could be used to evaluate the potencies of specific flu neuraminidase inhibitors (NAI) in inhibiting the release of the pseudotyped viral vector. In addition, using the HA-pseudotyped vectors, we developed a cell-based assay system to evaluate the flu HA-mediated entry using luciferase as a reporter. This assay was studied using A549, 293T, and H460 cells and validated by the inhibition using HA blockers such as specific HA antibodies, oligosaccharides of α-2,3-SiaGal, and synthetic HA-binding peptide, EB. A natural product extract library consisting of fermentation broth and herbal extracts was used to screen for HA antagonists by transducing A594 cells using pseudotyped vector in 384-well microplates. Hit extracts with inhibitory effect due to toxicity were excluded by a screening against the inhibition of luciferase expression in cells transfected with the reporter construct only. Specific HA antagonists were defined as those that were inhibitory to the flu HA-mediated transduction but were non-inhibitory to the VSV-G-mediated transduction.

Abstract P911

Evaluation of Intravenous (IV) Zanamivir Against H5N1 Infection in the Cynomolgus Macaque

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Infection of cynomolgus macaques with influenza A/Hong Kong/156/97 (H5N1) develops into acute respiratory distress syndrome similar to that seen in man. Studies were undertaken in this primate model to examine antiviral efficacy using IV zanamivir. The IV formulation of zanamivir has been evaluated previously in man and prevented experimental infection with influenza A (A/Texas, H1N1) at 600mg twice daily (plasma half-life 1.5-2.1 hours). Further evaluation of IV zanamivir against a highly pathogenic H5N1 virus was merited. The primate model was chosen based on the IV zanamivir plasma half-life of 2.67 hours in the macaque, which is similar to man, compared with just 15mins in the mouse and rat. Two experiments evaluated the prophylactic efficacy of IV zanamivir at 2, 10 and 20 mg/kg BID and of therapy at 20mg/kg BID (n=6 per group) starting either 12 hours before or 4 hours after intratracheal infection with A/Hong Kong (H5N1) at $10^{4.5}$ TCID50 of virus. Treatment was continued twice daily for 4 days. The most valuable markers of infection were virus lung titres and gross and microscopic lung pathology on day 5 after challenge. Antiviral efficacy appeared related to dose with no effect at 2mg/kg and efficacy (≥ 1 log 10 reduction) observed in 50% of animals at 10mg/kg (log 10 reductions, 1.95, 4.53 and 4.98) and at 20mg/kg (=600mg BID in man), in both prophylaxis (log 10 reductions, 1.5, 2.5, 3.4) and therapy (log 10 reductions 1.9, 3.7, 4.1) groups. Evidence of pneumonia was reduced in treated animals. At 10mg/kg prophylaxis, only 1/6 animals had mild pneumonia compared with 2 moderate and 1 mild in the controls. Variability observed within controls and treated groups may result from sampling due to irregular distribution of infection in the lungs, and to genetic diversity of the animals. In this new animal model for evaluation of influenza antivirals, IV zanamivir showed evidence of efficacy against a highly pathogenic H5N1 virus.

Abstract P912

Amantadine-resistant Influenza A (H3N2) Virus in Argentina, 2001-2006

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Introduction: Strains of influenza A (H3N2) virus with a specific mutation (Ser31Asn) associated with amantadine resistance have recently shown a dramatic increase in communities in Asia and North America. Adamantane virus resistant data available from South America are poor. In this study we report preliminary results of a surveillance study for resistance to adamantanes among circulating influenza viruses collected in Argentina from 2001 to 2006. Besides, we chose some of these strains to study the HA1 segment and try to match all the information available in order to know the evolutionary pattern of these viruses. Material and methods: Samples coming from the national network were collected from pediatric and adult outpatients and inpatients presenting respiratory infection. Viral isolation was performed in MDCK cells and the isolates frozen at –80 ºC (as stock). For this special work; we selected up to now 50 Influenza A H3 strains isolated between 2001 and 2006 to study the M2 gene. From them, we chose 20 strains to study the HA1 portion of the HA segment. We undertook reverse RT-PCR of the M2 gene (286 bp product encompassing nucleotides 741-1027) and HA1 portions of the HA gene (929 bp encompassing nucleotides 55-984). The analysis of the genomic data was made by the construction of phylogenetic trees. Results: A total of 50 influenza A H3 field isolates were screened for specific aminoacid substitutions in the M2 gene known to confer drug resistance. Of these viruses, 16 (32%) were identified with the resistance-conferring substitution S31N. It was not detected any resistant virus between 2001 and 2005, while all the 2006 strains studied were resistant. The analysis of the molecular study of the hemagglutinin gene showed that there are two changes only present in the resistant strains that are not observed in the sensible ones. Those changes are: S193F and D225N, which falls into the receptor-biding region. Conclusion: This study reveals an alarming increase in the incidence of amantadine-resistant influenza A H3 viruses since 2006 in Argentina. The complete analysis of the emagglutinin gene results would contribute to a better understanding of the evolutionary characteristics of the resistant and sensible strains circulating in the country.

Abstract P913

Prevalence of Amantadine Resistant Influenza A in Japan and Vietnam, and Clinical Courses Of Amantadine Resistant Patients

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Strains of influenza A/H3N2 virus with a specific mutation (S31N) have recently shown a dramatic increase in resistance (70 to 90%) to amantadine in communities in Asia and North America. We conducted a multicenter study to assess the prevalence of such resistance in Japan and Vietnam. The study included molecular analysis of the hemagglutinin gene of resistant and sensitive H3N2 viruses. We also investigated clinical courses of primary and emerged resistance patients who were treated with amantadine. Our study conducted in six prefectures in Japan in 2005-06 season showed that 231 (65.3%) of 354 H3N2 isolates were amantadine resistant, with S31N change in the M2 gene. None of 61 influenza H1N1 was resistant. The phylogenetic tree analysis of the HA gene showed that all amantadine resistant strains grouped in one, and sensitive to another. The resistant viruses had specific double mutations of S193F and D225N in the HA. Investigation in Okinawa Prefecture showed this peculiar virus started to circulate in May 2005 there, before the circulation in the mainland Japan. Of 108 influenza A isolates collected in Vietnam, the prevalence of amantadine resistance in H3N2 was 0-6% during 2001- 2003, but increased to 17.5% in 2004, and 47.6% in 2005. All resistant strains collected in 2005 carried the same mutations in M2 and HA as in the Japanese strains. Clinical courses of children who shed amantadine resistant viruses after treatment (n=19) had recrudescence of fever (>37.5 °C) on Days 4 and 5, while patients shedding sensitive viruses (n=54) had no resurgence. Of 11 patients shedding primary resistance strains treated by amantadine, 3 of 4 adults, and 5 of 7 children showed alleviation of fever (< 37.5 °C) by day 3. It was suggested the clinical courses of emerged amantadine resistance was prolonged, but that of primary resistance was not. Antiviral resistant monitoring should be intensified and maintained for rapid feedback into treatment strategies, and selection of alternative therapeutic agents.

Abstract P914

Surveillance of Resistance to Adamantanes Among Influenza A(H3N2) and A(H1N1) Viruses Isolated Worldwide (2005-2007)

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Background: Since 2003, an alarming rise of adamantane resistance among influenza A viruses was reported by CDC and other influenza surveillance laboratories (Bright et al., 2006a, b). During the 2005-06 and 2006-07 influenza seasons, we continued screening for mutations in the M2 protein associated with resistance to adamantanes using pyrosequencing as a primary method. Methods: Influenza A(H3N2) and A(H1N1) viruses (n=2845) isolated globally from 2005-2007 and submitted to the CDC were tested. Confirmatory sequence analysis of the entire M gene and yield reduction assay were performed on a subset of isolates. We also investigated possible correlation between the appearance of drug resistance and the genetic evolution of the hemagglutinin (HA) gene. Results: The results indicate that 96% (n=766) of the A(H3N2) viruses isolated in the United States during the 2005-2006 season were resistant. Resistance among A(H3N2) viruses has reached 100% in isolates received from some Asian countries. Genetic analysis suggests that at least two separate introductions of resistant viruses into the human population occurred. During 2006-07, 3/10 (33 %) A(H3N2) viruses collected in the US were resistant. Globally, the frequency of resistance in A(H3N2) viruses has decreased from 92% in 2005-2006 (n = 2014) to 44 % (n = 55) in recent isolates (October, 2006 – January, 2007). The frequency of drug resistance among A(H1N1) viruses isolated worldwide increased from 0.3% in 2004 to 15.5 % in 2005-2006; (4.0% in the U.S. alone) but has decreased during the 2006-07 influenza season to <3% (n=108) worldwide (1.0% in the U.S.; n=91). Phylogenetic analysis of the HA gene of A(H1N1) viruses suggests that acquisition of resistance can be linked to a specific genetic group. Genetic analysis showed that appearance of resistance in A(H1N1) viruses was not a result of reassortment between viruses of A(H3N2) and A(H1N1) subtypes. Conclusions: Influenza virus resistance continues at high rates among circulating H3N2 viruses with less resistance among H1N1 viruses. Our findings emphasize the necessity of close monitoring of resistance to existing antivirals and the need for development of new antiviral agents.

Abstract P915

Influenza Viruses Sensitivity to Antivirals in Russia

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Background: Two prescription medications that are active against influenza viruses are approved, commercially available and widely used in Russia: Rimantadine™ (blocks the function of the M2 protein) and Arbidol™ (inhibits the lipid membrane of influenza virus fusion with host cell). Our interest was to study the spectrum of epidemic influenza viruses (2002-2006) sensitivity to these antivirals. Methods: MDCK cell line was infected by influenza viruses with/or without antivirals, and then results were obtained by titration in HA-test, ELISA and Plaque assay. Results: The high percent of resistant to rimantadine (5.0 µg/ml) for influenza A viruses (2002-2003) was found (9.5%). The tendency to its increasing was detected for the consecutive seasons and during 2005-2006 this index was equal to 38.0%. Earlier we found, that arbidol supressed the plaque formation for all types of influenza viruses and its concentration for 50 percent of plague formation decreasing in comparing with control monolayer was between 5 and 10 µg/ml. Our results showed that all influenza A viruses including rimantadin-resistant mutants were sensitive to arbidol (10 µg/ml). Meantime among influenza B viruses we found some differences, which may be caused by their strain specificity to arbidol: only 57.0% among B/Shanghai/361/02-like epidemic viruses during 2004-2005 and all of B/Hong Kong/330/01-like epidemic viruses during 2005-2006 were sensitive to arbidol. Conclusions: Received results determinate the recommendations for antivirals using in annual epidemic preparedness and allow to use both of them (rimantadine and arbidol) for influenza treatment and prophylaxis in Russia now. The investigations were supported by ISTC project 3070.

Abstract P916

Pharmacokinetics and Safety of Peramivir by Intramuscular Administration

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Peramivir is a potent neuraminidase inhibitor with potential efficacy in treatment of human influenza infections. Two Phase 1 studies were performed to investigate the pharmacokinetics (PK) of i.m. peramivir. In Study A (n=24) subjects received i.m. doses of peramivir or placebo on two successive days (PK assessed on day 2); in Study B (n=27) subjects received peramivir administered by both i.v. and i.m. routes in a randomized crossover design. In Study A, the C_{max} (± SD) (ng/mL) after the second dose of 75 mg, 150 mg, and 300 mg were 3594±330, 7594±1424, and 13275±2438, respectively, and the terminal half-life ranged from 17. 1±3.1 to 18.4±2.0 hr. In Study B, the C_{max} (± SD) (ng/mL) after single i.v. administration of 75 mg, 150 mg, and 300 mg doses of peramivir were 4652±606, 9401±1962, and 17167±1482, respectively, and the terminal half-life ranged from 14.4±2.8 to 25.6±6.6 hr. In this crossover study, the mean bioavailability (F) of i.m. peramivir ranged from 98.9% to 105.1% (based on $AUC_{0-\infty}$). Safety: Across the two studies, no SAEs were reported. All adverse events reported were rated as mild in severity with the exception of 4 episodes of headache rated as moderate. No significant changes in clinical laboratory test results for hematology, liver function, or renal function analytes were noted. Elevations in total CPK following i.m. injections appeared related to the dose of peramivir administered; no CPK elevations exceeded Grade 1 (3-5.9 X ULN). Conclusion: The pharmacokinetics of i.m. peramivir appear to be dose-proportional and the bioavailability of single doses of i.m. peramivir is similar to i.v. peramivir. The relatively high plasma levels and long terminal half life of peramivir suggest that the potential for efficacy of single dose treatment of acute influenza should be evaluated.

Abstract P917

SCHOLARSHIP International Scholarship

The Multi-Country Emergence of Adamantane-Resistant Influenza A(H3) Viruses is Due to a Single Lineage That Possesses Distinct Internal Genes

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The amantadine-resistant influenza A (H3) viruses resulting from the mutation of the M2 protein at amino acid residue 31 (M2 (31N)) have become alarmingly prevalent in multiple countries since 2003. However, the M2 mutations per se, though conferring resistance to amantadines, were shown not to significantly increase the fitness of influenza A viruses. The sudden uprising of these viruses, which could not be attributed to the drug usage, remains mysterious. Here we analyzed the whole genome of 346 influenza A (H3) viruses that were isolated from Taiwan, New Zealand, New York, and Australia from 2004 through 2006 during which the frequency of amantadine-resistance rapidly increased. We found that in all four countries most of the M2 (31N) isolates possess internal genes with distinct amino acid substitutions. Moreover, our phylogenies of amino acid and nucleotide sequences of the M1, PA, NP, PB1, and PB2, inferred by at least two methods, all show a distinct clade of these amantadine-resistant M2 (31N) isolates, termed Linko-M2 (31N). In addition, the associated HA1 (193D/225N) and NA (93N) antigenic drift appeared to occur in Taiwan after the Linko-M2 (31N) had emerged. Our analysis suggests that the multi-country emergence of the M2 (31N) influenza A (H3) viruses is due to the expansion of one single lineage, and their apparent selective advantage can be attributed to, besides the major HA antigenic drift, altered functions pertaining to the amino acid substitutions in multiple internal genes. Furthermore, our preliminary data suggest that the replication rate of the amatadine-resitant Linko-M2 (31N) virus is higher than amantadine-senstive virus in MDCK cells. The result will be confirmed by further experiments in different mammalian cell lines, e.g., Vero. Therefore, these data suggest that the importance of the internal genes in steering the fitness of influenza viruses should be investigated and monitored more systematically.

Abstract P918

Evaluation of Anti-influenza Activity of Green Tea Extracts In Vitro and In Vivo

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Ongoing outbreaks of H5N1 highly pathogenic avian influenza virus threat the world since 1997. However, utility of most antiviral agents is limited by the inherent toxicity of the compounds and the appearance of drug resistant virus following their continued use. Although animals play an important role of influenza transmission, treatment or prevention of influenza using antiviral agents is not focused on animals, but on human. Furthermore, incomplete efficacy of inactivated avian influenza vaccines indicated that infection will be accompanied with continuously excretion of influenza virus. Besides the vaccines, therefore, alternative control measures in animals are needed to prepare next pandemic influenza. Green tea has been popular historic beverage of Asian populations for a long period. The major active ingredients of green tea is polyphenolic compound catechins ((-)-epigallocatechin gallate(EGCG), (-)-epicatechin gallate(ECG), (-)-epigallocatechin (EGC) and (-)-epicatechin(EC)), which have various biological and pharmacological activities. There is an extensive literature documenting the anticancer, antibacterial, antiparasitic, anti-inflammatory, antioxidative and antiviral activity of catechins. Among antiviral activities, anti-influenza activities of catechins were performed by blocking virus to be absorbed to cells, preventing acidification of endosome or lysosomes resulting in inhibition of growth of virus, and inhibition of neuraminidase and viral RNA synthesis. Based on anti-influenza activity, we investigated green tea-based treatment as alternative controls of influenza in chickens. Green tea extracts infused in water, extracts infused in 60% EtOH and byproducts were examined to evaluate antiviral activities and showed 50% effective concentrations of 39.57ug/ml, 6.36ug/ml and 6.49ug/ml respectively in MDCK cells. Green tea byproduct extracts were evaluated in vivo using BALB/c mouse and SPF chickens. A large scale consumption of green tea in Asia generates plenty of green tea byproducts. About 50 tons of green tea byproducts are produced in the biggest green tea production area of Boseong in Korea in a year. Considering their plenty resources and cheap price, feed additives using green tea leaves seems to be possible to contribute to control influenza infection in poultry productions.

Abstract P919

Cinnamon Extracts Inhibits Avian Influenza H9N2 Both In Vitro and In Vivo

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Influenza is one of the most prevalent and significant viral infections. The aim of this research was to examine the ability of the Cinnamon Extract (CE) and its fraction CEppt to inhibit avian influenza H9N2, after we previously demonstrated this antiviral activity against human influenza H1N1 (Barak, I. and Ovadia M., 2005, Antiviral Research, 65(3): 65). The ability of CE to neutralize the avian influenza virus was tested in-vitro on human erythrocytes by the hemolysis assay and in-ovo on chick embryos. Both activities of the virus were inhibited. A dose of 50-100 micrograms of the crude extract was sufficient to achieve total neutralization of 256 HAU of the virus within one minute. CE has a long shelf-life of at least two years in the refrigerator or at room temperature. It still retained its antiviral activity after dialysis in bags with a cut-off of 10 KD or after heating at various temperatures up to 121° C. The antiviral activity was also stable at a wide range of pH between 1 and 12. Moreover, the antiviral agent neutralized the virus after it was already attached onto the erythrocytes and prevented its subsequent hemolytic activity. CEppt decreased the viral infectivity in-ovo by 5 Logs when injected together into the allantoic cavity of 11 days chick embryos. In conclusion, CE has exhibited an effective antiviral activity against influenza virus both in-vitro and in-ovo. The cinnamon has been used in the human diet for thousands of years and should therefore not be an obstacle to introducing the isolated antiviral fraction for human and animal use. International Patent Application PCT/IL2004/001161.

Abstract P920

Antiviral Activity of Brazilian Propolis Against Influenza Virus In Vitro and In Vivo

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Propolis has been used worldwide as a folk medicine and a dietary supplement to maintain or improve human health since ca. 300 BC. It is a resinous material collected from various plant sources by honeybees and contains many kinds of compounds that vary in the areas collected. Propolis has been reported to exhibit various biological activities. Its anti-influenza virus activity was also reported in an in vitro-study. However, the anti-influenza virus activity in vivo is not clear. In this study, we examined the anti-influenza activity of Brazilian propolis in vitro and in vivo and found that one of 13 propolis exhibited therapeutic efficacy against influenza virus infection in mice. Thirteen propolis used were collected by Africanized Apis mellifera bees in different parts of southern Brazil, extracted with ethanol, and dried. The 13 ethanol extracts were screened for their antiviral activity against influenza A/PR/8/34 (H1N1) virus by the plaque reduction assay using Madin-Darby canine kidney (MDCK) cells. The cytotoxicity of extracts was evaluated by the trypan blue dye exclusion assay and MTT assay using MDCK cells. Among the 13, an extract (AF-08) exhibited a potent anti-influenza virus activity and did not inhibit influenza virus adsorption to cells. AF-08 also possessed antiviral activities against influenza A/Ishikawa/7/82 (H3N2), B/Fukushima/15/93, and oseltamivir-resistant A/PR/8/34 (H1N1) viruses. AF-08 was further examined for its therapeutic efficacy in an intranasal influenza virus infection model in mice. Its oral administration (0.4, 2, and 10 mg/kg three times daily for 7 days) prolonged the survival times of infected mice in a dose-dependent manner without toxicity. The dose of 10 mg/kg was significantly effective in reducing the body weight loss of infected mice and virus yields in the bronchoalveolar lavage fluids of lungs compared with those of controls. These anti-influenza efficacies at the 10 mg/kg corresponded to that of oseltamivir at 1 mg/kg twice daily as the dose for mice corresponding to a human dose. Thus, a Brazilian propolis, AF-08, was a possible candidate for anti-influenza dietary supplement and suggested to contain some compound that shows different anti-influenza action from oseltamivir.

Abstract P921

Similarity in the Pharmacokinetics of Oseltamivir and Oseltamivir Carboxylate in Japanese and Caucasian Children

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Background: The pharmacokinetics (PK) of oseltamivir and oseltamivir carboxylate (OC) in Japanese and Caucasian adults were shown to be similar in a previous study. Objective: To compare the concentrations of oseltamivir and OC achieved in Japanese and Caucasian children from studies conducted throughout the oseltamivir development program. Methods:

Raw and body-weight normalized time-scaled concentrations were compared from four pediatric studies in which PK samples were obtained. These included three Caucasian pediatric studies [PP16351: 24 children, uninfected, aged 1–5 years, intensive PK (up to 7 post-dose samples) following single dose treatment; WV15758: 99 children, flu infected, aged 1–5 years, intensive (up to 9 post-dose samples) and sparse PK during 5 days b.i.d. treatment; NP15826: 18 children, uninfected, aged 5–16 years, intensive PK (up to 11 post-dose samples) following single dose treatment] and one Japanese pediatric study [JV16284: 18 children, flu infected, aged 1–12 years, sparse PK (up to 2 post-dose samples) during 5 days b.i.d. treatment]. In these studies, mean doses and mean doses per kg body weight ranged from 41 to 74mg and from 1.80 to 2.89mg/kg, respectively. Results: Concentrations of oseltamivir and OC were comparable across Japanese and Caucasian children. After normalization to a 2mg/kg dose, inter-quartile ranges for oseltamivir concentrations were 3.63 to 26.75ng/mL in Caucasian children and 3.95 to 22.05ng/mL in Japanese children. Inter-quartile ranges for OC concentrations were 139 to 274ng/mL in Caucasian children and 167 to 298ng/mL in Japanese children. Conclusion: Available PK data for oseltamivir and OC from studies in Japanese and Caucasian children are similar. This investigation further supports similarity in PK of oseltamivir and OC across the age ranges in these ethnic groups.

Abstract P922

The NA-Star® Influenza Neuraminidase Inhibitor Resistance Detection Kit: Chemiluminescence Assay for Detection and Quantification of Influenza Neuraminidase Activity

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The NA-Star® Influenza Neuraminidase Inhibitor Resistance Detection Kit provides the NA-Star® 1,2-dioxetane chemiluminescent neuraminidase substrate, together with all necessary assay reagents and microplates, to measure the resistance level of influenza virus isolates to neuraminidase inhibitor antiviral therapeutics. The NA-Star chemiluminescent substrate provides highly sensitive detection of neuraminidase enzyme activity from influenza virus types A and B, including human, avian, porcine and equine viruses. Neuraminidase assays performed with the NA-Star 1,2-dioxetane chemiluminescent substrate provide approximately 50-fold higher sensitivity than neuraminidase assays with the MUNANA fluorescent substrate. The chemiluminescent assay with NA-Star substrate provides linear results over 3-4 orders of magnitude of neuraminidase concentration compared to 1-2 orders of magnitude achieved with the fluorescent assay, providing a greater assay dynamic range. Virus dilutions are briefly incubated with neuraminidase inhibitor, and then the two-reagent detection assay is performed. The entire assay is completed in approximately one hour. Data analysis, using non-linear curve fitting dose response analysis software (not provided), is performed to determine the IC50 value of the neuraminidase inhibitor with each viral isolate. The NA-Star chemiluminescence assay has been compared to fluorescence assays performed with MUNANA with isolates of the major influenza types, H1N1, H3N2 and influenza B strains, including NI-sensitive and resistant strains. Results obtained with both oseltamivir and zanamivir inhibitors will be presented. The NA-Star Influenza Neuraminidase Inhibitor Resistance Detection Kit combines highly sensitive and rapid chemiluminescent quantitation of neuraminidase activity from flu virus isolates using supplied reagents and a simple assay protocol to provide a convenient method for use in research laboratories to monitor the resistance levels of both human and animal influenza virus isolates to neuraminidase inhibitors.

Abstract P923

A Prospective Study To Assess the Emergence and Transmissibility of Resistance To Oseltamivir Following Treatment of Children With Influenza

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Background: Resistance to oseltamivir at a high frequency (18%) has been reported in children with influenza treated using a weight adjusted daily dose of 4mg/kg. A prospective study during 2005/06 and 2006/07 assessed the emergence and transmissibility of drug resistance following treatment of acute influenza in children. Children were treated with weight based unit dosing of oseltamivir where consequently smaller children receive higher doses of drug. Methods: Children (1-12 years) with a positive influenza rapid test presenting to Leicester Children's Hospital within 48 hours of onset of symptoms were prescribed oseltamivir. Nasopharyngeal swabs were obtained on days 0, 6 and 9 and on day 9 from household contacts (2006/07 season). Quantitative RT-PCR was carried out on original clinical material and susceptibility

of virus isolates to neuraminidase inhibitors was assessed using IC_{50} determination. Genotypic evaluation of original clinical material and virus isolates is underway. Results: In 2005/06, 46 children positive by rapid influenza test received oseltamivir. 40 (87%) were positive for influenza by RT-PCR. Virus isolates were recovered from 39 (85%) samples. Subtype analysis indicated B (20, 50%), A/H1 (12, 30%), A/H3 (6, 15%) and mixed A/B infections (2, 5%). No phenotypic resistance of any viral subtypes was observed in pre-treatment isolates. On day 6, 25/38 (66%) samples were RT-PCR positive with 16 (42%) culture positive. On day 9, 10/32 (31%) samples were RT-PCR positive and 2 (6%) remained culture positive. No phenotypic resistance among post treatment B isolates was observed. Sequencing of influenza B NA from original clinical material on day 9 has not identified any known mutations associated with NA resistance. Phenotypic and genotypic evaluation of 05/06 influenza A isolates at days 6 and 9 is in progress. During 2006/07, 30 children have so far been enrolled. Discussion: During the 2005/06 season, no phenotypic resistance was observed in community pre-treatment isolates of influenza H3N2, H1N1 and B. No phenotypic resistance was identified in post treatment isolates from 20 children who had influenza B infection suggesting that weight based unit dosing of oseltamivir may be superior to simple weight based dosing in preventing the emergence of antiviral resistance.

Abstract P924

Pre-Exposure Oseltamivir Prophylaxis Permits Protective Antibody Response To Influenza Virus

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Introduction: It is unclear whether pre-exposure oseltamivir prophylaxis during the first wave of an influenza pandemic would permit antibody response to virus protective against subsequent waves. We conducted mouse prime/challenge to assess this experimentally. Methods: In pilot studies we demonstrated that mice given 10^5 pfu A/FM/1/47(H1N1) (FM) intranasally survived whereas mice given $5X10^3$ pfu of a mouse-adapted (MA) variant died by day six. Oseltamivir at 10mg/kg/day in mice yields plasma levels equivalent to human treatment doses of 75 mg bid; human prophylaxis doses are 75 mg od. The study model included unprimed BALB/c mice and groups that were primed by FM infection at two dosages (10^3 or 10^5 pfu). Primed groups received no drug or two dosage regimes of oral oseltamivir suspension (5 mg/kg/day or 10 mg/kg/day) starting at day -7 before virus prime and continued until 10 days after prime. Mice were monitored for seroconversion by hemagglutination inhibition (HAI) assay and for protection against a lethal dose of MA virus. All groups were challenged with lethal doses of MA on day 35 and monitored for temperature, weight and survival. Virus replication in the lung was measured in three mice per group euthanized on days 2 & 37 and all remaining at 45. Results: Parameters of weight loss, survival and viral load were consistent with induction of protection by priming during oseltamivir use. Lung virus titres two days after priming showed lower trend among mice receiving oseltamivir but there was no difference in HAI titres at any time point by oseltamivir dosing level. Whereas all unprimed mice died following lethal challenge, all primed mice resisted lethal infection whether receiving oseltamivir or not. No virus was detected among primed mice two days after challenge in contrast to the unprimed group where titres exceeded 10^6 pfu. Conclusion: Using mice as surrogates for humans, this study suggests that pre-exposure oseltamivir prophylaxis is permissive of a protective antibody response to influenza virus, with implications for pandemic stockpile use.

Abstract P925

The NISN Influenza Virus Panel for Use in Detecting Neuraminidase Inhibitor Resistance

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The emergence of influenza viruses with resistance to the neuraminidase (NA) inhibitor (NAI) drugs oseltamivir and zanamivir has been limited to date. Reports of increased frequency of oseltamivir resistance in children treated for seasonal influenza in Japan, and the detection of resistant isolates of A(H5N1) following treatment indicate that the possible emergence and transmission of resistant influenza variants should be closely monitored through national and regional surveillance programmes, particularly in light of extensive stockpiles of NAIs held by many countries as part of pandemic preparedness plans. To assist with the development of global surveillance capacity for the measurement of NAI susceptibility it is important to have well characterised panels of reference virus strains, with known NA resistance mutations, and related sensitive parental strains to act as quality assurance reagents in laboratories undertaking surveillance work. On

behalf of the Neuraminidase Inhibitor Susceptibility Network (NISN), the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne, Australia, has produced a panel of reference influenza viruses for use in the quality control of NAI susceptibility testing of influenza viruses. The panel of viruses will be available to WHO national influenza centres and other interested laboratories and consist of parental sensitive viruses and NAI resistant variants which have defined NA mutations: influenza A(H3N2) - (E119V and R292K), A(H1N1) - (H274Y), and influenza type B (D198E) virus. All of the strains included in the panel have been plaque purified in MDCK cells, sequence confirmed and are stable following expansion in tissue culture. IC_{50} values for the reference viruses against zanamivir, oseltamivir carboxylate and peramivir in both a fluorescence-based and chemiluminescence-based NA enzyme inhibition assay are described. Use of the panel of NAI resistant and sensitive viruses will help laboratories currently performing NA enzyme inhibition assays to harmonise data and will be an important set of reference reagents to assist laboratories wishing to develop surveillance programmes. Use of well characterised reference viruses should be the basis of a standardised approach to global NAI susceptibility testing in the future and aid the comparison of all data estimating the frequency of drug resistance.

Abstract P926

Mutations in Non-conserved Residues Outside the Active Site Can Confer Resistance to the Influenza Neuraminidase Inhibitors

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Two recently developed drugs zanamivir and oseltamivir are effective against all strains of influenza, including human and avian strains. While no resistant viruses have been isolated from immunocompetent patients treated with zanamivir, resistant viruses have been isolated from a small number of patients treated with oseltamivir, including from oseltamivir treated H5N1 infected patients. Most of the mutations are in highly conserved residues in or around the active site. As part the Neuraminidase Inhibitor Susceptibility Network (NISN) global surveillance program we have identified an H1N1 isolate from an untreated Japanese patient, A/Hokkaido/15/2002 which demonstrated resistance in the enzyme inhibition assay to both oseltamivir and zanamivir. The isolate was plaque purified and was found to contain different populations exhibiting either drug sensitivity or drug resistance in the enzyme and plaque inhibition assays. Decreased sensitivity in the enzyme assay correlated with a Y155H mutation in the NA, confirmed by expression as a recombinant protein. Surprisingly this residue is not conserved, since the A/Tokyo/67 NA used for structural studies and design of zanamivir has H155, yet is clearly sensitive to the inhibitors. Furthermore H155 does not confer resistance in all N1 strains, since the H5N1 strains currently circulating have H155, but are sensitive to the inhibitors. Molecular modeling shows that this residue is remote from the active site, hence there are clearly unknown secondary structural effects outside the active site which impact on drug binding. The mutation affects enzyme activity and the pH optimum of the enzyme, the first report of a non-conserved residue having such a direct effect on the active site. Progeny revealed varying degrees of resistance in the plaque assay in either MDCK or SIAT-MDCK cells. The greatest resistance correlated with a mutation in the HA gene at residue D225, which forms part of the receptor binding site of the HA. This would be the first demonstration of a specific HA mutation from a human isolate correlating with drug resistance in a plaque assay.

Abstract P927

The Emergence of Adamantane Resistance in Influenza A(H1) Viruses in Australia and Regionally in 2006

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The adamantanes (amantadine and rimantadine) were the first antivirals licensed for use against influenza A viruses and have been used in some countries to control seasonal influenza. While increasing resistance of A(H3) viruses to this class of drug has been reported in recent years, only low levels of resistance were seen with A(H1) viruses until the 2005-6 influenza season in the USA. In this study we analysed 101 human influenza A viruses isolated in 2006 that were referred to the WHO Collaborating Centre for Reference and Research in Melbourne, from Australia and the surrounding regions, for evidence of resistance to adamantanes by sequencing of the M2 gene. We found that whereas previously A(H1) resistant viruses were rare, 21.8% of the 2006 viruses had a resistant genotype. By comparison, 58.6% of influenza A(H3) viruses isolated in 2006 that were tested at the Centre, had a resistant genotype. The A(H1) resistance was not as widespread as A(H3) resistance with several countries appearing to have no resistant viruses amongst the samples tested. Interestingly practically all resistant viruses of both A(H3) and A(H1) subtype

had the same S31N mutation in the M2 gene. Clearly both the A(H1) and A(H3) resistant viruses have no lack of viral fitness and are easily transmissible in man. While there are high levels of adamantane resistance in circulating influenza A viruses, the newer neuraminidase inhibitor drugs are the preferred drug of choice for the treatment or prophylaxis of influenza. While resistance levels appear to be falling in Northern America in 2007, ongoing surveillance and monitoring will be required for a number of years in order to determine if adamantanes can be used effectively in individual countries in the future.

Abstract P928

Catalytic Nucleic Acids Mediated Gene Silencing of Influenza AVirus

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Background: Influenza virus infects thousands of people each year, causing epidemics with high mortality. Moreover, there is an increasing concern about a potential influenza pandemic, as highly virulent avian influenza strains are spreading from South-East Asia, with a high risk to cross species-specific barriers. Recent advances in antisense technology have emerged as a ray of hope against many pathogens. The aim of this study to use the catalytic nucleic acids like Ribozymes and DNAzymes as a candidate to have a control on the spread of the influenza virus in the host cells. Methodology: The M1 and PB1 gene of A/PR/8/34 (H1N1) and A/Udorn/307/72 (H3N2) strains were cloned in pcDNA. The computer based secondary structures of RNA were analyzed to design the DNAzymes with 10-23 catalytic motifs and the hammerhead Ribozymes. These DNAzymes and Ribozymes were used for in vitro cleavage of RNA of the corresponding M1 and PB1 gene in the presence of different concentration of $MgCl_2$. The cleaved product were separated by PAGE and analyzed by autoradiography. The DNAzymes and Ribozymes were also used in combination. Result: The DNAzymes were able to cleave the M1 RNA at 137 nt position whereas Ribozymes targeted at163 nt position in the same target. These catalytic nucleic acids were highly efficient under the simulated physiological conditions. When DNAzymes and Ribozymes were used in combination, the cleavage was enhanced as compared to when they were used alone. Conclusion: This combinatorial strategy can be used to design multi target DNAzymes and Ribozymes to delay the appearance of escape mutants because of the low probability of simultaneous mutations in both the target RNA sites. This antiviral approach suppresses the production of influenza virus.

Abstract P929

High Incidence Of Adamantane Resistance, But No Evidence Of Neuraminidase Inhibitor Resistant Influenza A Viruses Isolated In Germany From 2002 To 2007

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Background: The high frequency of adamantane resistant influenza viruses isolated worldwide and the appearance of neuraminidase inhibitor (NAI) resistant viruses are cause for concern. In view of a possible wider application of antiviral drugs for therapy and prophylaxis of influenza infections, we developed and established rapid methods for resistance testing and investigated the frequency of resistant viruses circulating in Germany. Methods: Human influenza A viruses isolated in Germany from October 2002 through February 2007 were examined for antiviral resistance with pyrosequencing (PSQ), confirmatory sequence analysis, and phenotypic testing. We designed a new PSQ-PCR for NAI resistance testing, and established the PSQ-PCR according to Bright et al. 2005 for analysing the M2 gene. For phenotypic analysis the neuraminidase inhibition assay with pretitrated virus suspensions and the fluorometric substrate Munana (Sigma) was performed. Results: We developed a high-throughput and time-saving method using pyrosequencing, which allows for the screening of single nucleotide substitutions in the neuraminidase gene known to confer drug resistance. Using site-directed mutated neuraminidase genes as positive controls, we analysed for amino acid substitutions E41G, E76K, E119V, Q226H, R292K, and N294S in neuraminidase of subtype H3N2, and the H274Y, R292K, N294S substitutions of H1N1 neuraminidase. During the last four influenza seasons no resistance-associated mutations in the neuraminidase gene were detected (n=177 isolates collected from 2002 to 2006). Currently circulating viruses were analysed from nasal swabs collected from December 2006 through March 2007. Also no neuraminidase inhibitor resistance was detected, neither in genotypic (n=225) nor in phenotypic analysis (n=86, 50% inhibitory concentration for oseltamivir IC_{50}=0,43 ± 0,57 nM). On the other hand, a high incidence of influenza A viruses carrying the resistance-associated substitution S31N of the M2 gene was observed (n=118 isolates investigated). The prevalence of resistant viruses increased from 15% in 2004/2005 to 83% during the 2005/2006 season. For the current season a decrease in adamantane resistance was noted (29% of n=49 isolates, so far). Conclusions: In view of using neuraminidase inhibitors for stockpiling, therapy, and prophylaxis, monitoring the emergence and spread of resistant human or avian influenza viruses should be included as part of the ongoing influenza surveillance.

Abstract P930

Decreasing Sensitivity of Influenza B Viruses to Neuraminidase Inhibitors

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The neuraminidase inhibitor (NI) class of anti influenza drug was introduced in 1999. Global surveillance of influenza virus isolates in the three years preceding this showed a lack of primary resistance in all human influenza virus subtypes. Studies in the years after introduction of drugs have shown a seasonal fluctuation in the mean levels of susceptibility of influenza B viruses to oseltamivir which is not matched by influenza A viruses. The clinical significance of this evolution is not clear, as so far in the UK it is not associated with reports of treatment failure. We describe here a targeted study of susceptibility to both oseltamivir and zanamivir of influenza B isolates collected as part of UK national influenza surveillance from 1998, prior to NI introduction, to 2007. We find that the mean IC50 for influenza B isolates has increased by greater than 50% for both oseltamivir and zanamivir since 1997. A marked increase in IC50 to both drugs was seen between winter seasons in 2003 and 2004 with further smaller fluctuations since then. There has been considerable genetic and antigenic heterogeneity in influenza B viruses during this time since both Yamagata and Victoria lineage viruses have circulated in the UK. Alteration in IC50 is associated with genetic drift in viral neuraminidase rather than specific sequence changes associated with drug resistance. The UK has very low prescription rates of neuraminidase inhibitors in direct contrast to Japan with the highest per capita use. The observed fluctuations in drug susceptibility are unlikely to be attributable to high levels of prescribing, but are likely to be related to natural evolution of influenza viruses, emphasising the importance of continued surveillance of drug susceptibility.

Abstract P931

SCHOLARSHIP Promising Junior Investigator Scholarship

Persistent Influenza Infection and Antiviral Drug Resistance in Immunocompromised Patients

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Persistent infection with seasonal influenza virus can occur in immunocompromised patients resulting in prolonged shedding of virus over several months and significant morbidity. Extended virus shedding can continue despite treatment with either M2 channel blockers or neuraminidase inhibitor (NI) drugs, which leads to conditions favouring the emergence of drug resistant viruses associated with treatment failure and potential transmission of resistant virus strains. Here, we describe four cases of persistent influenza virus infection in immunocompromised patients treated using single or dual therapy: three influenza A infections (two H3N2 and one A H1N1) and one influenza B infection. In all four cases drug resistant viruses emerged during or after initial treatment and continued to be shed for several months. Shedding of drug resistant viruses was maintained, even after cessation of drug treatment. Viruses identified include strains with dual resistance to amantadine and NI inhibitors, deletions at the active site of viral NA and novel mutations conferring resistance to NI drugs. Early detection of drug resistant mutants is essential in order to review the treatment strategies. In addition to traditional methodology including enzyme inhibition assays and plaque reduction assays to measure phenotypic resistance we have developed pyrosequencing techniques allowing rapid and early identification of neuraminidase inhibitor resistance and enhanced methods for amantadine resistance detection allowing quanititation of viral quasispecies directly in clinical material.

Abstract P932

A European Surveillance Network for Influenza Antiviral Resistance (VIRGIL)

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Since the widespread emergence of antiviral resistance would likely have a significant impact on clinical effectiveness of antiviral therapy and prophylaxis, it is important to track detection of resistance through regional and national surveillance programmes. The European surveillance network for vigilance against viral resistance (VIRGIL), funded by the EC through the Sixth Framework Research Programme (contract 503359) in collaboration with the European Influenza Surveillance Scheme (EISS), has been working since 2004 to develop capability and capacity for surveillance of influenza antiviral resistance within Europe. This includes the creation of reference laboratories for influenza antiviral susceptibility measurement, development of testing protocols for phenotypic and genotypic analysis, training for national reference laboratories and linkage of susceptibility testing to clinical and virological surveillance activities in Europe. A representative subset of influenza virus strains submitted for antigenic analysis in winter seasons 04/05 (N=187), 05/06 (N=371) and 06/07 (N=97 to date) from 22 countries in Europe have been evaluated for susceptibility to neuraminidase inhibitor drugs (oseltamivir and zanamivir) using a fluorescence IC50 assay. During this period of time there has been circulation of influenza B, H3N2 and H1N1 strains. Less than 1% of strains show an alteration of susceptibility greater than 3SDs and less than 7% greater than 1.6SD from the mean IC50 for each subtype for each season, indicating that over this period of time there has been no measurable increase in detection of virus isolates with reduced susceptibility to NAI drugs. Genetic characterisation of strains with reduced IC50 values is underway. The presence of amantadine resistance in H3N2 viruses has been particularly marked in recent influenza seasons. In 05/06 season, the majority of circulating A/Wisconsin/67/2005 like H3N2 viruses (Clade 1 HA) in Europe possess the S31N amantadine resistance mutation. However, viruses from 06/07 winter season genetically more related to A/California/7/2004 like (Clade 2 HA) viruses possess an amantadine sensitive M2 gene indicating that amantadine resistance is not fixed amongst human H3N2 viruses and is virus clade dependent. This suggests that there is co-segregation of amantadine resistance with mutations elsewhere in the genome and that maintenance of amantadine resistance in circulating human H3N2 is unrelated to drug pressure.

Abstract P933

Evolution of Human Influenza A Virus Neuraminidase Gene and Evaluation of Susceptibility to Antivirals

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Background: Neuraminidase (NA) is the second major surface glycoprotein of Influenza virus and is responsible for facilitating the release and preventing the self-aggregation of newly synthesised virus and for allowing the access to target epithelial cells. The scientific interest and the amount of information available about this protein have only increased in the last few years, especially since the development of neuraminidase inhibitors. The study of neuraminidase through the analysis of antigenic drift and the emergence of reassortant strains and, most importantly, through the evaluation of susceptibility to antivirals is of great importance as it contributes to a better knowledge of the epidemiology of Influenza virus. Objectives: Analysis of the genetic variability of Influenza A virus N1 and N2 neuraminidase and evaluation of the susceptibility to NA inhibitors. Methods: Neuraminidase gene sequences were obtained using the dideoxynucleotide cycle sequencing method after PCR amplification. Phylogenetic trees were constructed using the neighbour-joining method and bootstrap analysis. The presence of positive selection was analysed by the maximum-likelihood method (codeml programme). Susceptibility to antivirals was evaluated by a chemiluminescent assay. Results and Discussion: Sixty six strains of Influenza A viruses isolated in Portugal between the 2000/2001 and 2005/2006 Influenza Winter seasons were analysed in this study, 26 belonging to N1 neuraminidase subtype and 40 to N2 subtype. Phylogenetic homogeneity was observed between all N1 isolates, as expected, given the lower incidence of these viruses in the population. On the contrary, N2 isolates formed two distinct genetic groups characterized by the vaccine strains A/Fujian/411/2002 and A/Panama/2007/1999. This N2 genetic heterogeneity is in conformity with the annual rates of amino acid substitution and with the statistical confirmed existence of positive selection obtained for the N2 gene. The occurrence of non-homologous recombination between different viruses was observed in several N2 isolates. The results concerning the evaluation of NA susceptibility to antivirals of AH3 viruses in the current on-going study are expected to be a contribute to enhancing the European surveillance in this field.

Abstract P934

An Anti-Influenza Peptide That Inhibits Attachment Through Aggregation

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Influenza viruses continue to cause widespread morbidity and mortality on a yearly basis. Protection is often afforded by vaccination programs and treatment with either of two classes of antivirals: adamantanes or neuraminidase inhibitors. In recent years, the highly pathogenic avian H5N1 influenza viruses have displayed characteristics suggesting increasing resistance to both classes of antivirals, supporting the need for development of new alternatives. We previously reported that a 20 amino acid peptide, Entry Blocker (EB), inhibited the attachment of influenza viruses to host cells at low micromolar concentrations (3 to 30 μM). Furthermore, we showed that the EB peptide interacts with hemagglutinin (HA), possibly facilitating the inhibition of attachment. In vivo, the EB peptide prevented clinical signs of infection when mice were administered EB-treated H5N1 influenza virus, and morbidity was delayed in mice given EB post-infection. Based on these studies, we hypothesized that the binding of EB to HA leads to viral aggregation. In support of this hypothesis, we demonstrate that the EB peptide directly interacts with HA and inhibits the attachment of HA to red blood cells. The binding of the EB peptide is independent of the carbohydrate moieties present on HA. Further, we demonstrate that treatment of virion with the EB peptide led to delayed mobility though a continuous 30-60% sucrose gradient during ultracentrifugation. Aggregation of virion was confirmed by electron microscopy. Our studies suggest that the binding of EB to HA leads to viral aggregation and decreased attachment to host cells. Aggregation is a common theme in pathogen clearance, with molecules such as surfactants facilitating aggregation mediated phagocytosis of influenza virions. Ongoing studies will continue to examine the mechanism of EB antiviral activity, in addition to understanding the effect of EB induced aggregation on viral clearance in vivo.

Abstract P935

Synergistic Inhibition of H1N1 and H3N2 Influenza Viruses by Aurintricarboxylic Acid With Amantadine, But Not With Interferon

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Aurintricarboxylic acid (ATA), a potent inhibitor of cellular nuclease, kinases and phosphatases, is known to have inhibitory properties against the growth of several families of viruses, including human immunodeficiency virus, vaccinia virus and coronavirus (SARS-CoV). The mechanisms involved in the antiviral activities of ATA are not well understood but might be related to disruption of both cellular and viral factors. Here we investigated the potential anti-viral effects of ATA against two strains of influenza A viruses, namely A/Puerto Rico/8, A/New Caledonia/20, (both H1N1 subtype) and A/New York/55 (H3N2 subtype). The mean 50% cytotoxic concentration (CC50) of ATA in MDCK cells was approximately 0.2 mg/mL as measured by neutral red uptake assay. At 0.01 mg/mL, ATA was found to significantly protect Madin Darby Canine Kidney (MDCK) cells against the aforementioned influenza virus subtypes as determined by electron microscope, neutral red cytotoxicity assay, and antigen quantitation using enzyme linked immunosorbent assay (ELISA). In addition, amplification of viral NP mRNA using RT-PCR revealed a substantial reduction of the viral transcripts, suggesting a block at the viral transcriptional level. Importantly, treatment of H1N1-infected cultures with non-toxic doses of ATA resulted in defective viral particles characterized with irregular matrix protein shells and RNP structures, strongly suggesting a direct effect of ATA on the viral structural proteins. We further determined whether ATA could synergistically inhibit influenza virus replication with other known anti-viral agents including amantadine and interferon gamma. ATA was found to synergistically inhibit virus infectivity with amantadine, but not with interferons. These novel findings could shed light on the mechanisms underlying the morphology and structure of the viral particles upon exposure to the aforementioned anti-viral agents. Furthermore, the defective viral particles generated in the presence of ATA might also be worthy of being investigated for its potential as candidate vaccines.

Abstract P936

Failure of Zanamivir Therapy for Pneumonia in a Bone Marrow Transplant Recipient Infected by a Zanamivir-Sensitive Influenza A (H1N1) Virus

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Influenza A viruses are responsible for significant morbidity and mortality after bone marrow transplantation. Early administration of an effective anti-influenza drug such as the neuraminidase inhibitors zanamivir (Relenza®) or oseltamivir (Tamiflu®) is essential for the outcome of the disease. Emergence of drug-resistant viruses is one possible limitation of treatment efficacy. Here we report failure of treatment with amantadine and inhaled zanamivir in a bone-marrow transplant recipient with pneumonia caused by an influenza A (H1N1) virus. On day 74 post transplant, the patient showed signs of pneumonia and an influenza A (H1N1) virus was isolated from a bronchoalveolar lavage sample taken 2 days after the onset of symptoms. The patient was first treated with amantadine alone (200 mg daily) for 3 days, and was further treated with both amantadine (up to day 111) and inhaled zanamivir (days 80 to 102). Nasopharyngeal and throat specimens remained positive for 3 weeks then became negative. Clinical recovery was complete but pulmonary abnormalities persisted. On day 144 post-transplant a clinical relapse occurred and an influenza A (H1N1) virus was isolated again. The viruses isolated from the bronchoalveolar lavages before and after treatment were sensitive and resistant to amantadine, respectively. However, both viruses were clearly found to be sensitive to zanamivir using cell-based and enzymatic assays. Treatment after the relapse with zanamivir did not result in any significant improvement, whereas subsequent oral treatment with oseltamivir allowed complete recovery. Poor bioavailability of zanamivir in the peripheral lungs might have been limiting treatment efficacy in such an immuno-compromised patient.

Workshop Topic #10

Innate and Adaptive Immunity

Poster Abstracts

Abstract P1001

Antiviral Activity of the Long Chain Pentraxin PTX3 Against Influenza Viruses

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Innate inhibitors of influenza virus represent important antiviral molecules that act to limit spread of virus in the respiratory tract prior to the induction of adaptive immune responses. In this study we describe the antiviral activity of the long chain pentraxin PTX3 against influenza viruses. Human PTX3 bound to influenza virus and inhibited haemagglutination, neutralized virus infectivity and inhibited the enzymatic activity of the viral neuraminidase in a Ca2+independent manner. Influenza viruses of the H3N2 subtype (1968-1973) were sensitive to inhibition by PTX3 while a range of H1N1, H5N1 and H3N2 (1975-2005) viruses were found to be resistant. To gain insight into the mechanisms underlying PTX3 sensitivity we generated a PTX3-resistant mutant of influenza virus strain X-31 (X-31-PTX3R) and examined the pathogenesis of wild type X-31 in PTX3-/- mice. Using these approaches, we report that the antiviral activity of PTX3 is associated with binding to the haemagglutinin of susceptible influenza virus strains. PTX3-/- mice are more susceptible than wild type mice to infection by strain X-31 and treatment of PTX3-/- mice with purified PTX3 promoted survival and reduced viral load in the lungs. Together, these studies describe a novel antiviral role for PTX3 in early host defence against influenza infections both in vitro and in vivo.

Abstract P1002

Immune Response to Experimental Influenza Infection and Vaccination in Mouse Nasal-Associated Lymphoid Tissue (NALT)

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The local immune response of mucous membranes of an organism are the first and most significant barriers preventing many virus infections, including influenza. The barrier against influenza infection is the mucosal-associated lymphoid tissue of the upper airways. It is considered that rodent nasopharyngeal-associated lymphoid tissue (NALT) is equivalent to the lymphoid tissue of human Valdryer's ring. This investigation is the first attempt to analyze and compare the development of cellular and antibody immune responses in NALT in a mouse model of influenza infection using a pathogenic for mice influenza virus A/PR/8/34 (H1N1) and an attenuated reassortant virus A (H1N1). The same parameters of the system immune response in the spleen were in parallel tested. Materials and methods: CBA mice were inoculated with A/PR/8/34 (H1N1) and attenuated reassortant 2/6 (R 2/6) containing HA and NA from A/PR/8/34 and all the internal gens from cold-adapted master strain A/Leningrad/134/47/57 (H2N2). Local and serum Abs, lymphoproliferation and apoptosis of NALT and spleen lymphocytes, induction of CD4+, CD8+, CD19+, IFN-γ, IL-2, IL-4, IL-6 were evaluated. NALT separation was performed using procedure described by Asanuma H., Thompson A.H. et al., 1997. Results: Local immune response. It was shown that the vaccine strain inherited the ability to induce high-grade local antibody responses from the virulent virus. However the vaccine strain had less capacity to stimulate production of circulating antibodies. Both strains had an equal ability to induce a lymphoproliferative immune response in NALT lymphocytes. The attenuated reassortant virus was able to stimulate proliferation of Th ($CD4^+$), B-cells ($CD19^+$) and CTL ($CD8^+$) in NALT. According to the cytokine assay (IFN-γ, IL-6, IL-4) the attenuated reassortant virus activated both Th1- and Th2-lymphocytes in NALT. System immune response. It was shown that R 2/6 had less ability than the pathogenic parental virus to activate circulating IgG-antibodies, secretion of Th1-cytokine IFN-γ by splenocytes and production of CTL ($CD8^+$) in the spleen. On the other hand, R 2/6 was highly competitive with pathogenic parental virus as regards quantitative characteristics of lymphoproliferative activity of splenocytes, the stimulation of Th ($CD4^+$), B-cells ($CD19^+$) and Th2-cytokine IL-2. Both viruses poorly induced IL-6 production in spleen. Apopthosis and lymphoproliferation. Significant differences in physiological AP intensity were observed in splenocytes and NALT lymphocytes (20 - 30 % and 6 - 9 % respectively). Non lethal influenza infection and single vaccination with R 2/6 were accompanied by a short-term (24 h) increase in the levels of lymphocyte AP in both NALT and spleen. A second introduction of pathogenic virus or R 2/6 did not intensify lymphocyte apoptosis in these organs. Secondary cellular lymphoproliferative immune responses in the spleen were more expressed than in NALT. Conclusion: This data suggest that intranasal immunization with live attenuated reassortant viruses results in a balanced stimulation of both Th1-and Th2-immune responses in the primary site of infection (NALT) as well as influenza infection. Attenuation of influenza A (H1N1) virus by 2/6 genetic reassortment influences the induction of systemic immunity constituents differentially. Some parameters can be reduced, others not. When preparing vaccine strains for LIV, attention should be given first of all to

increased induction of circulating antibodies - one of the major parts of antiviral immunity. Vaccination with R 2/6 and do not influence apoptosis of lymphocytes of NALT and spleen.

Abstract P1003

Influenza Infection and Vaccination With Live Attenuated Influenza Vaccine in the Experimental Model of Allergic Bronchial Asthma

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Influenza virus continues to be an important contributor to exacerbations of asthma and elevates morbidity and mortality in all age categories of asthmatic subjects during epidemics. It has been shown that inactivated influenza vaccine is safe in this high-risk population, but the safety of live attenuated reassortant influenza vaccine (LAIV) is still being discussed. Objective: To investigate the allergoimmunological consequences of influenza infection and immunization with LAIV in murine model of asthma. Methods: We used a model of ovalbumin (OVA)-induced bronchial asthma followed by immunization with pathogenic and attenuated reassortant viruses during two pathogenetic phases of asthma: acute allergic inflammation and remission (48 hours or 10 days following last challenge with OVA respectively). The levels of OVA- and virus-specific IgE as well as cellular immune responses to viruses and OVA were measured and inflammatory histopathology of lungs was observed. Results: Influenza infection significantly increased the levels of OVA-specific IgE and airway eosinophilic infiltration when pathogenic virus was administered in acute allergic respiratory inflammation. We found that attenuated virus also was capable to elevate the production of IgE and airway inflammatory changes when administrating in acute asthma but in smaller extent than pathogenic one. Immunization with viruses during remission of asthma didn't increase the levels of OVA-specific IgE. However, significant airway inflammatory damage remained in infected but not in vaccinated mice. We supposed that additional exposure to allergen (OVA) after influenza infection or vaccination may contribute to exacerbations of asthma. The additional challenge with OVA on day 14 after virus immunization resulted in outcome of asthma exacerbation only in mice infected with pathogenic virus both in acute phase and remission of allergic respiratory inflammation. We observed only increase of IgE-levels without inflammatory histopathology in lungs of vaccinated mice even those were immunized in acute asthma. Conclusion: The pathogenic virus provoked asthma exacerbation both in acute phase and remission of allergic airway inflammation. Vaccination of mice with attenuated reassortant virus during the remission of asthma is safe. Thus our results indicate that it is possible to find the optimal approach to administration of LAIV to subjects with respiratory allergy.

Abstract P1004

Characterization of Endogenously Loaded HLA Class I Peptides During Influenza Infection

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The influenza virus attacks epithelial cells of the lower and upper respiratory tract. During infection, the virus induces cell stress and anti-viral responses and directly interferes with normal cellular processes. Changes in the proteome of the infected cell are mimicked at the cell surface by the HLA (Human Leukocyte Antigen) class I molecule, positioned on the surface of virtually all nucleated cells. The class I molecule displays endogenously loaded peptides, approximately 8-12 amino acids in length, allowing cytotoxic T lymphocytes to distinguish influenza infected cells from surrounding healthy tissue. Our hypothesis is that during influenza infection, the HLA class I molecule presents viral-derived peptide epitopes as well as an altered repertoire of host-derived peptides. In order to test this hypothesis we first gathered peptide:HLA class I from infected and uninfected cells by constructing an expression vector for soluble forms of HLA molecules A*0201 and B*0702. These soluble HLA (sHLA) class I constructs were expressed in influenza susceptible cells lines and these cells were infected with influenza PR8. Peptides were eluted from the HLA molecules by an acid boil, separated by RP-HPLC, and mapped by mass spectrometry. Class I HLA presented peptide epitopes unique to infected cells were and are being further characterized by T-cell immunogenicity assays, Western blots, real-time PCR, and ELISpots. To date, we have identified two viral-derived HLA B*0702 ligands from the Influenza A/PR/8 hemagglutinin and nucleoprotein molecules as well as several self-derived HLA B*0702 and A*0201 ligands that are either unique to influenza infected cells or exhibit increased presentation during infection. These data indicate that HLA-B molecules present viral-derived peptides while both HLA-A and –B molecules present changes in the proteome of the infected cell. Future experiments include the characterization of additional strains of influenza and the formation of TCR mimics to determine the copy number and timing of peptide presentation on the surface of influenza infected cells.

Abstract P1005

Cross-Reactive Humoral and Cell-Mediated Immunity With an Adjuvanted H5N1 Candidate Vaccine

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Background: The use of a pandemic vaccine just before or just after pandemic onset is considered to be one of the most effective measures capable of reducing substantially the overall infection rate and consequently morbidity and mortality within the population. However, such a vaccine also needs to be able to confer cross-protection against potential drift pandemic strains. Genetic studies have enabled maps to be drawn which detail the natural evolutionary relationship of the H5N1 strains, linking groups known as clades to a common ancestor. Reciprocal cross-reactions in hemaglutininination inhibition (HI) tests demonstrated antigenic similarity of HAs within the same genetic clade and distinguished antigenic characteristics from HA representatives of different clades. To document the breadth of the immune response induced by vaccination with this adjuvanted H5N1 split vaccine and to make sure cross-reactivity would be evaluated in sufficiently stringent conditions, the present study was performed using virus strains of different clades, the vaccine strain (clade 1: H5N1-A/Vietnam/1194/04) and a heterologous clade 2 strain (H5N1-A/Indonesia/5/05). Methods: The present results have been obtained from a phase I study (106750/NCT00309634) conducted in 400 adults (18 to 60 years) having as primary objective the evaluation of the reactogenicity and the immunogenicity of the GlaxoSmithKline Biological's pandemic influenza split virus candidate vaccine administered at 4 antigen concentrations (3.8µg, 7.5µg, 15µg or 30µg HA of H5N1 A/Vietnam/1194/04 split virion) adjuvanted or not with a fixed volume of a novel oil-in-water based proprietary adjuvant system. Two doses were administered intramuscularly 21 days apart. At days 0, 21 and 42, blood samples were collected. Since dose-sparing strategies are crucial for the development of a pandemic influenza vaccine, the cross-protective potential of the candidate vaccine was investigated for the two vaccine formulations containing the lowest amount of adjuvanted antigens (3.8µg and 7.5µg). Cross-reactive HI antibody titers and neutralizing (NT) antibody titers against A/Indonesia 5/05 strains were measured using horse red blood cells. Geometric mean titers (GMTs), seroconversion rates (SCR) and seroprotection rates (SPR) for HI and GMTs and SCR for NT were calculated. A HI titer>1:40 was considered to be seroprotective. The conventional 4-fold increase in neutralizing titers was used to assess seroconversion. The vaccine's ability to prime for T- and B-cell mediated immunity against strains heterologous to the vaccine strain is important to allow for better responsiveness upon revaccination or infection with drift pandemic strain. We thus evaluated T- and B-cell responses against both the vaccine strain (clade 1: H5N1-A/Vietnam/1194/04) and a heterologous clade 2 strain (H5N1-A/Indonesia/5/05). T-cells were restimulated in vitro using either the H5N1 split antigen (vaccine strain) or with a pool of peptides covering the conserved and non-conserved amino acid sequences shared by H5 hemagglutinin from both studied strains. Influenza-specific T-cells were enumerated by flow cytometry following conventional immunofluorescence labeling of intracellular cytokines. Results obtained at day 42 were expressed as percentage of T-cells with 95% confidence interval (95%CI). Enumeration of H5N1 specific B-cells was carried out by the B-cell Elispot method. Results: Cross-reactive neutralization results showed very high SCR at day 42, of 77.1% [62.7-88.0] and 67.4% [52.0-80.5] against the Indonesia strain in the 3.8µg and 7.5µg HA adjuvanted vaccine groups, respectively. In the corresponding non-adjuvanted vaccine groups the SCR were < 3%. Furthermore, despite the much lower sensitivity of the HI assay, HI seroprotective titers against the A/Indonesia 5/05 strain were detectable at day 42, in 20.0% [95% CI: 10.0-33.7] and 32.0% [19.5-46.7] of subjects in the 3.8µg and 7.5µg HA adjuvanted vaccine groups but in none of the subjects in the corresponding non-adjuvanted groups. Before vaccination, frequencies of influenza-specific CD4 T-cells were comparable in all groups. After two vaccine doses, these frequencies were significantly higher after vaccination with adjuvanted formulations than with non-adjuvanted ones. A marked increase of the frequency of H5N1-specific CD4 T-cells was observed after dose 1 and were boosted after dose 2. Additionally, the frequencies of CD4 T-cells specific for the conserved sequence between the H5-A/Vietnam/1194/2004 and the H5-A/Indonesia/05/2005 strains were higher in the groups that received the adjuvanted formulation of the vaccine than in the groups receiving unadjuvanted vaccines. Both IL-2 and CD40L were shown to be produced by H5-specific CD4 T-cells which is supportive of memory T-cell phenotype. Consistently, putative memory B-cells were also found at significantly ($p<0.001$) higher frequencies in the groups of subjects that received the adjuvanted vaccine. Conclusion: These results demonstrate that GSK's candidate pre-pandemic adjuvanted H5N1 (clade 1) influenza vaccine induces cross-reactive humoral immunity and suggest B- and T-cell memory responses against a substantially drifted (clade 2) strain of H5N1. Taken together, these data provide evidence that this vaccine would immunologocally prime for better responsiveness upon revaccination with an actual pandemic H5N1 vaccine or infection with pandemic H5N1 virus. The use of such a prepandemic vaccine prior to the onset of a pandemic might be a key asset in minimizing the impact of a pandemic.

Abstract P1006

Antibodies Induced by the Light Chain of Influenza A Haemagglutinin Improve the Course of In Vivo Influenza Infection

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We followed the effect of polyclonal antibodies induced by the light chain of influenza A virus haemagglutinin (HA2 gp) on the course of in vivo infection. BALB/c mice were immunized with recombinant vaccinia viruses expressing chimeric molecule of haemagglutinin, composed from HA1 and HA2 gp of influenza A virus of different subtypes, originated from A/PR8/34(H1N1) and A/NT/60/68(H3N2) viruses. IgG were purified from sera of immunized mice by affinity chromatography on protein A-Sepharose. The titers of antiviral and HA1- resp. HA2- specific antibodies were determined by Western-blott and by ELISA. The IgG fraction from sera of immunized mice at defined concentration of specific antibodies was passively transferred to 6-week-old BALB/c mice which were subsequently infected with influenza A virus. We showed that the antibodies induced by two-step immunization with recombinant vaccinia virus (10^6 PFU/mouse) expressing the HA2 gp of homologous subtype (and HA1 gp of heterologous subtype) to the challenge influenza virus A/Mississippi/1/85(H3N2), lowered the mortality of infection with dose $2LD_{50}$ by 28% in comparison to the control group of mice. Control mice were immunized with wild type vaccinia virus which did not expressed any influenza virus protein. The protection effect mediated by specific IgG was confirmed also by the lower levels of infectious virus in lungs and by earlier elimination of virus and vRNA from lungs of immunized mice than in the control. Though this way of immunization stimulates the complex immune response, the passive transfer of IgG fraction containing the specific antibodies of defined titer showed that antibodies induced by expressed HA2 gp moderate the influenza A infection and cause earlier recovery from the lethal infection. These studies confirmed our previous observation that intravenously applicated HA2-specific monoclonal antibodies contribute to the protection of mice against lethal influenza A infection (Gocnik et al., J Gen Virol, 88(2007) 951). Supported by The Scientific Grant Agency of Ministry of Education of Slovak Republic and Slovak Academy of Sciences, grants Nos. 2/6077/6, 2/7065/7 and by The Slovak Research and Development Agency under the contract No. APVV/51 – 021605.

Abstract P1007

Influenza Virus Infection Enhances Expression of Epithelial Defensins and Surfactant Proteins in Tracheal, Nasal and Paranasal Sinus Mucosa

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In naive hosts, protection against IAV infection is dependent on innate immune mechanisms. However, little is known about the role of innate responses in the upper airway. Recently, defensins have been shown to be active against IAV in vitro. However, their in vivo role in IAV infection is unknown. Here we investigated the effect of IAV infection on the in vivo expression of several components of the innate immune system including a surfactant protein (SP-D) and antimicrobial peptides (β-defensins MBD-1, MBD-2, MBD-3, MBD-4). We infected mice with either influenza A/PR/8/34 (H1N1) or A2/Hong Kong/8/68 (H3N2) using small droplets (10-microliters) that are thought to be more similar to natural infection and collected tissues at various times after infection. Tracheal and micro-dissected nasal and paranasal sinus mucosa were analyzed for viral titers and total RNA were analyzed for gene expression using quantitative RT-PCR assays. Mock infected animals were used as normal control. Our results showed minimal expression of MBD-1 and MBD-2 in nasal and tracheal mucosa. Importantly, the level of MBD-3 and MBD-4, which are the murine homologs of human beta-defensin 2 and 3, were enhanced by 5-6 folds in the tracheal and nasal mucosa by day 3 post-infection. Although these defensins were induced at a lower level on day one post-infection, it suggested that they were expressed during early event in host response to IAV infection. Immunohistochemical staining showed that both MBDs were localized in the epithelial cells of airway mucosa. SP-D expression was enhanced by ~2 folds by day 3 post-infection. We conclude that IAV infection upregulated the expression of β-defensins in the upper airway. These novel findings suggested that β-defensins might contribute to innate and adaptive immune responses targeted against IAV infection. Our demonstration of the upregulation of SP-D in the tracheal, nasal and paranasal sinus mucosa provides evidence that SP-D plays a role in IAV infection of the upper airway in complement to its previously reported role in lung infection.

Abstract P1008

Analysis of Virus-Specific CD8 T Cells in a Successful Immune Response to Primary Avian Influenza Virus Infection in Mice

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The high fatality rate associated with H5N1 avian influenza virus (AIV) infections in humans shows that these viruses are of unusually high virulence. Although significant strides have been made in the identification of viral molecular determinants of pathogenicity, a major gap in our understanding of the pathogenesis of H5N1 infections is the lack of information on the development of adaptive immune responses during the disease process. A unique feature of human H5N1 isolates is their ability, without prior adaptation, to replicate to very high levels in lungs of other mammalian species, including mice. Since the disease outcomes in mice and humans are quite similar, studies of H5N1 virus pathogenesis in mice are likely to provide important insights into the genesis of disease in humans. Development of effective vaccines requires a thorough understanding of the nature of the immune response that is required to successfully control a pulmonary AIV infection. Following adoptive transfer of naive influenza virus-specific TCR transgenic (tg) CD8 T cells (CL-4) into BALB/C mice, we investigated the kinetics of CD8 T cell activation in the draining lymph nodes (DLN) and bronco-alveolar lavage (BAL) during a sub-lethal intranasal infection with AIV, A/Hong Kong/486/97 (H5N1; HK486). The activation of CL-4 CD8 T cells in the DLN as evidenced by upregulation of cell surface LFA-1 did not occur until day 4 post-infection (PI). However, by day 6 PI all CL-4 CD8 T cells upregulated LFA-1, differentiated into effectors, and migrated to the infected lung. At day 7 PI, CL-4 CD8 T cells expressed high levels of granzyme B, and constituted ~87% of CD8 T cells in the BAL. Adoptive transfer of Carboxyfluorescein diacetate succinimidyl ester-labeled CL-4 CD8 T cells showed that proliferative expansion and differentiation of virus-specific CD8 T cells into effectors occur in the DLNs between days 4 to 6 PI. Importantly, T cell deficiency increased susceptibility of mice to lethal infection with a sub-lethal dose of HK486. In summary, we show that successful recovery of mice from a pulmonary H5N1 AIV infections not only correlated with strong virus-specific CD8 T cell responses, but required full complement of T cells.

Abstract P1009

Analysis of the Cellular and Humoral Immune Response in Mice Following Intranasal Administration of Live Attenuated Influenza A Virus Reassortants

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Cold adapted live-reassortant influenza A vaccines can stimulate T cell immunity to shared determinants and an antibody-mediated response to HA and NA viral surface glycoproteins in situations of antigenic drift. However, it is not known whether the HA and NA surface antigens influence cell mediated responses (CMIs) induced by the NP, M1, P and NS internal proteins of vaccine reassortants. The aim of this study was to investigate the immunogenicity of live attenuated influenza A reassortants viruses possessing the same internal proteins but different surface glycoproteins. The immune response elicited by the cold-adapted influenza donor strain A/Ann Arbor/6/60 (H2N2) and six of its reassortants; CR6 (H3N2), CR18 (H3N2), CR29 (H3N2), CR35 (H1N1), CR6-35 (H1N2), CR6-35* (H3N1) were studied in the BALB/c (H-2^d) and C57BL/6 (H-2^b) mouse animal models. Antibody responses to the HA and NA surface antigens after vaccination were investigated by the measurement of virus-specific antibody secreting cell (ASC) responses using the ELISPOT assay. Serum antibody levels were measured by HI assay. The ability of the individual reassortants to induce cytotoxic T cell (CTL) responses that cross-react with heterologous strains was determined by MHC-tetramer (H-$2D^b$ / h b_2 m /NP_{366} and PA_{244} -PE) staining of lymphocytes from the lungs and spleens of infected mice. Pathological changes in the lungs were investigated in parallel. All six reassortants elicited a significantly greater systemic humoral response in BALB/c mice than in C57BL/6 mice. However, a much weaker CMI response was induced in BALB/c mice infected with each reassortant. In BALB/c mice, the reassortant CR35 elicited a significantly higher serum antibody response and a lower level lung ASC response than the other viruses. By contrast, in C57BL/6 mice, CR35 induced a lower humoral response and a higher lung CTL response, in which 2.9% of lung $CD8^+$ T cells were virus-specific. This study has also demonstrated that the CTL responses induced by live cold adapted influenza A vaccine virus cross-react with different subtypes. These results suggest that viral growth characteristics and the serum antibody level play a role in determining CMI responses and that the role of T and B effector cells in animal models used could be a useful predictor of the efficacy of live attenuated influenza vaccines.

Abstract P1010

Pre-existing Immunity Against Influenza Virus Suppresses Immune Responses to HA/SIV Chimeric VLPs

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Mucosal immunity plays an important role in preventing infection by viruses such as HIV and influenza, which can initiate infection through mucosal surfaces. Previous studies have shown that chimeric HA/SIV virus-like particles (VLPs) containing membrane-bound influenza HA proteins exhibit enhanced immunogenicity in intranasal immunizations. In this study, we investigated the impact of pre-existing mucosal immunity against influenza virus on induction of immune responses by HA/SIV chimeric VLPs. Mice were immunized by intranasal instillation with inactivated influenza viruses PR8 (H1N1) or Aichi (H3N2). At 4 weeks after the pre-immunization, mice were immunized intranasally with SIV VLPs or HA/SIV chimeric VLPs containing the influenza virus PR8 HA proteins. Blood and mucosal samples were collected at 2 weeks after the pre-immunization and the final immunization and analyzed for antibody responses against the influenza viruses (PR8 and Aichi) as well as the SIV Env protein. Our results show that the pre-existing immune responses against influenza virus greatly inhibited the induction of antibody responses against the SIV Env protein, and this inhibitory effect was observed for both PR8 and Aichi influenza virus immunized mice. On the other hand, immune responses to influenza virus PR8 were effectively boosted by HA/SIV chimeric VLP immunizations in mice pre-immunized with influenza virus PR8. Induction of immune response to influenza virus PR8 by HA/SIV chimeric VLPs was also inhibited in mice pre-immunized with the H3N2 influenza virus Aichi. These results indicate that pre-existing mucosal immune responses against one influenza virus subtype may suppress induction of immune responses to non-influenza related antigens as well as the HA proteins from a different influenza virus subtype in HA/SIV chimeric VLPs.

Abstract P1011

Identification of T-Cell Epitopes of Influenza Virus By Stable Isotope Tagging of Epitopes (Site); An Essential Platform Technology for Vaccine Development

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Avian influenza H5N1 changes rapidly, requiring new strategies for vaccination that are based on cross protective immune responses. The cross-specific responses are predominantly generated by T cells, necessitating the further identification of T cell epitopes for H5N1 avian influenza, since only few are known. To date, T cell epitopes have been defined based on detection of responses by peripheral blood mononuclear cells of individuals after natural infection. However, only few individuals were infected with avian influenza. In addition, frequencies and responses of T cells may significantly vary between donors. It is therefore not conceivable to identify the T cell epitopes of avian influenza (H5, H7, H9) for the major HLA class I supertypes by detection of leukocyte responses of previously infected individuals. We developed a new platform technology Stable Isotope Tagging of Epitopes (SITE), which is based on detection of CD8 T cell epitopes on virus-infected cells, in vitro. Virus-infected cells were cultured with stable-isotope-labeled amino acids and mixed with non-infected cells, cultured in normal medium. Viral epitopes were acid-eluted from immunoprecipitated MHC molecules and were identified based on characteristic ratios of labeled and non-labeled peptides by Liquid Chromatography/Mass Spectrometric analysis. Importantly, SITE allows detection of epitopes on different cell types, which is essential since it was found that differential expression of T cell epitopes of influenza on dendritic cells and epithelial cells may have severe implications for protective immunity. Applying SITE, we identified several novel epitopes of respiratory viruses for HLA-supertypes HLA-A2 and HLA-B7 in virus-infected epithelial cells and dendritic cells. Subsequently, we determined the immunodominance of the epitope-specific CD8 T-cells based on functional responses and T-cell frequencies. Recently, we applied the technology for identification of influenza epitopes, results of which will be presented. The findings are a major contribution to the identification of human correlates of protection for pandemic influenza and demonstrate the importance of this platform technology for avian influenza vaccine development.The SITE technology was recently published and will be included in 'Current Protocols in Immunology' as a standard.

Abstract P1012

Sublingual Vaccination Against Influenza Virus

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The sublingual route has been used for many years to deliver drugs and small molecules to the bloodstream. Surprisingly, the potential of this route for delivering vaccines has received very little if any attention. We have now documented the effectiveness of sublingual immunization for inducing a broad range of immune responses against a variety of antigens, including soluble proteins, inert particulate antigens (killed viruses) as well as live-attenuated viruses (influenza virus, adenovirus). In all cases, systemic and mucosal immune responses, including humoral and cytotoxic T cell responses were induced in both mucosal (lungs) and extra-mucosal tissues. Sublingual vaccination of mice with killed or live influenza virus conferred full protection against a lethal respiratory challenge with virus. Overall, sublingual immunization was comparable to nasal immunization regarding the magnitude, breadth, and anatomic dissemination of the induced immune responses. Importantly, and contrary to nasal administration, sublingual administration did not redirect antigens and/or adjuvants to the brain. These promising results have prompted us to initiate a phase 1 clinical study to evaluate the safety and immunogenicity of sublingually delivered prototype protein vaccine in healthy human volunteers. The results of this clinical study support the notion that sublingual immunization offers a viable alternative for non-invasive delivery of vaccines.

Abstract P1013

Evaluation of the Cell-Mediated Immunity of Two Influenza Subunit Vaccines, Combined and Not With MF-59 Adjuvant Emulsion, Administered to HIV-1 Seropositive and Seronegative Adults

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T helper cells control whole immune responses, by secreting a multitude of cytokines and by expressing surface molecules for activation of B cells, antigen-presenting cells, cytotoxic T lymphocytes and several other cells of the immune system. A central component of vaccines are immune stimulating agents called adjuvants that enhance both magnitude and duration of immune responses. An open, randomized, comparative clinical trial was performed during the 2005-2006 season. A total of 100 subjects, randomized 1:1 in two groups, by their HIV-1 seropositive status, received intramuscularly one dose of the WHO recommended formulation of two influenza vaccines, adjuvanted (Fluad) and not (Agrippal). To evaluate the protective immune responses generated after vaccination, we used the multicolour flow cytometry to study the T cell memory by functional approach. The quantitative assessment of the proliferative potential by the flow dye dilution assay to estimate the precursor frequency of cells proliferating in response to specific antigens utilizes vital fluorescent dyes, such as carboxyfuorescein diacetate succinimidyl ester (CFSE), that are equally divided between daughter cells during the successive generation of expanding cells. With mathematical deconvolution of the fluorescence histograms, informations can be derived about the precursor frequency of cells in the original population that responded to the specific stimulus. Moreover this test compared to traditional methods for assaying antigen-specific proliferation offers the additional ability to phenotype the responding cells and determines their rate of proliferation. In HIV-1 seronegative subjects, presenting an intact immune system, the analysis of the precursor frequency (PF) regarding the proliferating T CD3+CD4+ cells did not show substantial differences between the two vaccination groups. On the contrary, the presence of the adjuvant demonstrated a positive effect in HIV-1 infected patients: in fact, in these subjects, the adjuvanted vaccine globally demonstrated a faster lymphocytes proliferation

in HIV-1 infected patients. Furthermore, in HIV-1 infected subjects, no significant changes in CD4+ cell-count were detected, demonstrating the good safety of both vaccines under study. Depending on the innate immunity ?sense? the vaccine, the data collected by this approach can be relevant about the dynamics of adaptive immune responses.

Abstract P1014

Comparison of Fresh and Frozen Influenza-specific Plasmablasts Post Vaccination

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Introduction: Increase in neutralizing antibody titers is the conventional humoral immune markers of influenza vaccine responses. Antibody secreting cells are responsible for the rise in antibody levels. Plasmablasts or antibody secreting cells (ASC) are increased in the blood of human subjects after trivalent inactivated influenza (TIV) vaccination. We hypothesize that plasmablast responses may be better immune markers of vaccine responses. Methods: We compared influenza Hemagglutinin A H1, H3, and H7-specific ASC IgG Elispots frequencies from fresh and frozen human peripheral blood mononuclear cells (PBMC) after 2006 TIV vaccination in 10 young healthy subjects. Blood was drawn on day 0, 7, and 28. Elispots and 11- color flow cytometry on the LSRII was performed on each blood sample (CD19, CD20, CD10, CD27, IgD, IgG, CD38). Results: H1-, H3-, and H7-specific IgG ASC spots/million PBMC on day 7 were 174±236, 77±62, and 6 ± 11 respectively. Percentage H1-, H3-, and H7-specific of total IgG ASC spots were 9.2±10.3, 4.4±3.2, and 0.3 ±0.7 respectively. Total IgG ASC spots/million PBMC pre- and day 7 post-vaccination were 290±188 (0.029% PBMC) and 1691±836 (0.17% PBMC) respectively. No H1- and H3- specific IgG ASC were detected in any of the subjects on day 0 and 28. H7 antigens were used as negative controls. H1 -H3-, H7- and total specific ASC IgG Elispot frequencies in the fresh vs frozen PBMC on day 7 were similar. Frequencies of total plasmablasts by flow cytometry (0.21±0.19% of PBMC) were similar to total IgG ASC spot frequencies suggesting most plasmablasts secreted IgG. Plasmablasts from frozen samples were not detected by flow analysis but were detected by Elispot. Conclusions: H1- and H3-specific ASC IgG spots/million PBMC can be detected (d7) after TIV. In contrast, no H7-specific ASC IgG spots were detected. Fresh vs frozen H1-, H3-specific and total IgG ASC spots/million were similar. Fresh flow analysis of plasmablasts compared to total IgG ASC Elispots demonstrated similar frequencies in the blood (d7). These results provide proof of principle that these assays measure early immune responses to vaccination. Whether they will function as early biomarkers of long-term protective responses remains to be established. Supported by: Rochester Center of Biodefense of Immunocompromised Populations.

Workshop Topic #11

Mathematical Modeling

Poster Abstracts

Abstract P1101

Estimating Sensitivity of Laboratory Confirmations for Influenza in Canada Through Modelling

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Background: The Respiratory Virus Detection Surveillance System (RVDSS) collects, collates and reports on weekly respiratory virus identifications from a sentinel network of Canadian laboratories. As the sensitivity of influenza testing is known to vary due to various factors, we aimed to estimate the test sensitivity for influenza at the population level, using a modelling approach previously employed to estimate influenza-attributed hospitalizations based on all respiratory admissions in Canada. Methods: The total number of specimens tested each week from 1998 to 2006, along with positive viral identifications, was obtained from the RVDSS. The number of influenza negative specimens was modelled as a function of viral identifications for influenza, Respiratory Syncytial Virus, adenovirus and para-influenza, seasonality, and trend using Poisson regression. The model estimate of the number of false negative specimens was calculated as the difference between the model predicted and baseline. Sensitivity was calculated as the number confirmed positive for influenza divided by the sum of influenza confirmations and model-estimated false negatives. Results: The sensitivity of influenza testing was estimated to be 33% (95%CI 32-34%). The proportion of samples confirmed in laboratory testing to be positive for influenza generally peaks each year at 20-30%. After correcting for the model-estimated test sensitivity, the proportion of specimens attributable to a recent influenza infection ranged from 50-80%. Conclusions: Factors leading to false-negative influenza testing include inadequate specimen procurement or handling, testing too late in illness and test characteristics. Laboratory confirmations have routinely been expressed as proportion-positive, which normalizes for a variable number of total specimens tested, and has been used as an indicator of the level of influenza activity. However, these results imply that proportion positive is a potentially misleading representation of annual influenza epidemic; tending to overstate activity during periods of low activity of other viruses, and understating the relative level of activity during peak periods.

Abstract P1102

Searching of Molecular Targets for Influenza A Virus Subtyping Using Profile Hidden Markov Models

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Influenza A virus classification is based on its surface antigenicity of HA and NA proteins. Standard HI or NI test, however, requires a comprehensive collection of antiserums, which are often of limited supply during outbreaks. With the advancement of RT-PCR and sequencing techniques, viral RNA can now be determined in a rather cost-effective and high-throughout manner. Previously we reported using profile hidden Markov models in molecularly detecting enterovirus type 71 (EV71) strains. In this work we have expanded this approach in influenza HA subtyping, and found it useful in revealing HA fragments that are abundant with genomic characteristics to differentiate one subtype from the others. A total of 6,239 HA coding sequences were retrieved from GenBank and analyzed. Non-redundant amino acid sequences no less than 600-aa were collected and aligned for each HA subtype. Instead of constructing a single model upon the entire HA (as we had done previously for EV71 VP1 gene), we used a sliding window of 100-aa with 10-aa increment in building many profiles with the package HMMER (Washington University at St. Louis), followed by evaluating what 100-aa segment(s) yield best overall differentiation power between a subtype and other subtypes. Taking H3 for example, we utilized 729 sequences to build 48 H3-specific models, and found approximately the first 2/3 (equivalent to HA1 domain) of the HA gene capable of unambiguously differentiating H3 from non-H3 strains. This observation is inline with wet-lab findings that HA1 is more an antigenicity determinant than HA2 is for H3 strains. With these we are developing a web-based application in detecting the association of an unknown influenza A strain to a given subtype, provided a partial or complete HA protein sequence. We are also expanding this method in lineage (strain-like)-based detection as well as NA subtyping.

Abstract P1103

Modeling of Bio-aerosol Transport and Dispersion in a Ventilation Room

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The transport and dispersion of aerosol imbedded with influenza virus in a positively or negatively pressurized ventilation room is investigated by Lagrangian stochastic flight model and computational fluid dynamics (CFD) methods. Real influenza, caused by a specific group of viruses, can actually be dangerous. These viruses naturally spread through the air via turbulent suspension and transport and infect others who walk in. The virus-containing bio-aerosols originate from the respiratory activities of a patient such as coughing and the water droplets evaporate rapidly after these germ-laden droplets are released. Since the size of these droplets are very small it is justified to model these aerosols as passive scalars such as carbon dioxide (CO2), as a first approximation Given the location of source (standing, sitting, and sleeping position) of an index case in an enclosed space (the release location of tracers), the mathematical model can be sued to track the transport and dispersion of these aerosols, and calculate the probability density function (PDF) or influence area of the aerosols in the ventilation room for flu viruses and other contaminants. This modeling can also provide valuable information for ventilation control strategies with respiratory protection, such as enhanced air exchange (with higher ventilation rate), air filtration rate, and improved airflow patterns to reduce indoor airborne infection risk. In this study we focus on the influences of source levels and strength on the contaminant concentration and spatial distribution by comparison to available experimental data. Finally, the modeling compares vertical profile of bio-aerosols vs. Gaussian profile for passive scalar (tracers) dispersion. Future study will expand into effects of droplet evaporation on dispersion patterns and droplet residence (flight) time before it hits a target. Key words: Aerosol; Influenza virus; Lagrangian stochastic; Indoor air.

Abstract P1104

Control of Pandemic Influenza Using Antiviral Drugs–Some Logistical Constraints

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Antiviral drugs will play an important role in the control of pandemic influenza, particularly in the early stages of the pandemic where no effective vaccine would be available. However, antiviral programmes may be severely hampered by logistical constraints. We consider the effects of two such constraints using a compartmental modelling approach. First, with a limited stockpile, is it preferable to adopt a drug-sparing policy to avoid runout, or an aggressive one? Second, if there is a maximum antiviral distribution rate, due to limited manpower, is this likely to have a significant effect? We find that it is almost always preferable to adopt the most aggressive possible treatment policy, even if this should lead to runout. Such a policy minimises the final epidemic size, and, in most cases of practical interest, delays and reduces the epidemic peak. Moreover, if R_0 is not too large, it may even be possible to avoid stockpile runout by a sufficiently aggressive programme. We derive corresponding conditions on R_0. Concerning antiviral distribution, our results suggest that, as long as treatment courses sufficient to treat a minimum of 4% of the population can be dispensed per day, there will be no significant impact on the disease dynamics.

Abstract P1105

Scenarios of Diffusion and Control of Influenza Pandemic in Italy

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We developed a SEIR deterministic model with a stochastic simulation component to investigate the spread of pandemic influenza in Italy. We evaluated the impact of vaccination prioritizing different categories, antiviral prophylaxis -AVP- and social distancing measures, listed in the Italian national plan for preparedness and response to an influenza pandemic.

We assumed an R_0 of 1.8, with a cumulative attack rate (AR) of 35%. As standard parameters, we considered that the target population would receive the first dose of vaccine 8 or 12 weeks after the onset of the index case in Italy, and the second dose four weeks after that, with a vaccine efficacy of 70%. Vaccine coverage was fixed at 60%. AVP was assumed to reduce susceptibility to infection by 30%. We limited the use of AVP to household contacts, for a maximum of 8 weeks after the onset of illness in the first national case. Closure of schools was assumed to last 3 weeks, public offices for 4 weeks, and recreational venues for 8 weeks. In absence of control measures, the epidemic peak would be reached approximately 4 months after the first case onset. The epidemic would be over in 7 months, with a total of approximately 20 million of cases). The interventions considered, when singly implemented, would reduce the cumulative AR to, at best, approximately 32% (vaccination only). Using either AVP or social distancing measures alone would have no effect in reducing the cumulative AR, but would delay the epidemic peak by approximately one and three weeks, respectively. Multiple interventions involving vaccination starting 8 weeks after importation of the first case, AVP and social distancing measures, would reduce the cumulative attack rate to 10% at a minimum (range: 10-12%), with 14 millions of cases avoided. Modelling results corroborate the need to respond to a pandemic with multiple preventive measures. Measures aimed to increase social distancing, could be extremely useful for delaying the epidemic peak, allowing time for greater availability of a vaccine, and thus optimising its impact.

Abstract P1106

Curtailing Pandemic Influenza in Facilities That Provide Care for Those Requiring Assistance With the Activities of Daily Life

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Can nursing homes and residences for the mentally developmentally challenged survive pandemic influenza? Several groups have modelled influenza transmission in communities and various modes for mitigating the consequences. These models suggest that antiviral agents and vaccines would be the most effective interventions, and non-pharmaceutical interventions (NPIs), both direct infection control and alternative configurations of working, will play a secondary role. However, the levels of antivirals and vaccines required are unlikely to be available at the beginning of a pandemic, even in affluent societies. In less economically developed settings, the bulk of control efforts available will be NPIs. A recent report explored the extent to which NPIs might enable acute-care hospitals to effectively control a pandemic. It demonstrated that the tight coupling between acute-care hospitals and the surrounding community limits NPI effectiveness. For even moderately severe pandemics, no level of within-hospital transmission control will prevent internal propagation of infection. However, other healthcare and social institutions have the potential to restrict community access. These include ~16,000 institutions within the US that care for individuals who require assistance with activities of daily living (ADL), the disabled (mostly elderly) and mentally developmentally challenged. We studied whether and to what extent such institutions might attain effective internal transmission control with NPIs, alone and when combined with antiviral and/or vaccine resources. We found that combinations of NPIs and the stringency of application required to reduce influenza transmission below epidemic level could be gauged against a threshold probability of risk of introduction of the virus. This risk threshold decreased as the transmissibility of the virus, R_0, increased. Of course, any combination of NPIs, has intrinsic limitations. Therefore, different, combinations of NPIs must be chosen to meet the challenge of higher levels of severity. Our model indicated that to prevent internal propagation of an influenza pandemic an ADL-institution must rigorously control visitor access, implement non-conventional ways of working, and impose stringent infection control measures. In particular, for pandemic severity above moderate, non-conventional staffing arrangements appeared to be essential to reduce risk of infection introduction below the level at which there was internal propagation of pandemic influenza.

Abstract P1107

Aerosol Generation Model for Cough Simulations

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The airborne transmission of disease is of great concern to the public health community because of the pandemic potential of newly-emerging diseases like avian influenza. The possible spread of infectious disease by aerosols is of particular concern among health-care workers and emergency responders, who

face a much greater risk of exposure to these hazards than does the general public. Influenza is thought to spread in part by dissemination and inhalation of aerosols of small droplet nuclei that are produced by coughing and remain airborne for an extended time. For that reason a better understanding of the generation of aerosols by humans is important. The goal of this study is to investigate the air-flow dynamics and the generation of aerosols during coughing. A fairly simple yet accurate model has been developed to simulate the flow inside the upper respiratory tract, focusing on the larynx and its vicinity, and to predict the number and size distribution of the aerosols generated during coughing. The aerosol generation and entrainment model (AGEM) is composed of droplet entrainment, generation and break-up models. The flow model solves for the velocity, shear stress and pressure distribution, as well as the turbulent kinetic energy. These, in turn, are used as input parameters in the AGEM to calculate the aerosol formation during a cough. The size distribution of the aerosol droplets after coughing is calculated and compared with experimental results. It is shown that, with appropriate model coefficients, it is possible to calculate size distributions of aerosols that are consistent with the experimental findings. A parametric study of physical properties of the mucus has also been carried out. This study provided a size distribution with trends that are compatible with experimental observations. (The findings and conclusions in this abstract have not been formally disseminated by NIOSH and should not be construed to represent any agency determination or policy.)

Abstract P1108

Validation of the Moving Epidemic Seasons Method for Detecting Influenza Epidemics in Europe

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Introduction: The beginning, duration, intensity and geographical spread of seasonal influenza epidemics are unpredictable. In Spain, a method for detecting the seasonal epidemics has been used since 2003 with reliable results[i]. A modified approach named the "Moving Epidemic Season Method" is being implemented for systematic use in the European Influenza Surveillance Scheme. The aim of this work is to assess whether this method was able to detect the beginning of the influenza epidemic based on consultation rates for Influenza-Like-Illness. Method: Basically, the Moving Epidemic Season Method consists of estimating the epidemic period for each season as the minimum number of weeks with the maximum cumulated rate. The seasons are then moved to match all the epidemic periods in the same position. The threshold is the upper 95%CL of the geometric mean of the highest rates in the pre-epidemic period of each season. The method detects the beginning of the epidemic when a weekly incidence rate exceeds the pre-epidemic threshold. The method was applied to historical data from eight European countries registering weekly ILI consultation rates over the last 5 to 10 influenza seasons, i.e. Denmark, England, Ireland, Italy, Portugal, Spain, Switzerland and the Netherlands. For each season and country, a threshold was estimated. The start week (alert signal) was confirmed when the weekly rates do not drop below the threshold until the epidemic peak was reached. The positive predictive value of the alert signal is the percentage of seasons with the start week confirmed (true positive alerts). Results: Positive predictive value of the alert signal is 60% or more in most of the countries. Switzerland has a 100%, England 89% and Portugal 85%. Ireland and Spain have the lowest. Most of false alerts occur in weeks with incidence rates around the threshold or in early season weeks. Discussion: The estimated threshold is conservative. The model aims to minimize "false alerts". A false positive (false alert) could appear if the reported ILI cases are attributable to other viruses (increased acute respiratory infections or respiratory syncytial virus cases before the influenza epidemic) or if the ILI rates are unstable. [i] Vega Alonso T, Lozano Alonso JE, Ortiz de Lejarazu R, Gutiérrez Pérez MS Modelling influenza epidemic—can we detect the beginning and predict the intensity and duration? International Congress Series 1263 (2004) 281-283.

Abstract P1109

Hierarchy in Influenza Circulation: From Immunological to Epidemiological Dynamics

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A good classification of the circulations of influenza viruses is based on their genotypes (type, subtype and strain). During pandemics, new influenza A subtypes always replaced the pre-circulating subtypes, but not the other type: influenza B. Since H1N1 reemerged in 1977, it co-circulates with H3N2 and the compositions of H1 and H3 (dominant strains) continuously change, however, those strains (belonging to H1/H3) fail to replace the other subtype (H3/H1). In order to understand these observations and the classification of the circulation

based on genotypes, we construct an ecological model to investigate the complex but interesting epidemiological dynamics of influenza. Our model suggests that the observed hierarchical structure in the circulation of influenza viruses has its origin in the difference in the mechanisms of broad cross-protective immunity. Respectively, short-lived T cells (CD4+, CD8+) and specific antibodies (IgA) play a causal role in providing cross protection against heterosubtypic infection and infection with drift viruses within a subtype of A-type virus, as well as within B-type virus. Our model provides a comprehensive scenario for the emergence of new viruses, their subsequent circulations and eventual replacements. This scenario casts light on our understanding of epidemiological dynamics of influenza, which is necessary for preparedness for influenza pandemics.

Abstract P1110

HI Antibody Response and Clinical Protection Against Influenza: A Model and Application

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Serum haemagglutination inhibition (HI) antibody titers of 40 are considered "protective". However, the exact relationship between HI titer and clinical protection against influenza is poorly documented. Using published data, we modeled the relationship between HI titer and clinical protection and used this model to extrapolate vaccine efficacy (VE) from new immunogenicity data. We identified articles providing data on both post-vaccination HI titer and occurrence of influenza illness and retained those with sufficient internal validity (scored based on population size, number of antibody classes and drop-out rate). The titer-protection relationship was modeled using a scaled logistic function. Parameters were estimated using the Maximum Likelihood method and confidence intervals (95%CI) were obtained using Naïve Bootstrap. This model was then applied to immunogenicity data from a clinical study for the registration of Vaxigrip® for the 2006–07 season to estimate the VE. VE confidence intervals were determined using resampling and Monte Carlo simulations. Nine papers were retained (6 challenge studies, 2 clinical trials and 1 cohort study), representing 1306 observations, among which there were 245 influenza cases or positive reactions to challenge. The estimated level of protection was 23% [95%CI: 8-40] for an HI titer of 10, 69% [54-79] for a titer of 40, and to 94% [91-98] for a titer of 160. When applied to the annual vaccine registration clinical trial results, VE among 18-60 year olds (N=62) was estimated to be 77.3% [68.1-85.3] against the H1N1 strain, 83.9% [76.3-90.6] against H3N2, and 60.6% [50.3-69.9] against the B strain. VE was lower among adults aged 60+ (N=58). It was, respectively, 60.0% [48.5-69.9], 71.9% [60.3-82.5], and 55.9% [44.6-66.1] against the three strains. The reliability of the VE estimate depends on the quality of data used for both the estimation and calculation phases. Our results should be considered as preliminary results meriting further investigation with more robust data. While questions remain concerning the relevance and clinical accuracy of the approach, our analysis confirms that it is possible to model the relationship between HI titers and clinical protection against influenza and ultimately estimate the efficacy of influenza vaccination.

Abstract P1111

Modeling the Population Benefits of Various Pediatric Influenza Vaccination Strategies

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Background: Evidence suggests that vaccinating children against influenza may have a greater impact on disease incidence in the whole population than current strategies of vaccinating people at risk of complications. We investigated the role of children in influenza transmission and the potential benefit for the US population of vaccinating children in addition to current target groups. Methods: Using a stochastic model, we simulated influenza transmission in a representative community of 2000 persons of different risk statuses and ages (9 age groups). Individuals were characterized by a probability of infection and a probability of transmission depending on vaccination status and the risk of meeting infectious individuals. We evaluated the clinical consequences of an influenza epidemic in different childhood vaccination scenarios, without modifying coverage rate in adults. Baseline vaccination coverage was that estimated for 2006 by the CDC. Results: Increasing vaccination coverage among 5-10, 11-14 or 15-18 year olds (yo) to 30% reduced the global disease burden in the community by 22.8%, 22.9% and 25.1% (≈ 4.5, 4.6 and 5.0 million fewer cases/year in the US) respectively. The number of cases avoided per additional vaccinee was 1.17 and 1.60 to 1.96, respectively for 5-10, 11-14 or 15-18 yo. The indirect benefit for the elderly (no increased coverage in this age group) was 22–25% fewer cases compared to baseline.

Conclusion: Our results suggest that vaccinating children in addition to persons in high risk groups would extensively prevent influenza morbidity and mortality in the whole population.

Abstract P1112

Quantification of the Link Between Immunogenicity and Protective Efficacy of Influenza Vaccines in the Older Population

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Introduction: Vaccine efficacy can be evaluated on the basis of clinical endpoints and/or validated serological surrogate endpoints. In the case of influenza, protection against serious illness is generally associated with HI titres of ≥40. However, while the link between immunogenicity and protection against influenza is accepted, there is no formal quantification of the relationship between them. Objectives: The objective was to predict the potential advantages of adjuvanted influenza vaccines in terms of case reduction. This required the development of an empirical model to quantify the link between immunogenicity and protection. Methods: Three steps were necessary: the assessment of the infection rate from historical trials; the development of a predictive model of the likelihood of infection based on antibody titre; and the comparison of clinical trial data measured by changes in antibody titre with adjuvanted and non-adjuvanted vaccines. The number of subjects infected following exposure to a virus has a binomial distribution. Using large sample theory, the likelihood of infection with approximate 95% confidence intervals was generated. Based on this model, raw data from a meta-analysis comparing adjuvanted with standard vaccines were used to estimate the likely infection rate based on the differences in antibody titres pre- and post-vaccination in the older / high risk older populations. Case reduction was estimated as follows: 1 – (Cases with adjuvanted vaccine/Cases with standard vaccine). Results: On the basis of our model, the published superior immunogenicity of MF59 adjuvanted vaccines translate into additional case reductions by influenza strain in the older population of 23.8% in A/H3N2, 11.8% in A/H1N1 and 19.6% in B, compared to non-adjuvanted influenza vaccines. For the high risk older population, additional case reductions by strain were 26.6%, 10.4% and 23.8% respectively. These results are in line with published clinical data on the superior effectiveness of MF59 adjuvanted influenza vaccines in this population. Conclusions: The results of this model confirm that the protective efficacy of influenza vaccines is directly related to the increase in antibodies induced in the older population. Findings also suggest that the enhanced immunogenic properties associated with adjuvanted vaccines are beneficial for the older population, particularly those with chronic disease.

Abstract P1113

Patterns of Diversifying Selection in the Avian Influenza A H5N1 Strains Belonging to the Genotype 'Z'

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The avian influenza A H5N1 genotype Z has emerged as the dominant circulating strain since 2002, and has been responsible for outbreaks in several countries. The genotype has evolved into two major clades, viz. Clade 1 and clade 2. Earlier studies (Li, 2004), based on the analysis substitution rates in genes of genotype Z viruses isolated in 2002-04, suggested that the gene constellation of the genotype had not fully adapted to poultry and may continue to evolve through mutation or reassortment to achieve greater viral fitness. We undertook to study the genes of the avian influenza H5N1 strains of the Z genotype, circulating in 2005 and 2006 to investigate molecular evolution through diversifying selection analysis. MEGA was used to calculate the synonymous (dS) and nonsynonymous (dN) substitution rates and the average ratio d_N/ d_S (=w). So also, maximum likelihood models of codon substitution that allow for heterogeneous selection pressures among sites were implemented using PAML. Tests for positive selection based on M7 and M8 codon substitution models were applied to sequences representative of the clades 1 and 2. Though the mean w values for all the genes were less than 1, suggestive of their overall conservative evolution, the tests for positive selection indicated that the HA1, M2, NA and NS1 genes showed significant evidence of positive selection. Bayesian models identified several specific sites under positive selection with a high posterior probability. Of the 14 sites in HA1, six sites (129, 138, 140, 141, 156 and 209) are retained when compared to the sites observed in a study of H5N1 viruses of the 2004 outbreaks. In addition, 6 unique sites were observed. The site at 156 (T, A, S), is the potential glycosylation site at the globular tip of the haemagglutinin. The physiochemical properties of the candidate positive selection sites such as polarity and solvent accessibility were significantly different when compared to the variable sites. The positive selection sites could be mapped on to

validated immunogenic epitopes. The study would be useful in understanding the evolutionary patterns within the clades of the Z genotype.

Abstract P1114

A Kinetic Mathematical Model of Adaptive Immune Responses to Influenza A Virus Infection

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The primary cellular immune response to influenza is complex. Although some response functions can be measured directly, many others cannot. In addition, it is often difficult to determine how viral clearance, T and B cell responses, and antigen presentation in different compartments interact with each other in a dynamic fashion. Therefore, we developed a two-compartment set of time-delay ordinary differential equations for airway/lung and lymph node/spleen, forming a global flu model. Our objective is to use a mathematical model to predict the characteristics of primary influenza response when virulence or certain arms of the immune system are altered. By comparing our global flu model with experimental data on the kinetics of the cellular responses, we initially addressed the dependence of viral clearance on CD8 T cell and antiviral B cell responses in primary influenza infection, and the role of CD4 T cell help in the persistence of antibody after primary infection. The developed mathematical model was used to guide the design of new experiments quantifying different arms of the immune response to influenza infection. This work is part of the multidisciplinary efforts of investigators from the Center for Biodefence Immune Modeling and funded by by NIAID/NIH contract no. N01-AI-50020.

Abstract P1115

Collection Efficiency of Personal Aerosol Sampler

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Public exposure to viral agents, especially influenza, is of great concern in environments such as schools, office buildings and hospitals. The assessment of personal exposure risk factors in such environments requires efficient and accurate sampling methods valid for both short term and long term area samples. The National Institute of Occupational Safety and Health (NIOSH) recently developed a prototype personal aerosol sampler that works on the principles of cyclone. The innovative design makes use of commercially available microcentrifuge tubes of various designs that can be attached to the prototype sampler. With the actual collection of spores taking place in the microcentrifuge tubes, the design has the added advantage of zero transfer losses by eliminating the sample transfer step. In the current study Computational Fluid Dynamic (CFD) simulations are performed on the prototype design to assess the collection efficiency of the sampler for various particle sizes injected at the inlet at different flow rates. A CFD grid was built to the exact design parameters of the sampler. The grid was modified accordingly for various collection tubes. Initial validation studies were conducted with particle diameters ranging from 0.5 to 6.0 μm with two flow rates of 2.0 L/min and 4.0 L/min to match the conditions of the experimental studies performed on the prototype at NIOSH. Preliminary analysis of the CFD results showed good agreement with the experimental results. The reported collection efficiency data for the sampler for various conditions will be verified using the CFD study. Further simulations will be conducted to generate detailed efficiency maps for a wide range of particle diameter and flow rates. A two-stage sampler design with the potential of collecting size segregated samples in two different microcentrifuge tubes was introduced recently. CFD simulations will also be performed for this two-stage design.

Workshop Topic #12

Virus Host Interaction/Pathogenesis

Poster Abstracts

Abstract P1201

Pathogenesis of H5N1 Avian Influenza Virus Gene Reassortants in Chickens

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Highly pathogenic H5N1 avian influenza viruses produce severe disease and mortality in chickens. Identification of viral genes important for cell tropism and replication efficiency helps identify and target virulence factors. To determine which viral gene or genes contribute to the virulence of H5N1 avian influenza viruses in chickens, we used reverse genetics to generate single-gene recombinant viruses and examined their pathogenicity in chickens. Intranasal inoculation of two week-old chickens with the recombinant avian influenza virus rEgret/HK/02 resulted in 100% mortality and high viral titers in tissues. Inoculation of chickens with rCk/Indonesia/03 produced only 50% mortality with significantly less viral replication in tissues. Reassortants combining genes of the mentioned viruses demonstrated that exchanging the hemagglutinin and PB2 genes considerably affected pathogenicity; this was reflected in increased mortality and increased viral replication and spread in tissues. The NS gene also had an effect on viral replication; however, no effect on mortality was observed. Differences in mRNA cytokine levels in lung and spleen were observed among the different groups, indicating a role of the innate immune response on the outcome of infection.

Abstract P1202

Inefficient Transmission of H5N1 Influenza Viruses in a Ferret Contact Model

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The ability to infect and transmit efficiently among humans is essential for a novel influenza A virus to cause pandemic. To evaluate the pandemic potential of widely disseminated highly pathogenic avian influenza H5N1 virus, a ferret contact model that comprised of one inoculated and two contact ferrets was used to study the transmissibility of four humans H5N1 viruses isolated during 2003–2006. The effects of viral pathogenicity and receptor binding specificity on transmissibility were assessed. A/Vietnam/1203/04 and A/Vietnam/JP36-2/05 viruses, which possess "avian-like" α2,3-linked sialic acid (SA) receptor specificity, caused neurological symptoms and death in ferrets inoculated with 10^3 $TCID_{50}$. A/HongKong/213/03 and A/Turkey/65-596/06 viruses, which show binding affinity for "human-like" α2,6-linked SA receptors in addition to their affinity for α2,3-linked SA receptors, caused mild clinical symptoms and were not lethal to ferrets. No transmission of A/Vietnam/1203/04 or A/Turkey/65-596/06 viruses was detected. One contact ferret developed neutralizing antibodies to A/Hong Kong/213/03 in the absence of clinical signs or detectable virus shedding. In two groups, one of two naïve contact ferrets had detectable virus after 6 to 8 days housing together with the A/Vietnam/JP36-2/05 virus inoculated ferrets. Infected contact ferrets showed severe clinical signs including pneumonia and neurological symptoms, although little or no virus was detected in nasal washes. This limited virus shedding explained the absence of secondary transmission from the infected contact ferret to the other naïve ferret that was housed in the same cage. Our results suggest that despite their receptor binding affinity, circulating H5N1 viruses retain molecular determinants that restrict their spread among mammalian species.

Abstract P1203

Transmission of Influenza Virus A is More Efficient by Aerosol Spread Than by Fomites

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The modes of transmission of influenza A virus amongst humans is currently under debate. Optimal use of healthcare and other government resources in controlling influenza virus spread during pandemic and interpandemic periods relies on resolution of this discussion. Limited evidence exists to support infection control guidelines, and the relative contributions of fomites (inanimate objects and substances) and aerosols (large droplets and droplet nuclei) to transmission remains unknown. We recently developed a novel mammalian model of influenza virus transmission using the guinea pig. We have established that the guinea pig is highly susceptible to unadapted human influenza viruses and

capable of guinea pig to guinea pig transmission by direct contact and aerosol. Using this model, we investigated the role of fomites in influenza virus transmission. Twelve Hartley guinea pigs were placed in cages vacated by influenza A/Panama/2007/1999 (Pan99)-inoculated guinea pigs. Only two out of twelve exposed animals became infected and seroconverted, while aerosol transmission from guinea pig to guinea pig was 100%. In an additional experiment, none of six Hartley guinea pigs placed in cages to which Pan99 virus was directly applied became infected or seroconverted. To pursue the role of autoinoculation via fomites, we applied Pan99 to the paws of the superior extremities of 4 guinea pigs. None of these animals shed virus and only one seroconverted. Similar results were obtained using strain 13 (inbred) guinea pigs. This demonstrates that compared to aerosol spread, contamination of environmental surfaces does not contribute to efficient transmission of influenza viruses in this animal system.

Abstract P1204

Genetic Evolution of Human Influenza H3N2 Virus in a Mouse Pneumonitis Model: Insights Into Viral Virulence and Host Pathogenesis

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Human strains of influenza A virus are generally non-pathogenic for mice, but may be adapted to virulence by serial lung-to-lung passage in mice. The lung pathology induced by the adapted strains resembles that of human primary influenza pneumonitis. The changes that occur in the virus during mouse adaptation may provide insights into the virulence factors that contribute to severe lung infection. We have developed a mouse model in which the A/Aichi/2/68 (H3N2) strain was mouse-adapted by passaging through several batches of mice via intranasal administration. Briefly, influenza virus was initially administered intranasally to six week-old female BALB/c mice. Two days later, the animals were sacrificed, lung homogenate was prepared to infect a second batch of mice, and the process of passaging was repeated ten times. By assaying its infectivity in MDCK cells and by real-time RT-PCR, the virus titer increased progressively with each passage. The highly pathogenic, late-passage H3N2 virus induced considerable weight loss and high mortality in infected mice. Histopathologic analysis of their lungs revealed bronchopneumonia, alveolar damage and pulmonary edema with an intense inflammatory infiltration of neutrophils and activated macrophages which can trigger a cytokine storm. Elevated levels of inflammatory cytokines (e.g. MCP-1, IL-12 and IL-6) were detected in the broncho-alveolar lavage fluid. A major determinant for mouse lung virulence, the viral hemagglutinin of the hypervirulent strain harbored significant mutations including a non-conservative Gly218Glu substitution within the receptor-binding domain of the HA1 region. This mouse model can be employed to study pulmonary damage and repair, and to test the protective efficacy of candidate vaccines and antivirals.

Abstract P1205

The Use of ex-vivo Tissues for Infection by Pathogenic and Non-pathogenic Influenza Viruses

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Background: A proper understanding of the disease severity of various viral respiratory pathogens relies on knowledge of the cell tropism and anatomical basis of infection by the pathogen. At present research is mainly directed towards using animal models, cell line cultures or retrospective analysis of human autopsy tissues to determine why emerging viral pathogens such as SARS or H5N1 lead to disease severity and mortality. Unfortunately animal models of these diseases do not mimic the human situation and cell culture models also may not faithfully recreate disease models. The use of ex vivo freshly removed tissue biopsy fragments from surgical material represents a compromise in determining cell infection and pathogenesis without risk to the patient, and also allows an assessment of potential hazard from newly emerging pathogens. Methods: Fresh biopsies were obtained from the normal nasopharynx of 15 individuals being screened for nasopharyngeal carcinoma and 6 biopsies were obtained from healthy volunteers. In addition, fragments of tonsillar or adenoidal tissue were obtained from patients undergoing elective tonsillectomy and adenoidectomy. The biopsies were infected with influenza A viruses of subtypes H5N1, H3N2, H9N2 or H5N8 within three hours of collection. Results: We found that H3N2 and H5N1 viruses readily infected epithelial tissues of the nasopharynx, adenoid and tonsil. No viral antigen detected in mock infected tissues, or tissues infected with UV-inactivated H5N1. Productive viral infection in the tissue fragments from the nasopharynx was demonstrated by increasing viral yield in culture supernatants and viral antigen was detectable from 16- 72 hours after infection. We also carried out parallel experiments using ex-vivo cultures of lung tissue from 5 patients. We identified viral antigen in ex-vivo infected lung tissues and dual labeling of these tissues showed that the virus infected cells were pneumocytes and alveolar macrophages. Increasing viral yield in culture

supernatants provided evidence of productive viral replication. Viral antigen was also readily detected in H1N1 infected lung tissues. Conclusion: The use of ex-vivo biopsies from the upper and lower respiratory tract is a feasible and safe method for determining respiratory viral pathogenesis in emerging infections and should be utilized more in research laboratories that have access to clinical material

Abstract P1206

Promising Junior Investigator Scholarship

Polarity of Influenza H5N1 Virus Infection in Respiratory Epithelial Cells and the Impact of Basolateral Release of Cytokines in Disease Pathogenesis

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Background: Highly pathogenic avian influenza virus (H5N1) is the first avian influenza virus that documented to cause respiratory disease and death in human. The biological basis for the severe human disease and high fatality rate remains unclear. We previously demonstrated that H5N1 viruses were more potent inducers of pro-inflammatory cytokines from non-polarized respiratory epithelial cells and macrophages, which suggest that cytokine dysregulation may play a role in the pathogenesis. As respiratory epithelial cells are the primary target cell for replication of influenza viruses, a full understanding of how influenza virus interacts with respiratory epithelium is vital to advance our knowledge of its tropism and pathogenesis. Method: Primary human respiratory epithelial cells were isolated and seeded onto collagen-coated transwell filters and cultured under air-liquid interface until the cells were polarized and differentiated. The replication kinetic of influenza virus was assessed by virus yield titration ($TCID_{50}$) in MDCK cells. The cytokines profile induced by H5N1 virus (A/HK/483/97 and A/Vietnam/3046/04) was compared with human H1N1 virus by quantitative RT-PCR and ELISA. Results: We demonstrated that influenza A viruses (H5N1 and H1N1) can infect and productively replicate in the bronchial and alveolar epithelial cells leading to cytopathic effects. We found that cells infected on the apical rather than the basolateral surface showed high levels of viral replication. Progeny virus was released into the apical chamber at titers up to 3 log higher than those recovered from the basolateral surface of polarized cell cultures. Influenza H5N1 viruses were more potent inducers of cytokines in human respiratory epithelial cells. We now show that these cytokines are released preferentially on the basolateral aspect in the polarized epithelial cells. Conclusion: Our data suggest that influenza H5N1 viruses enter and are released from the apical domain of respiratory epithelium, while the pro-inflammatory cytokines are released preferentially from the basolateral aspect of the respiratory epithelium. These finding may provide important insights into the mechanism of replication and pathogenesis of influenza H5N1 virus in humans.

Abstract P1207

Pathogenesis of Influenza A Infection of Mice With Viruses of Different Virulence

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Three influenza A viruses originally isolated from humans were adapted to mice: A/Dunedin/4/73(H3N2), A/Mississippi/1/85(H3N2), and A/PR/8/34(H1N1). In vitro infectivity of the viruses on MDCK cells was proved to be similar: $1.2x10^7$ to $2.2x10^7$ pfu/ml. However, these viruses considerably differed in virulence for mice, i.e. in pfu representing 1 LD_{50}: 79 000 pfu of Dunedin, 5 000 pfu of Mississippi, and 65 pfu of PR/8. Ratio of their virulence therefore can be expressed as 1 215:77:1 (D:M:P) which qualifies Dunedin as low virulent, Mississippi medium, and PR/8 highly virulent. Virus spread in intranasally infected mice with dose of 0.5 LD_{50} was evaluated. Lungs, heart, thymus, liver, spleen, brain and blood were tested for presence of infectious virus by rapid culture assay (RCA) and for presence of vRNA by RT-PCR on days 0,5, 1, 2, 4, 6, 8 and 10 post infection. Viral antigen was immunohistochemically stained in organ sections and histopathological changes of tissues were simultaneously evaluated. All three viruses were detected in lungs, heart, and thymus by cultivation in RCA. Maximum virus titer in the lungs was reached on the 1st day p.i. for Dunedin, on the 2nd day p.i. for Mississippi, and on the 6th day p.i. for PR/8. Viral RNA of all viruses detected by RT-PCR was found also in liver and spleen. Moreover, vRNA of Dunedin and PR/8 was present in kidneys and vRNA of Dunedin and Mississippi in blood. Only vRNA of Dunedin was demonstrated in the brain. Histology of lungs sections after infection with all three viruses proved exsudative bronchitis and interstitial pneumonia which in milder form (Dunedin) changed to the peribronchitis combined with the onset of regeneration process. Lung damage was most extensive after the infection with PR/8 virus in spite of the lowest viral titer reached in lungs and the lowest amount of influenza antigen-positive lung cells in histological sections. It is notable that the low virulent virus Dunedin exhibited the quickest onset of infection in various organs and its vRNA was observed in brain, while the highly virulent PR/8 virus spread to the more limited variety of organs and reached lower titer.

Abstract P1208

Identification of the Molecular Determinants Required for Duck H9N2 Influenza Virus Replication in Chickens

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H9N2 influenza viruses have been frequently isolated from chickens in Asia since 1994. The natural reservoir of the viruses is wild waterfowl such as migratory ducks. It is believed that duck influenza viruses are the origin of H9N2 viruses that cause epidemics in chickens. However, we found that H9N2 viruses isolated from ducks (A/duck/Hokkaido/9/99) hardly replicated in chickens, whereas another H9N2 virus isolated from chickens (A/chicken/aq-Y-55/01) efficiently replicated in the respiratory tissues of chickens. To identify the determinants restricting the replication of H9N2 viruses in chickens, we established a reverse genetics system for these duck and chicken H9N2 viruses (RG-Dk/Hok and RG-Ck/Y55, respectively) and reassortant viruses in various combinationsbetween RG-Dk/Hok and RG-Ck/Y55, and compared their abilities to replicate in chickens. We found that HA was the most critical component for the replication of H9N2 virus in chickens. No difference in hemagglutinating activities for duck or chicken red blood cells between these viruses was found. We then examined the susceptibility of RG-Dk/Hok and RG-Ck/Y55 to proteases in the mucosal secretions of chickens. RG-Ck/Y55 replicated in MDCK cells with media supplemented with chicken tracheal or intestinal wash instead of trypsin, but RG-Dk/Hok did not, suggesting that RG-Dk/Hok HA had lower susceptibility to the proteases of chickens. However, mutant Dk/Hok whose cleavage site was replaced with that of Ck/Y55 grew neither in cultured cells with the mucosal secretion nor in respiratory tissues of chickens. A molecular modeling study revealed three different amino acids near the cleavage sites of Dk/Hok and Ck/Y55 HAs, all of which were highly conserved in duck and chicken H9N2 viruses, respectively. Accordingly, the charge distributions on the HA cleavage site regions of Dk/Hok and Ck/Y55 were mainly negative and positive, respectively. Taken together, these results suggest that the inability of duck H9N2 virus to replicate in chickens depends on its lower HA cleavability with chicken proteases, though it is defined not only by the cleavage site sequence itself but also by another structural restriction that may affect the interaction of HA with the HA-cleaving proteases of the chicken.

Abstract P1209

Identification of a Mutation in the Hemagglutinin That Promotes Efficient Transmission of Avian H1N1 Influenza Viruses

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Influenza pandemics occur when a new strain emerges possessing the ability to spread globally and cause high rates of illness among serologically naïve humans. The studies presented here are aimed at the identification of the genetic determinants that govern transmissibility of influenza viruses among humans, a key pandemic trait. We have investigated the ability of four avian H1N1 viruses to transmit by either respiratory droplet (spread of influenza virus through the air) or direct contact in a ferret model. The replication kinetics of all four avian viruses in the upper respiratory tract of inoculated ferrets were comparable to the replication observed with a human H1N1 virus (A/Tx/36/91) that transmitted efficiently by respiratory droplet. Nevertheless, none of the avian viruses examined were capable of respiratory droplet transmission between ferrets. However, two of the viruses (A/Dk/Alb/35/76 and A/Dk/NY/15024-21/96) were able to transmit between ferrets in direct contact experiments, albeit inefficiently. A natural variant of the A/Dk/Alb/35/76 virus isolated from one of the contact ferrets in this experiment possessed a novel mutation in the hemagglutinin that altered the receptor binding specificity from the parental avian α2,3 sialic acid (SA) receptor preference to a virus with both human α2,6 and avian α2,3 SA specificity. This virus was found to transmit efficiently between ferrets via direct contact, but still lacked the ability to transmit by respiratory droplet spread. These results highlight the influence of receptor specificity on the transmission of avian influenza viruses but indicate that additional genetic changes are required for efficient respiratory droplet transmission in mammals. The identification of molecular determinants of influenza virus transmission in an animal model may provide a framework for the future identification of influenza viruses with the potential to cause a pandemic.

Abstract P1210

Cytokine Induction Profile of Avian and Human Influenza Viruses–A Preliminary Report

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Introduction: The proinflammatory cytokine dysregulation is thought to contribute to the severity of human H5N1 infection. This study compared the profiles of cytokine/chemokine gene expression induced by avian and human influenza viruses. Methods and Materials: Quantitative real-time RT-PCR was used to compare the profiles of cytokine/chemokine and chemokine receptor gene expression in human lung epithelial cells (NCI-H292) following infection with influenza A/HK/483/97(H5N1/97), A/Thai/KAN1/04(H5N1/04), A/Canada/504/04(H7N3/04), and circulating H1N1. Results: The quantitative real-time RT-PCR results showed that during early post-infection, there were greater up-regulation of mRNA for TNF-alpha, RANTES, IP-10, CXCL-9, and IL-10 in H5N1/97, H5N1/04, and H7N3/04 infected cells than those infected with H1N1. During late post-infection, the mRNA level for TNF-alpha, RANTES, IP-10, CXCL-9 remained high in H5N1/97, H5N1/04, H7N3/04 infected cells. However, in the late phase, the IL-10 mRNA level dropped in H5N1/97 and H7N3/04 infected cells, but not in H5N1/04 infected cells. Furthermore, during the late phase, IFN-gamma, CCL-3, IL-6, IL-8 and BCA mRNA levels were also up-regulated in H5N1/97, H5N1/04 infected cells. The levels of CCR2 and CXCR5 receptor gene expression were elevated in H5N1/04 in late post-infection. However, there was a decrease in CCR2, CXCR4 and CXCR5 gene expression in H5N1/97 and H7N3/04 in late post-infection. In contrast, the H1N1 infected cells showed a decrease in CXCR4 mRNA level in the early phase, and a decrease in CCR2 mRNA level in the late phase after infection. Conclusion: There is a difference in cytokine/chemokine response of NCI-H292 cells to human and avian influenza virus infection in vitro. The H5N1/97, H5N1/04 viruses are more potent inducers of proinflammatory cytokine/chemokine, particularly TNF-alpha, RANTES and IP-10.

Abstract P1211

Identification of Virulence Determinants of H5N1 Influenza Viruses Isolated From Humans in Thailand During 2004

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Highly pathogenic avian influenza H5N1 viruses have had devastating effects in poultry and wild bird populations across three continents. Since early 2004, over 270 human cases with a fatality rate of approximately 60% have occurred, primarily as a result of direct exposure to H5N1 virus-infected poultry. Because information about the biologic and molecular factors affecting the virulence of H5N1 viruses in humans is limited, animal models are needed to investigate the basis of virulence of emerging H5N1 strains. Previously, we used the ferret model to evaluate the relative virulence of several H5N1 viruses and identified two strains isolated from humans in Thailand during 2004 that exhibited markedly different levels of virulence in ferrets but differed by only 16 amino acids (PB2, 2; PB1, 1; PA, 4; HA, 2; NP, 1; NA, 5; NS, 1). A/Thailand/16/04 (TH16) was highly virulent for ferrets, with severe morbidity and 100% mortality, while A/Thailand/SP/83/04 (SP83) exhibited low virulence with minor morbidity. To identify the molecular determinants responsible for these significantly different disease states, we used reverse genetics to generate TH16-SP83 reassortant viruses and evaluated their virulence in ferrets. Our results suggested that, for this H5N1 virus pair, one or more of the polymerase genes is a major determinant of virulence. The role of PB2, PB1 and PA in virulence of TH16 and SP83 for ferrets is currently under investigation. These results have public health importance by improving our understanding of the mechanisms involved in the pathogenesis of H5N1 viruses in humans.

Abstract P1212

PB1-F2 and the Change in Virulence of Influenza A(H1N1) Viruses

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Can actions of mankind act to fix mutations in influenza viruses? PB1-F2 is translated from an alternate reading frame in the PB1 gene of most influenza A viruses. A functional polypeptide, 79 to101 amino acids, induces apoptosis in infected cells, especially tissue monocytes. PB1-F2 is said to be under strong positive selection. Viruses with a functional PB1-F2 are more virulent that those without. Apoptosis of cells directly affected by influenza infection, lung epithelium and alveolar macrophages, delays viral clearance and facilitates secondary bacterial pneumonia. By report, PB1-F2 of all influenza A(H1N1) viruses isolated before 1947 was full-length; but after 1956, a mutation became prevalent containing 57 codons. A(H1N1) viruses were replaced by A(H2N2) viruses in 1957, and have exhibited virulence similar to B viruses since re-emergence in 1977. Did truncation of PB1-F2 reduce the virulence of A(H1N1) viruses and trigger the pandemic of 1957/58? What would be the evolutionary advantage of fixing such a mutation? Gene sequence reports were silent on the interval 1947-1956, so we searched the ISD database at Los Alamos National Laboratory. Eighteen complete sequences were reported for 1918-1947. All were full-length except one, from 1947 of length 57. Only six sequences, all of length 57, were isolated 1947-1956. Of 351 PB1-F2 sequences isolated after 1977, 347 were 57 codons long. Thus, the now dominant truncated PB1-F2 appeared a decade before the A(H2N2) pandemic. After the two catastrophic seasons of emergence of the 1918 virus, the pattern of pneumonia & influenza (P&I) mortality for the US from 1920/21 to 1936/37 consisted of 5 similarly large peaks spaced 3-4 years apart. Over the next decade, P&I mortality dropped by two-thirds. In 1937, the first year of commercialization of antibiotics, over 90% of all-cause excess mortality was attributable to P&I. Rapidly incorporated, antibiotic usage dramatically reduced mortality associated with secondary bacterial pneumonia. Complicating pneumonia delays viral clearance; a benefit to the virus. There must be an evolutionary cost associated with preserving a full-length PB1-F2 gene in an alternate frame. The erosion of benefit may have opened the pathway to mutation, reduced virulence and possibly the next pandemic virus.

Abstract P1213

Host Immune and Cell Death Responses Induced by Highly Pathogenic Influenza A Viruses

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Highly pathogenic avian influenza A (H5N1) virus causes a severe viral pneumonia leading to acute respiratory dysfunction syndrome. The pathogenicity of influenza A viruses varies depending on the species of host animals and virus strains. Infection with A/PR/8/34 (H1N1), but not with A/Aichi/2/68 (H3N2) strain of influenza A virus causes severe mortality in mice. However, the plausible explanation for their difference of pathogenicity is still unknown. To identify the critical determinants for the pathogenicity of these viruses, we examined the immunological responses to the infection of these viruses. Influenza virus A/PR/8/34 or A/Aichi/2/68 was inoculated into mice and the lung, spleen, and sera from the mice were obtained on 3, 5, and 7 days after the infection. Infection with A/PR/8/34, but not with A/Aichi/2/68 virus results in severe mortality and tissue damages of the lung and spleen. It is also noted that the weight of the spleen and the number of splenocytes in mice infected with A/PR/8/34 virus was decreased, whereas those with A/Aichi/2/68 was increased. Furthermore, we found that A/PR/8 virus infection strongly induced cell death in the lung and spleen and changes of cellular profile such as macrophages and T cell populations in the spleen. Taken together, cell death induction in the spleen and lung in addition to the change of the composition of splenocytes could be critical characteristics for the severe pathogenicity determined by influenza virus infection. Furthermore, to clarify determinants for the virulence of influenza A H5N1 virus, we screened the binding proteins of the host cells with PB2 polymerase, which is involved in viral replication. Using the antibody array analysis, we found that some proteins critical for the regulation of apoptosis or transcription factors of host cells were associated with PB2 protein of highly pathogenic influenza virus (H5N1) A/HK/483/97. We study the functional roles of these proteins and the contribution to the pathogenicity of influenza viruses.

Abstract P1214

Low Pathogenicity Avian Influenza (LPAI) Infection Provides Limited Immuno-Protection Against Highly Pathogenicity (HPAI) Virus Challenge in Chickens

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The generation of HPAI from LPAI in poultry has been demonstrated on a number of occasions including USA in 1983-84, Mexico 1994-95 and Italy in 1999-2000. This transition involves molecular evolution at the amino acid level within the haemagglutinin (HA) cleavage site. The roles of the innate and adaptive immune responses in the process are less well understood. One important question is: Are birds previously infected with LPAI immune to subsequent challenge with HPAI from the same lineage? Chicks (n=50, 3 weeks old) were inoculated with LAPI H7N1 (A/Ck/It/1279/99, 100MID) and were subsequently challenged at 6 weekly intervals (n=10 at 6, 12, 18, 24 & 30 wks) with HPAI H7N1 (A/Os/It/984/00, 100LD) from the Italian epidemic. Antibody (HI) and viral shedding (rt-qPCR) levels were determined prior to and daily for 3 weeks post challenge in addition to survival rates. LAPI primary infection resulted in varying antibody levels (titre of 2-256) and viral shedding, buccal 7-10 and cloacal 3-21 days, all birds survived. Following HPAI infection 100% of animals in each challenge group became infected, the survival rates were 80, 70, 40, 40, and 70% respectively. There appeared to be no substantial link between survival and antibody level pre- or post-HPAI challenge. Survivors shed less virus (1.5 logs compared to 3.5 logs) and excretion was more intermittent compared to those that died. Glycosylation typing and innate immune responses marker analyses are currently underway. HPAI can become established in birds previously infected with antigenically homologous LPAI, however mortality rates are reduced from 100% (HPAI alone) down to 20-60% depending on the interval between challenges (6-24 weeks). Antibody levels were enhanced by dual virus infection and shedding reduced in those that survived. However, HPAI virus did continue to be shed and therefore the transmission to naïve birds may have been possible. Indicating that whilst primary experienced birds (LAPI and HPAI) survived they may have gone on to promote occult dispersal of pathogenic disease.

Abstract P1215

Does H9N2 Avian Influenza Virus Acquire High Pathogenicity for Chicken?

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Objective: H9N2 avian influenza virus strains have caused outbreaks in Eurasian countries, giving serious economic losses. Since H9N2 viruses have been isolated not only from domestic birds but from pigs and humans, H9 virus is one of the candidates causing next pandemic in humans. It is presently believed that virus strains with H5 and H7 subtype hemagglutinins (HAs) become highly pathogenic during extensive infection in chicken population. It is known that highly pathogenic avian influenza viruses (HPAIVs) possess HA with insertion of more than a pair of di-basic amino acid residues at the cleavage site, resulting in cleavage into HA1 and HA2 subunits by ubiquitous proteases such as furin and PC-6. In the present study, we investigated whether virus with H9HA become HPAIV strain for chicken by mutation to give a pair of di-basic amino acid residues at the cleavage site on the HA molecule. Materials, methods and results: We generated mutants of A/chicken/Yokohama/aq55/01 (H9N2) (Y55) to substitute the amino acids for basic amino acid residues at the cleavage site by site-directed-mutagenesis and reverse genetics. The amino acid sequences at the cleavage sites of their HAs are as follows: Y55 (PARSSR/G), mY55A (PARSKR/G), mY55B (PARKKR/G), mY55C (PRRKKR/G), and mY55D (RRRKKR/G). These mutant viruses were experimentally inoculated intranasally into chickens. Virus replication occurred only in the respiratory tracts of the chickens and the birds did not any severe disease signs. There were no difference in growth and infectivity in MDCK cells and chicken embryos between the original virus and mutant strains. Discussion: H9 mutant viruses required trypsin for efficient growth in MDCK cells as other non-HPAIVs. The mutant viruses with the HA with basic amino asid residues by substitution at the cleavage sites of the H9HA did not cause systemic infection in chickens. Pathogenicity of other mutant H9 viruses which acquired a series of basic amino acid residues at the cleavage sites by insertion are currently under investigation.

Abstract P1216

SCHOLARSHIP International Scholarship

Biphasic Effect of rTGF-β1 on Nitric Oxide Synthase (iNOS) and dsRNA Dependent Protein Kinase (PKR) During Experimental Influenza Infection in Mice

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Background: Influenza virus infection activates the interferon (IFN) inducible gene, nitric oxide synthase 2 (iNOS) and dsRNA dependent protein kinase (PKR). PKR activates cytokine induced apoptotic pathway, however the production of nitric oxide (NO) inhibits the apoptosis via nitrosylation of caspase 3 an executioner capase. This process is tightly regulated by a complex network of cytokines. Among these, transforming growth factor-β1 (TGF-β1) is known to suppress IFN expression. NO has been shown to contribute to the pathogenesis of influenza virus via modulationg the apoptosis. Methods: Eight-week-old BALB/c mice were intranasally instilled with influenza A virus (A/Udorn/317/72/H3N2), $4.1x10^3$ PFU of virus in 50µl of allantoic fluid or mock infected 50µl of allantoic fluid. rTGF-β1 administered to mice by giving intravenous injection of rTGF-β1, 0.5µg/ Kg body weight of mouse. The mice were euthanized on days 3, 5 and 7 post infection (p.i.) for analysis of the parameters. Cytokine IFN-γ in BALF , iNOS expression in the lung homogenate and PKR expression in alveolar lavage cells. Apoptotic parameters (Caspase3 assay and DNA fragmentation) in alveolar lavage cells. Results: We observed an increase in caspase3 activity and DNA fragmentation on 3rd and 5th day p.i. however administration of rTGF-β1 with virus reduced the DNA fragmentation on day 3rd and 5th p.i. A significant increase of INF-γ was observed on 3rd and 5th day and decreased to basal lavel on 7th day p.i.. Simultaneous administration of rTGF-β1 with virus instillation inhibited release of INF-γ level on 3rd day and 5th day. The iNOS expression was detected on 3rd day p.i. and maximum level was observed on 5th day p.i in virus instilled group. However simultaneous administration of rTGF-β1 with virus significantly reduced the level of iNOS on 3rd, 5th and 7th day p.i.. The PKR expression also inhibited by rTGF-β1 administration. Conclusions: rTGF-β1 acts as an immunomodulatory cytokine and inhibits cytokine mediated apoptosis by modulating inflammatory cytokine INF-γ, which inturn down regulated the interferon inducible genes iNOS and PKR. Downregulation of PKR decreased the apoptosis.

Abstract P1217

Proteolytic Activation of Influenza A Viruses by Serine Proteases HAT and TMPRSS2 From Human Airways

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Cleavage of the hemagglutinin protein (HA) of influenza A viruses is a prerequisite of fusion of viral and endosomal membranes at low pH and therefore for the infectivity of the virus. However, relevant proteases in the human airway epithelium have not been identified so far. We have cloned two serine proteases, TMPRSS2 (transmembrane serine protease, S1 family member 2) and HAT (human airway trypsin-like protease), from cultures of human tracheobronchial epithelial cells. Co-expression of HA and either protease in mammalian cells revealed cleavage of the HA0 precursor protein into subunits HA1 and HA2. MDCK cells expressing TMPRSS2 or HAT supported multicycle replication of influenza viruses in the absence of exogenous trypsin, providing evidence for proteolytic activation of progeny virions in these cells. Addition of serine protease inhibitors such as aprotinin and soybean trypsin inhibitor prevented cleavage of HA and spread of viral infection in MDCK-HAT and MDCK-TMPRSS2 cells. Taken together, our results suggest TMPRSS2 and HAT as appropriate proteases for proteolytic activation of influenza viruses in vivo.

Abstract P1218

A Single Amino Acid Change in Influenza A Virus PB1-F2 Increases Pathogenicity in Mice

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The pro-apoptotic PB1-F2 protein of influenza A viruses has been shown to contribute to pathogenesis in the mouse model. Expression of functional PB1-F2 increases the pathogenesis of influenza A virus causing weight loss, slower viral clearance and increased viral titers in the lungs. PB1-F2 is an 87 amino acid protein translated from the +2 reading frame of the PB1 segment of influenza A virus, which causes apoptosis through the intrinsic pathway. Amino acids 45 through 75 contain a mitochondrial transmembrane sequence with amino acids

62-70 being essential for mitochondrial localization and apoptosis. To investigate the importance of the PB1-F2 amino acid sequence for its function during a viral infection an alignment was done between two similar viruses with different pathogenicities in mice, HK/156/97 and HK/483/97. We found one amino acid change in the PB1-F2 sequence of an N to S at the 66th amino acid. The presence of an N at position 66 is common to most PB1-F2 sequences, whereas an S at position 66 correlates with increased pathogenicity in other influenza A virus strains. Two recombinant chimeric viruses were created in a WSN background containing the PB1 segment from HK/156/97 (WH) and the other virus containing the change of N to S at position 66 in the PB1-F2 reading frame (WH N66S). In mice infected with WH N66S virus (with an S at position 66) there was increased pathogenicity as measured by weight loss and decreased survival when compared to mice infected with the WH virus (with an N at position 66). There was also a 1.5 to 2 log increase in virus replication and slower viral clearance from the lungs of mice infected WH N66S compared to those infected with WH virus. Together this data shows that a single amino acid change in PB1-F2 can increase pathogenesis and it further supports our previous data that PB1-F2 is an important contributing factor to influenza A virus pathogenesis.

Abstract P1219

Evolution of Interferon Resistance in the Influenza A Virus NS1 Gene

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Influenza virus replication is dependent on an ability to resist the antiviral effects of interferon (IFN) through the antagonistic properties of the NS1 protein. As the mutational basis for IFN resistance is unknown, a systematic assessment of evolution of IFN resistance was performed. An experimental murine model of evolution identified 5 adaptive mutations in the NS1 gene. Recombinant viruses that differed solely due to some of these NS1 mutations demonstrated increased resistance to IFN in mouse epithelium. Some mutations that increased IFN antagonism also inhibited viral replication indicating that defenses come at a cost which must be paid by compensating mutations. The NS1 mutations involved binding sites for translation initiation factors and RNA. Furthermore experimental evolution recapitulated natural evolution in birds where the Asian H5N1 lineage had independently selected 3 identical mutations. This demonstration of convergent viral evolution among species indicates that the molecular basis for modulations of influenza IFN antagonist function is similar among mammalian and avian species. Furthermore it can be concluded that sites of viral-host interaction modulating these IFN response elements are molecularly conserved and thus may serve as general targets of antiviral therapy.

Abstract P1220

Effect of Cyclosporine A on the Immune Responses to and Pathogenesis of a H9N2 Low Pathogenic Avian Influenza Virus in Specific Pathogen Free Chickens

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Avian H9N2 influenza viruses have become panzootic in poultry and have caused repeated human infections in Asia since 1998. Recent study showed that some Korean H9N2 influenza viruses isolated from domestic chickens in 2004 could replicate well in mice without prior adaptation. These H9 influenza viruses circulating in Korea seemed to have undergone antigenic and pathogenic changes. The H9N2 low pathogenic avian influenza (LPAI) viruses are able to produce a severe infection in the context of opportunistic secondary pathogens or immunosuppressive viruses in chickens. Similarly, fatal human infections caused by H1N1 low pathogenic influenza viruses are increased particularly in older patients and immunocompromised persons. Therefore, alternative prevention strategies may be needed and it is very important to evaluate the efficacy of influenza vaccine in those people. Research works on the pathogenesis of influenza infection and analysis of host immune response against the LPAI viruses are still needed. In this study, pathogenesis of and host immune responses to H9N2 LPAI virus in immunosuppressed chicken model were evaluated by virus shedding, histopathology, FACs analysis and real-time quantitative RT-PCR for cytokine gene expression. We demonstrated that pathogenicity of H9N2 LPAI virus was more enhanced in cyclosporine A (CsA)–treated SPF chickens than cyclophosphamide (CY)-treated or immunocompetent SPF chickens. CsA treatment induced increased pathogenic index (PI) value and mortality (1.225 PI and 25% mortality) as well as significantly increased virus shedding after infection. Influenza virus was immunohistochemically demonstrated

only in kidney of CsA-treated dead chickens. To define the influenza viruses induced kidney lesions, which were caused by either necrosis or apoptosis, TUNEL assay was carried out. The proportion of CD8+ T cells and expression of mRNA for IFN-γ dramatically increased in immunocompetent chickens at 5 days post infection. However, those of CsA-treated chickens significantly decreased. These results indicate that T cells and T cell mediated responses may be important in viral clearance and promoting recovery from H9N2 LPAI virus infection. Our data provide the insights into the immunopathogenesis of this virus, one of the candidate for pandemic influenza, with immunocomprimised human as well as poultry.

Abstract P1221

Interference-Dominance Properties of Cold-Adapted Influenza Viruses

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Live attenuated cold-adapted (ca) influenza vaccines are an effective means for the control of influenza, most likely due to their ability to induce both humoral and cellular immune responses. Recent evidence has demonstrated that cold-adapted (ca) influenza vaccines have the additional benefit of providing protection shortly after administration prior to the development of the adaptive immune response. This activity is probably due to the phenomenon of viral interference. The following paper addresses concerns that ca influenza virus vaccines may somehow interact with wild-type (wt) viruses including avian strains. In the present study we investigated the interaction of termo-sensitive ca reassortant vaccine viruses prepared using Russian cold-adapted master donor strain (MDS) A/Leningrad/134/17/57 (H2N2) over wild-type viruses and apathogenic avian virus. Several pairs of live cold-adapted vaccine strains prepared by genetic recombination with ca MDS A/Leningrad/134/17/57 (H2N2) and their wild-type parents A/New Caledonia/20/97 (H1N1), A/Sydney/5/97 (H3N2), A/California/07/2004 (H3N2), A/duck/Potsdam/1402-6/86 (H5N2) were tested in various susceptible systems: chicken embryos, MDCK cells and in mice. Experiments have been done under conditions of simultaneous infection and in cases when ca and wt viruses are given sequentially. Samples from both mixed infected cultures and single infected cultures have been tested for virus yield for both permissive and non-permissive temperatures. The replication of wild-type virus at high temperature in mixed infection in comparison with replication wild-type virus in single infection is used as the criterion of interference. In chicken embryos growth of wild-type viruses was significantly reduced after mixed infections with cold-adapted viruses. The levels of reduction depended upon wt virus dose and time after inoculation of ca virus. The interference had been observed if ca and wt viruses represent one subtype – homologous interference, and different subtypes - heterologous interference. The maximal reduction of wild-type virus yield have been observed when doses of both viruses were equal and time between inoculations is 24 hours. In MDCK cells the effect of interference was also observed. In case of mixed infection the replication of wt virus was reduced as much as 1000 times compare to yield from single infection at high temperature. Interference was more obvious if the proportion of ca virus in the inocula increased. Also the interference was observed both at low and at high multiplicities of infection. In mice replication of ca viruses in the lungs was diminished. Simultaneous inoculation ca and wt viruses do not lead to increasing pathogenicity over single infection of wt viruses. The reduction of wt virus titers in the lungs of mice infected by mix of viruses have been seen at both permissive and non-permissive temperatures in various degree, and was more dramatic if the time between inoculations exceeded 24 hours. The molecular mechanisms of interference are not completely understood and under investigation. Also the possibility of generation reassortants with mutant ts-phenotype during co-infection human cold-adapted and avian viruses will to be studied. It is important to remember that a key characteristic of live cold-adapted influenza vaccines is that they prevail wild-type viruses.

Abstract P1222

Restriction of Avian But Not Human Influenza Virus Infection at Temperatures of the Human Upper Airways

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Although avian influenza viruses infect and replicate in the ciliated airway epithelium of the human respiratory tract in vitro (Matrosovich et al. 2004; Thompson et al. 2006), efficient transmission from avian species to the human population rarely occurs. Recently, the lack of transmissibility of avian influenza viruses was attributed to low infection levels in the upper conducting airways due to a lack of the appropriate sialic acid residues that mediate infection (Shinya et al. 2006). Since avian influenza viruses are adapted to the temperature of the enteric tract of aquatic birds (40°C) we hypothesized that the cooler temperatures of the human upper airways (~32°C) may attenuate infection of these airway regions, thus

limiting zoonotic transmission. Using an in vitro model of human ciliated airway epithelium (HAE), we show that growth of low pathogenicity avian influenza (LPAI) viruses including A/Duck/England/62 (H4N6) and A/Duck/Singapore/97 (H5N3) is equal to that of representative H3N2 human influenza viruses at the temperature of the distal airways (37°C), reaching titers of 10^6 pfu/ml by 48hrs. At temperatures reflective of the upper airways (32°C), human influenza viruses grew equally well as compared to 37°C. In contrast, at 32°C LPAI titer was decreased by 3 logs at 48hrs. Rhinovirus 16 infection of HAE had a growth advantage at 32°C compared to 37°C, as predicted for a virus that is restricted to upper airway infection. These data indicate that LPAI viruses are not adapted for efficient infection/growth at temperatures reflective of the human upper airways. Temperature shift assays in which HAE were infected with LPAI viruses at 37°C and then shifted to 32°C revealed partial rescue of the low temperature attenuation, suggesting that early events during entry or replication of LPAI viruses at 32°C was compromised. Further characterization of the attenuated phenotype of LPAI viruses at 32°C will measure the extent of infection and the relative activities of virus NA and polymerase genes at different temperatures. In conclusion, LPAI infection of the upper but not lower airways is attenuated due to differences in regional airway temperatures, potentially limiting the likelihood of zoonotic and human-human transmission.

Abstract P1223

Analyses on Aerosol Particles From Sneezing and the Viability of Influenza Virus in the Air-Born Particles

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There is a dispute on the major possible modes of transmission of influenza: the infection through the large-droplet or through the aerosol which float relatively long time in the air. [1), 2)] It is prerequisite to know the nature of the aerosol from cough and sneezing and the survival of the virus in the aerosol from these physiological phenomena in several environmental conditions. Such kinds of analyses had been done intensively several decades ago with techniques of the age, but have not been done thence until today. We, therefore, performed a study using state-of-art techniques, on aerosols from human sneezing and an artificial aerosol-atomizing system and on the inactivation of the virus in the aerosol made by the latter system, under several atmospheric conditions. Viruses used in this study were A/WSN(H1N1), A/Aichi/2/68(H3N2), B/Lee and C/Taylor, all in the allantoic fluid except the last one in the amniotic fluid. The virus was atomized as aerosol by an electric nebulizer under the temperature and humidity from 15 to 30 degree C and from 30 to 70 % relative humidity, respectively, in a chamber room equipped with HEPA filtration system. The aerosol was collected using the electric impinger system or gelatin-filter membrane system, followed by virus titration. Sneezing of the volunteers were monitored with the Laser particle counter, and recorded with a digital high-speed video system, and analyzed by the image analysis method. Most of the mists from sneezing became air-borne particles of a few micrometer or sub micro meter- size and the large droplets disappeared within a half second, and the size population of these particles was very similar to that by the artificial atomizing system. Viability of the air-born virus was stable for a long time under certain conditions but decreased dramatically depending on the increase in temperature and/or relative humidity, suggesting that the control of the indoor environment like humidity and temperature may become effective means for the control of transmission of influenza if such active air-borne virus causes the infection. Ref: 1) Tellier R. EID 12, Nov. 2006. 2) Lemieux C et al. EID 13, Jan 2007.

Abstract P1224

The Genetic and Cellular Basis of Host Range Restriction Due to HA Receptor Binding

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Although avian influenza viruses replicate in the human airway, zoonotic and human-human transmission is rare without mutations that confer transmissibility. In primary cultures of human ciliated airway epithelium, the predominant cell types infected by avian influenza viruses are ciliated epithelial cells, whereas viruses that readily undergo transmission between humans infect predominantly non-ciliated cells thus providing a model system for predicting the transmissibility of viruses (Thompson et al. 2006). Differences in cell tropism have been attributed to the relative binding affinity of the haemagglutinin (HA) for host cell sialic acid residues (SA) with α2-3 or α2-6 linkages. Assessment of cell types susceptible to infection by avian influenza virus with variant HA proteins could identify which mutations enhance viral infection of non-ciliated cells and provide insights into the transmissibility status of evolving avian influenza virus strains. We have used a recombinant

baculovirus expressing H5 HA from A/Vietnam/1194/04 in insect cells and introduced mutations predicted to alter the receptor binding preference. Recombinant wild type and mutated H5 HA proteins were used in binding assays with synthetic polymeric carbohydrates that represent different SA bearing receptors. Mutations of H5 HA at amino acids 226 and 228, (H3 HA equivalent numbering), enhanced binding to the α2-6 SA on 6'SLN, but the level of binding achieved was much below that shown by H3 or H1 human HA proteins. Mutation of residue 228 alone enabled binding to selected α2-6 SA oligosaccharides included on a glycomic array whereas a S227N mutant showed a decrease in binding to 3'SL but did not show 'human virus'like levels of binding to 6'SLN. In a related reverse genetic approach, the α2-3 SA binding of a human H3N2 subtype influenza virus (A/Victoria/3/75) was increased by mutating HA residues 226 and 228. The ability of this virus to infect non-ciliated cells of human airway will be assessed as a predictor of the transmissibility of viruses that display avian virus-like receptor binding preference. Thompson et al. 2006. J. Virol. 80: 8060.

Abstract P1225

SCHOLARSHIP International Scholarship

Two-Phase Control of Apoptosis in Influenza Virus-Infected Cells by Up-Regulation of Akt and P53 Signaling

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PI3k-Akt and p53 pathways are known to play anti- and pro-apoptotic roles in cell death, respectively. Whether these pathways are recruited in influenza virus infection was studied here. Phosphorylation of Akt (Akt-pho) was found to occur early after infection (5-9 h.p.i). Nuclear accumulation and phosphorylation of p53 (p53-pho), and expression of its natural target p21/waf showed low constitutive expression levels at this period, whereas all three parameters were markedly elevated only at the late apoptotic stage (17-20 h.p.i.). Up-regulation of Akt-pho and p53-pho required virus replication and were accomplished by posttranslational mechanisms. However, p53 activation did not seem to play a leading role in influenza-induced cell death: (i) infection of CV1 and MDCK cells with recombinant NS1-deficient virus provoked accelerated apoptotic death characterized by the lack of p53 and Akt activation; (ii) virus-induced death developed in human bronchial H-1299 cells possessing a tetracycline-regulated p53 gene failed to depend on p53 gene activation by tetracycline. Virus-induced apoptosis and signaling of Akt and p53 developed in IFN-deficient VERO cells with similar kinetics as in IFN-competent CV1-infected cells indicating that these processes were IFN-independent. Apoptosis in influenza-infected cells was Akt-dependent and was accelerated by Ly294002, a specific inhibitor of PI3k-Akt signaling, and down-regulated by the viral protein NS1, an inducer of host Akt. The obtained data suggest that influenza virus (i) initiates NS1-dependent anti-apoptotic PI3k-Akt signaling at early and middle phases of infection to protect cells from fast apoptotic death, (ii) provokes both p53-dependent and alternative p53-independent apoptotic and/or necrotic cell death at the late stage of infection.

Abstract P1226

Biochemical Characteristics of Replication of Clinical Influenza Viruses in Human Intestinal Cell Culture CACO-2

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Influenza A viruses isolated from the respiratory tract of patients with influenza were cultured in human intestinal epithelium cells (CACO-2 line). The CACO-2 cells were found to be 100-fold more susceptible to the clinical viruses than MDCK cells and chicken embryos. On passaging in CACO-2 cells, clinical isolates of the subtype H3N2 retained the original "human" phenotype and agglutinated human but not chicken erythrocytes, whereas on passaging in MDCK cells the virus phenotype changed to the "avian" one. On comparison with laboratory strains (grown in chicken embryos or MDCK cells), the clinical viruses were characterized by higher stability of the anti-interferon protein NS1 but had a reduced synthesis of the matrix protein M1, and this could facilitate the virus adaptation and escape of the infected cells from immune attack in the human body. The increased tropism to the human CACO-2 cells correlated with higher adsorption of the clinical viruses on cellular receptors. However, in the CACO-2 and MDCK cells the ratio of sialyl-containing receptors of the 2-3 and 2-6 type was similar. These observations indicated that not only sialic acid residues were involved in the adsorption and penetration of the clinical viruses into human cells, but also the protein moiety of the cellular receptor itself and/or an additional cell coreceptor. Thus, clinical influenza viruses are shown to possess a specific mechanism of sorption and entry into human epithelial cells, which is responsible for their higher tropism to human cells and is unlike such a mechanism in canine cells.

Abstract P1227

Toll-Like Receptor 7 (TLR7) Genomic Locus of Ducks

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Toll-like receptors (TLRs) are a family of innate immune receptors that recognize conserved microbial patterns, triggering an inflammatory response. TLR7 and TLR8, in particular, recognize antiviral compounds and single-stranded RNA, and have been implicated in the immune response to viruses such as influenza. Some strains of influenza can be highly pathogenic in the chicken, while infection is generally asymptomatic in ducks. Philbin et al. (2005) identified a functional TLR7 gene but genomic disruption of TLR8 in chicken, and PCR evidence that this disruption did not exist in ducks. We speculated that differences in the TLR7/8 loci could contribute to the differential susceptibility to influenza between chickens and duck. To investigate this, we have isolated a duck genomic fosmid clone containing an insert of approximately 40kb, which contains the TLR7 gene. We have sequenced the duck TLR7 and report 85% amino acid identity to the chicken TLR7. We have also sequenced 15kb downstream of TLR7 to assess the presence of TLR8. Duck TLR7 is intact, while we can detect only fragments of the TLR8 gene, similar to chicken. We have examined the expression of cytokines and interferon alpha in response to stimulation of duck splenocytes and peripheral blood mononuclear cells with TLR7 ligands. We speculate that the function of TLR7 to elicit an interferon response differs between ducks and chickens.

Abstract P1228

Is H5N1 Influenza Virus A Novel Etiological Agent in the Development of Post-Encephalitic Parkinsonism?

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Influenza viruses essentially cause respiratory disease in humans, but occasionally the brain can be affected. During the great influenza pandemic of 1918, approximately 25-30% of the world population was infected. Starting in 1920, an unusual epidemic of neurological diseases was observed worldwide. Circumstantial and epidemiological evidence links several movement disorders, including post-encephalitic Parkinsonism (PEP), to the 1918 influenza pandemic; however, a causal link was never formally proven. Recent research suggests that the 1918 virus could have arisen directly from an avian influenza virus. This finding is worrisome considering the current global spread of H5N1, an avian influenza virus with considerable pandemic potential. H5N1 viruses can be neurotropic in different animal species- including mammals. If H5N1 were to cause the next human influenza pandemic, we fear that another epidemic of PEP could ensue. Currently we are investigating if infection with avian H5N1 influenza viruses can either directly or indirectly lead to neurodegeneration in the brain, including in the substantia nigra, the main structure that is lost in Parkinson's disease. Our specific aims are to 1) determine the time-course for H5N1 virus infection in the brain of mice after intranasal inoculation and 2) empirically determine if H5N1 virus infection results in a loss of neurons, concentrating on neuronal populations associated with Parkinsonism. Seven-week old female C57/BL6 mice were inoculated intranasally with 10^2 EID_{50} of virus A/Vietnam/1203/2004 (H5N1). At 1, 3, 7, 10, and 22 days post-infection, mice were transcardially perfused with formalin and brains were harvested. After fixation, brains were cryoprotected and serially-sectioned in the coronal plane. Immunohistochemical (IHC) detection was used to map the presence of H5N1 virus in the brain over time. H5N1 virus was detected in neurons throughout the brain with high levels seen in the olfactory bulb, midbrain, subthalamic nuclei and pontine nuclei in the brainstem. Activated caspase-3, a marker of apoptotic neurons, was seen in these same areas, indicating virus-associated neuronal cell death. Both brainstem and pontine nuclei are involved in motor control. These results indicate that H5N1 virus infection can directly lead to neurodegeneration in brain sections that could be linked upstream or downstream to PEP.

Workshop Topic #13

Clinical Guidance and Policies

Poster Abstracts

Abstract P1301

Influenza Vaccination Coverage Rate and Perceptions in Season 2005-2006 in Korea

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Objectives: To investigate influenza vaccination coverage rate and perceptions on vaccination in general population of Republic of Korea. Methods: Telephone surveys were performed to caregivers of children/adolescents of age 6 months to 18 years and adults of age > 18. Demographic data, history of vaccination in season 2005-2006, and perceptions on vaccination were asked. Results: 2018 care-givers of children/adolescents and 2017 adults responded and people in high risk group, defined as either having chronic illness or at the age of 6 months to 23 months in children and >= 65 in adults, were 242 (12.0%) and 475 (23.6%) in children and adults, respectively. Influenza vaccine coverage rate was 51.6% in total children/adolescents and 49.2% in high risk group. The most frequent reason for vaccination was to prevent influenza (90.2%) and the most frequent reason for non vaccination was perception of good health and there for do not need vaccination (54.6%). 27.7% of children/adolescents were recommended to receive vaccination while 28.0% in high risk group was recommended. 69.7% of caregivers of children/adolescents thought vaccine to be efficient and 67% thought vaccine to be safe. Influenza vaccination coverage rate in adults was 32.9% and in high risk group, 64.0%. 18.9% of adults were recommended to receive vaccination while 25.7% of adults in high risk group were recommended. The most frequent recommenders were friends and relatives (31.9%). 70.7% of total adults thought vaccine to be efficient and 66.7% thought vaccine to be safe. Influenza vaccination coverage rate in adults was 32.9% and in high risk group, 64.0%. 18.9% of adults were recommended to receive vaccination while 25.7% of adults in high risk group were recommended. The most frequent recommenders were friends and relatives (31.9%). 70.7% of total adults thought vaccine to be efficient and 66.7% thought vaccine to be safe. Conclusions: Influenza vaccine coverage rate is relatively high in Korea but it needs to be improved in high risk groups of children/adolescents. The rate of vaccine recommendation is low in all groups. Since most people agree on the efficacy and safety of influenza vaccine, health care workers should actively encourage and recommend vaccination to high risk groups.

Abstract P1302

Influenza Vaccination Coverage Rate and Perceptions of High Risk Group in Season 2005-2006 in Korea

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Objectives: To investigate influenza vaccination coverage rate and perceptions on vaccination of people in high risk group in Republic of Korea. Methods: Patients with diabetes, chronic renal failure and undergoing hemodialysis, and asthma were surveyed. Individual interviews were performed to patients with diabetes visiting endocrinology department in a university hospital, patients undergoing maintenance hemodialysis in 4 local clinics and 2 university hospitals. Questionnaires were mailed to patients registered to have asthma in a university hospital were asked to mail back the questionnaire. Demographic data, history of vaccination in season 2005-2006, and perceptions on vaccination were asked. Results: 506 diabetes patients, 260 chronic kidney disease patients and 123 asthma patients were analysed. Influenza vaccination coverage rate was 37.5% in diabetes patients and statistically significant factors associated with vaccination were as follows: higher age group, influenza vaccination recommended, perception on efficacy of vaccination. 13.9% of patients were recommended to receive influenza vaccine and the most frequent recommenders were doctors (43.6%). 61.3% of respondents believed in the efficacy of influenza vaccine and 61.5% believed in the safety. Influenza vaccination coverage rate was 81.9% in patients undergoing hemodialysis due to chronic kidney disease and statistically significant factors associated with vaccination were as follows: influenza vaccination recommended, perception on efficacy of vaccination. 73.7% of patients were recommended to receive influenza vaccine and the most frequent recommenders were doctors (45.3%) and nurses (43.6%). 77.3% of respondents believed influenza vaccine to be efficient and 75.4% believed it to be safe. 70.7% of patients with asthma received influenza vaccination. 46.3% were recommended to get vaccination and the most frequent recommenders were doctors (28.5%). 69.1% of respondents thought vaccine to be effective and 67.5% thought vaccine to be safe. Conclusions: Influenza vaccination coverage was considerable different among different chronic illness. The rate was relatively high in patients with diabetes and chronic kidney disease while it was low in patients with diabetes. The rate of vaccine recommendation is low in all patients and active recommendations by health care workers should be made.

Abstract P1303

Influenza and the Health Care Workforce: Identifying Barriers and Facilitators to Vaccine Uptake in British Columbia

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Introduction: Vaccinating health care workers (HCWs) reduces their personal risk of influenza and the probability of transmitting influenza to patients. Annual immunization rates among HCWs remain low despite promotional campaigns and provincial and workplace policies focusing on immunization as a duty of care. Few studies have explored the influence of workplace systems, culture, and environmental factors on HCW immunization decision-making. The objective of this qualitative study was to explore the contribution of both individual- and system-level factors influencing HCW influenza immunization. Methods: A semi-structured interview guide was developed to explore determinants and deterrents of influenza immunization. HCWs were selected from health care facilities across BC. Facilities were chosen to solicit input by facility type (acute/long-term care), size (large/small), location (urban/rural), and historic immunization coverage (high/low vaccine uptake). Program managers were also interviewed. Data were collected through 20 to 30 minute telephone interviews. Results: 103 HCWs from 12 facilities were recruited, including 79 vaccinated and 24 non-vaccinated staff. Eleven of 12 program managers were interviewed. The dominant theme emerging was that the decision to be immunized is primarily personal. Three sub-themes within the personal domain were identified as key influencers: 1) perceived personal health risk or benefit (self or family), 2) workplace policy as it overlaps with personal considerations (ability to work and be paid) and, 3) access to in-depth education, preferably one-on-one by an independent and knowledgeable expert. Conclusions: Further research should explore in greater depth the dominant personal theme that emerged in this study. Specific strategies incorporating HCW perspectives should be developed with individual-level education and personal benefits emphasized over mass promotional and patient-centred campaigns. Direct benefits to HCWs and their families need to be highlighted. Local expert champions and trusted opinion leaders ("train-the-trainer") may best address ongoing HCW questions and concerns.

Abstract P1304

Intention to Receive, and Uptake of, Influenza Vaccine by Immunization Providers

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Introduction: Health care workers are recommended to receive annual influenza vaccine to protect themselves and to reduce transmission of influenza to their patients. The objectives of this study were to determine 1) the proportion of immunization providers (nurses and physicians) who intend to receive and who have received influenza vaccine; and 2) factors associated with intention to receive, and uptake of, influenza vaccine by immunization providers in BC. Methods: A cross-sectional postal survey based on the Theory of Planned Behaviour was sent to all immunization providers in BC. Associations between intention to receive influenza vaccine and reported receipt of influenza vaccine and variables of interest were determined. Those achieving a significance of 0.1 or greater on univariate analyses were modeled using logistic regression. Results: Completed surveys were received from 335 nurses and 343 physicians (67% and 22% response rate respectively). Overall 89% (95%CI:86-92%) of respondents reported intending to receive the influenza vaccine every year; 78% (95%CI.74 -.82) reported they had received the vaccine =/>75% of the time. Those with positive direct attitudes (reported receiving the vaccine themselves to be important, beneficial and good) and direct social norms (feel they should receive the vaccine) compared to those without were 8.3 and 6.2 times respectively more likely to intend to receive influenza vaccine and 2.5 and 3.2 times more likely to report receiving the vaccine. Being a physician or a nurse and indirect social norms (opinions of colleagues and employers) was not related to intention or behaviour regarding influenza vaccine uptake. Conclusions: Almost 90% of immunization providers intend to receive annual influenza vaccine. Although uptake was closer to 80%, this rate is higher than found in health care workers in acute and long-term facility settings. Positive attitudes towards receiving the vaccine themselves are important. Health promotion initiatives should target individual perceptions as opinions of others may have little effect.

Abstract P1305

Influtec®–An Update on the Online Calculation Tool for the Assessment of the Cost-Benefits of Influenza Vaccination in the Workplace

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Background: In the healthy working population, influenza infections cause absenteeism from work and less productivity at work each winter. Although this influenza-borne absenteeism is associated with significant economic loss to industrialised economies every year, is not easily quantifiable for individual companies. Often the losses are underestimated and the economic potential of offering free-of-charge influenza vaccination to employees remains unrecognized. However, not only the employers' willingness to provide an influenza vaccination workplace program is pivotal; also the employees' motivation to get vaccinated–which may rest on the clarification of doubts and fears around efficacy and safety of influenza vaccination–is crucial for its success. Objectives: (1) To offer a user-friendly, web-based calculator for the country- and company-specific assessment of the cost-benefits of influenza vaccination in the workplace; (2) To increase the usefulness of Influtec® by providing pertinent literature, references and support materials to occupational health physicians, human resource managers and any individuals responsible for the management of staff shortages and productivity losses during the influenza season. Material and Methods: The decision-analytic model Influtec® enables the quantification of the "lost productivity" caused by influenza in any particular company in any of 18 country settings offered. Default values for the calculation have been collected from original research, systematic reviews and public databases. Allowance for company and country-related inputs has been included in order to individualize the assessment further. In a members-only section of Influtec®, information and support material is provided; especially sources and mathematical assumptions used in Influtec®, as well as non-economic arguments to motivate employees to be vaccinated. Results: The model calculates the following outputs: number of employees falling ill and productive days lost during a flu outbreak; cost and savings due to the vaccination program from the company perspective; cost-benefit ratio ("return on investment") of the vaccination program. The additional support can be accessed in the members-only section after a short registration procedure. Discussion: The default input values require regular updating and the results call for interpretation within the company and country-specific context. In order to realize the reduction of productivity losses, employers should inform the employees about the proven efficacy and safety of influenza vaccination which will lower the "barriers" to get vaccinated. Conclusions: The cost-benefit calculator Influtec® offers a rigorous and transparent assessment of the health and economic benefit of influenza vaccination of employees. The updated members-only section provides information and support for both employers and employees.

Abstract P1306

Clinical Effectiveness of Zanamivir for Both Influenza A and B Patients

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Background: We reported that oseltamivir was less effective against influenza B than against influenza A. Because, the effectiveness of another neuraminidase inhibitor, zanamivir, has not been compared for these infections, we investigated its effectiveness for the patients with influenza A and influenza B infection in the 2001/2002, 2002/2003, 2003/2004, 2004/2005, and 2005/2006 seasons. Patients and Methods: Family doctors, pediatricians, and physicians at local clinics participated in the study. Patients reporting with influenza like illness manifesting symptoms such as body temperature over 37.5 C, upper respiratory tract symptoms, and systemic symptoms were diagnosed as influenza A or B by commercial antigen detection kits. Influenza patients who received zanamivir within 48 hours of onset were enrolled for study with informed consent. The effectiveness of zanamivir in the treatment of 232 patients with influenza A and 105 patients with influenza B infection were analyzed. The date and time of the first inhalation of zanamivir and the disappearance of fever were recorded by the physician, the patient, or a family member. The time at which a body temperature of under 37.5 C was attained was defined the time the patient became afeblile. Virus isolation was done with throat swabs by standard methods using Madin-Darby canine kidney cells. Results: The mean duration of fever from the first inhalation of zanamivir was 32.1 hours in the patients with influenza A and 35.9 hours in the patients with influenza B. There was no significant difference between the patients with influenza A and influenza B. The mean duration of fever from the first inhalation of zanamivir was 23.7 hours in patients with influenza A/H1N1 and 33.3 hours in patients with influenza A/H3N2. The rate at which patients became afebrile at 18 hours after the first inhalation was 42.2 % for patients with influenza A and 35.2 % for patients with influenza B.

The respective frequencies of patients afebrile at 42 hours after the first inhalation were 81.0 % and 80.0 %. Discussion: In our previous study, the duration of fever after the first dose of oseltamivir was 31.2±23.7 and 47.1±30.8 hours in patients with influenza A and influenza B, respectively. In this study for zanamivir, the duration of fever and the percent of patients afebrile at 18 and 42 hours were not significantly different for influenza A and influenza B. The reported difference of the mean IC50 between the influenza A and B viruses was less for zanamivir (2.09 nM vs 4.15 nM) than for oseltamivir (0.73 nM vs 11.53 nM). These findings may explain our results showing that oseltamivir is less effective against influenza B infection than against influenza A and that zanamivir is equally effective against both. Conclusion: Zanamivir is equally effective for both influenza A and B.

Abstract P1307

A Retrospective Study of Mortality Following Influenza Infection Among Tamiflu Users

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Background: Influenza causes substantial numbers of deaths each year, primarily as a result of respiratory and cardiovascular complications. Vaccination is the primary approach to preventing influenza and its complications, and has been shown to decrease all-cause mortality during influenza seasons in the elderly and high-risk populations by 50% or more. Vaccination is limited, however, in that not all at-risk individuals choose to be vaccinated. Because the vaccine required for each year's influenza season takes several months to develop, the circulating influenza virus must be correctly identified long in advance of the onset of each year's epidemic, and inadequate supplies cannot be quickly remedied. In spite of the accumulation of evidence for the protective effects of oseltamivir against influenza complications, little evidence has been presented regarding the potential to reduce mortality associated with influenza. Clinical trials contain insufficient numbers of subjects to detect an effect, and they typically exclude those with underlying health conditions that place them at high risk of dying. In a small study conducted in nursing homes, an 85 percent decrease in risk of death associated with oseltamivir use was observed. In order to determine whether this finding holds in the general, non-institutionalized population, we conducted a large observational study with the ability to adjust for patient characteristics that may be associated with differential probability of both oseltamivir use and death. We used data from a large US health care insurer to perform a retrospective cohort study of oseltamivir use and mortality following a diagnosis of influenza, employing propensity score techniques to achieve balance between cohorts at baseline. Secondary outcomes investigated included two of the primary complications of influenza that can lead to death: pneumonia and myocardial infarction (MI). Methods: This retrospective cohort study used US health insurance claims data. Patients at least one year of age with at least 6 months continuous enrollment in the health plan were grouped into two cohorts: 1) those diagnosed with influenza (ICD-9 487.XX) at a physician visit and dispensed oseltamivir (n=60,267 individuals, 60,834 incident cases); and 2) those with the same diagnosis and no antiviral treatment (175,933 individuals, 183,786 incident cases). The study period ranged from November 1, 1999 through April 30, 2005. Individuals who were hospitalized on the day of diagnosis were omitted from the analyses. The core analysis involved creating strata (quintile) of a propensity score, a summary measure of factors that tended to distinguish oseltamivir recipients from other persons with a diagnosis of influenza. Within strata of the score, users and nonusers have very similar covariate distributions. To create the propensity score, we examined numerous a-priori variables including age, sex, health plan region, month and year of index date, as well as number of hospitalizations, home health services, and vaccination for influenza and pneumococcus during the six months prior to the index date (the baseline period). Health status variables from the baseline period included codes for the following conditions associated with either a physician visit or hospitalization discharge: diabetes, cancer, HIV, bronchitis, chronic obstructive pulmonary disease, asthma, hyperlipidemia/hypercholesterolemia, hypertension, cardiac conditions other than MI, stroke/transient ischemic attack, pneumonia, and MI. We also included general measures of healthcare utilization such as total medical costs, number of physician visits, and number of different prescription drugs received, and empirically derived variables obtained from the most common diagnoses, procedures, and drugs in the cohorts from the six months prior to influenza diagnosis. Each patient was assigned a predicted probability of starting oseltamivir, as estimated by the logistic regression model. The propensity score quintile was used in all analyses as a stratification variable and its components were entered into all outcome models to check for residual confounding within strata. Outcome measures: Outcomes included death within 30 days of the index influenza diagnosis from: 1) respiratory or circulatory causes; 2) all causes; 3) pneumonia or influenza. Secondary outcomes included a diagnosis of myocardial infarction or pneumonia. Results: There were 2 deaths from respiratory or circulatory causes in the treated cohort; 31 in the comparison group. There was no demonstrable difference in respiratory or circulatory deaths between the groups (odds ratio 0.38, 95% confidence interval 0.08-1.68) or any of the other outcome measures. There were

4 deaths from any cause within 30 days of the index date in the treated cohort; 41 in the no-antiviral cohort. All 13 of the deaths due to pneumonia or influenza occurred in the no-antiviral treatment cohort. Directionally, each of the estimates showed protective effects for oseltamivir. There were 3,339 cases of pneumonia observed in the 30 days following the index date (738 in the oseltamivir group, 2,601in the no-antivirals group). There were 43 myocardial infarctions in the same period (10 in the oseltamivir group, 33 in the no-antivirals group). There was no difference between the groups for the secondary outcomes, myocardial infarction (odds ratio 1.48, 95% confidence interval: 0.68 – 3.21) or pneumonia (odds ratio 1.01, 95% confidence interval: 0.92 – 1.11). Discussion and Conclusion: In this retrospective cohort study we found that the risk of death due to respiratory or circulatory causes in the period 30 days after the diagnosis of influenza was suggestive of a protective effect for the group treated with oseltamivir compared to the group not treated with antiviral medications. However, the confidence intervals were broad and the results did not achieve statistical significance. We also found that there was no statistically significant difference between these groups for the risk of death from any cause during the same period. However, for both outcomes, there were substantially fewer deaths in the oseltamivir group. The small number of events reduced the statistical power and may have prevented the detection of a protective effect.

Abstract P1308

Influenza Vaccination in Workers of a University Hospital 2005-2006

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Introduction: Administration of influenza vaccine to healthcare workers decreases morbidity and mortality from influenza at hospitals. Objective: Assess the coverage of the 2005-2006 vaccination campaign and the characteristics of staff vaccinated at Hospital Universitario 12 de Octubre. Material And Methods: A descriptive study. Hospital staff database was consulted to collect variables such as date of birth, sex, professional group, department, and influenza vaccination. The number of workers was 6443. Workers receiving influenza vaccine during the 2005-2006 campaign were considered vaccinated. Results: Vaccine coverage was 42.2% (2717), with no significant differences by sex (40.7% and 39% for males and females respectively) or between healthcare (physicians, nurses, auxiliary staff, etc.) and non-healthcare staff (porters, maintenance staff, etc.) (39.7% and 38.4% respectively). More workers were vaccinated in the group aged ≥ 44 years as compared to the group aged < 44 years (42.5% and 36.1% respectively, p<0.001)). Among healthcare staff, the highest vaccination rates were found in resident intern physicians (55.1%) and staff physicians (47.2%), while vaccination rates lower than the mean rate were seen for nurses (37.1%) and nursing auxiliary staff (33.4%). Healthcare departments considered to be of risk for patients showing a greater vaccine coverage were the HIV unit (71.4%), heart transplantation (50%), and hematology-BMT (38.5%), while the lowest coverages were found in departments such as radiotherapeutic oncology (24.7%), medical oncology (27.5%), and neonatology (27.4%). Vaccine coverage increased 38.4% as compared to the 2004-2005 campaign (30.5%). Conclusions: Influenza vaccine coverage (42.2%) in our hospital staff during the 2005-2006 campaign was higher among staff and resident intern physicians, while values lower than the mean coverage were found in nursing staff, nursing auxiliary staff, and workers from some of the departments where patients are considered to be at a greater risk (oncology and neonatology). This type of studies are helpful to increase influenza vaccine coverage in future vaccination campaigns.

Abstract P1309

Oseltamivir for Prevention of Seasonal Influenza in Children

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The impact of influenza in children is often underestimated. The disease course is often severe, typically with nausea and vomiting, and the risk of complications, such as febrile seizures and otitis media, high.[1] Attack rates from influenza are also higher in children than adults, as is the rate of viral shedding, making children a prime vector for the spread of disease in the community.[1] Preventing influenza infection in children is therefore an important health goal. Oseltamivir is an oral neuraminidase inhibitor, and has been shown to be effective against all strains of influenza so far tested, particularly when given early after symptom onset. By inhibiting viral infectivity and spread, the drug significantly reduces the severity and duration of influenza symptoms and the frequency of complications.[2] Oseltamivir, administered twice daily for 5 days, is currently approved for the treatment of influenza A and B infection in children older than 1 year. It is also approved for the prophylaxis of influenza in individuals exposed to infected contacts (post-exposure prophylaxis; once daily dosing for 10 days). A phase IIIb study is now under way to investigate a 6-week course of oseltamivir for the seasonal prophylaxis of

influenza in children up to 12 years of age. The study is being run in approximately 8–10 centers in the USA, Canada and Europe, using an open-label design. Children aged 1 to 12 years are eligible for entry into the study if, in the investigator's judgment, they, or someone in their household, are at risk of influenza infection or its complications. Approximately 50 children will be recruited during a period when influenza is known to be circulating in their community (as of 20 February 2007, 17 boys and 20 girls, aged 1.6 to 12.3 years, have been enrolled into the study). Children entering the study must be free from influenza at study baseline, based on a rapid diagnostic test and the absence of influenza-like symptoms. Participants will receive oseltamivir 30–75mg once daily according to body weight, either as a suspension (12mg/ml) or, for some children weighing >40kg, in capsule form (75mg). The prophylactic period is 6 weeks, with an additional 4-week follow-up. Safety in the study will be evaluated by adverse event incidence, vital signs and physical examination results, as well as serum chemistry and hematology results. Efficacy endpoints are the proportions of participants with either laboratory-confirmed clinical influenza (i.e. positive viral culture, a 4-fold or greater rise in antibody titer, fever, cough and coryza) or influenza (confirmed infection but no symptoms), and the proportions with asymptomatic influenza or influenza-like illness. Influenza symptoms and temperature will both be recorded on daily diary cards. Laboratory confirmation of infection will be by reverse transcriptase-polymerase chain reaction and viral culture of nasal and throat swab eluates, and measurement of hemagglutination inhibition titer in serum. Preliminary data are expected to be available in Q2 2007. References: 1. Heikkinen T. Acta Paediatr. 2006;95:778–84; 2. Oxford J. Expert Opin Pharmacother. 2005;6:2493–500.

Abstract P1310

The Impact of Rapid Antigen Testing for Influenza on Paediatric Hospital Admissions in a Large UK Emergency Department: A Retrospective Observation

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Introduction: Hospitalisation admission rates due to influenza in children pose significant demands on paediatric healthcare resources. As the majority of febrile children assessed in secondary care are managed without knowledge of the aetiology of the illness, influenza-related assessments may lead to unnecessary admission, investigations and treatment as opposed to outpatient treatment with antivirals. During a treatment study, we introduced influenza rapid antigen tests into the emergency units at Leicester Childrens' Hospital and observed a reduction in admissions compared to previous years. Methods: Leicester Childrens' Hospital typically assesses over 3000 children with acute respiratory illness during a winter season. Age-specific influenza-related assessment and admission rates are known from prospective surveillance studies using PCR detection conducted in 2002/03 and 2003/04. During 2005/06 and 2006/07, we introduced influenza rapid antigen tests to the same unit to identify subjects eligible for an oseltamivir treatment study. No children were excluded due to severity of illness. The rapid test result was recorded in the case-notes. The emergency paediatric admission team was responsible for all aspects of clinical management; the study team had no role in clinical decisions other than prescription of oseltamivir. Results: During 2002-03, although there was low national activity, influenza was responsible for 5-6% of all childrens' admissions. Overall, there were 14 (42%) admissions of 33 children who were later diagnosed to have influenza infection. During 2005/6, 55 children presenting to the same unit were positive by rapid antigen test. Two (3.6%) were hospitalised. The remaining 53 were discharged; 47 received oseltamivir. In 2006/07, 59 children were positive by rapid antigen test. Three (5%) were hospitalised. The remaining 56 were discharged; 22 received oseltamivir. Discussion: This suggests that early diagnosis and availability of antiviral therapy enables clinicians to safely discharge febrile children with influenza presenting to secondary care. This reduces hospital admissions among influenza-infected children decreasing the potential for nosocomial transmission and unnecessary invasive investigations. Formal randomised cost evaluation programmes of rapid antigen testing are needed.

Abstract P1311

Can Pharmacists Diagnose Influenza? Assessing the Accuracy of Influenza Diagnosis in the Community Pharmacy Setting

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Background: The British Department of Health is encouraging increased involvement of pharmacists in the provision of selected primary care services. Aim: To assess the accuracy with which community pharmacists are able to make a diagnosis of influenza. Design: Cross-sectional study. Pharmacists'

diagnoses of influenza in patients presenting to the pharmacy were compared with the results of nasopharyngeal swab PCR virology. Subjects and Setting: 22 community pharmacists in the UK. Results: In 217 subjects, pharmacists diagnosed influenza in 53 and of these 27 were confirmed virologically (positive predictive value 51%). A raised temperature measured at presentation did not significantly improve diagnostic accuracy. 21(40%) of the 53 confirmed influenza cases and 32% of all subjects presented within 48 hours of symptom onset. In subjects presenting within 48 hours a total symptom score exceeding 10 (39 subjects) was associated with confirmed flu in 38%, compared with a total score less than 10 (30 subjects) associated with confirmed influenza in only 20%. Conclusion: Despite a low level of circulating influenza over the study period, community pharmacists achieved a level of accuracy in diagnosing influenza (51%) which compares favourably with results achieved in clinical trials of neuraminidase inhibitors where recruitment was limited to early presentation. The data suggest that pharmacists could play an extended role in the management of seasonal influenza. Most patients did not present early enough to benefit from treatment with antiviral drugs. There is a need for better public understanding of the benefits of these drugs.

Abstract P1312

Seasonal Influenza Vaccination Coverage Rates and Drivers in 10 Countries from Africa, Asia Pacific, Europe, Latin America, and the Middle East

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Objective: To ascertain age-group-specific seasonal influenza vaccination coverage rates (IVCRs) in ten countries in relation to: local recommendations, immunisation programmes (IPs), and regional awareness of influenza severity. Method: Non-institutionalised individuals representative of the general population were interviewed in China, South Korea, Taiwan, Poland, Romania, and Turkey during February-April 2006; and Australia, Argentina, Chile and South Africa during August-October 2006. Interviewees included adults (18-64 years), elderly (65+ years), and children (0-17 years). Children were subcategorized as 0-3 years, 4-8 years and 9-17 years. Results: 24 400 individuals (16 983 adults and elderly, 7417 children) were surveyed. Global IVCRs were highest in Korea. In countries with fully reimbursed or free IPs, global IVCRs were 14-41%; without such IPs, IVCRs were around 10%. National IPs for the elderly were frequently associated with high IVCRs among the elderly (37%-74%), as well as apparently increased IVCRs among adults. Strong national recommendations for paediatric immunisation were associated with high IVCRs in children (23-62%). Vaccination recommendations or IPs for 6-23-month-old children were frequently associated with high IVCRs among 0-3-year olds (40-60%), as well as apparently increased IVCRs among 4-8-year olds (25-83%). Conclusion: Global and age-specific seasonal IVCRs varied across countries, with higher IVCRs generally observed in countries with ongoing recommendations or IPs. International and age-related differences may be partly attributable to disparities in the economic and healthcare-system maturity in relation to seasonal influenza perception and vaccination.

Abstract P1313

Influenza Antiviral Prescribing Practices in the United States, 2004 and 2005

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Background: During the fall of 2005, there were reports of personal stockpiling of oseltamivir for use during a possible influenza pandemic. We undertook this study to examine if stockpiling was occurring, to understand the magnitude of the practice, and to discern who was receiving and prescribing these drugs. Methods: We analyzed a pharmacy benefits manager database serving more than 50 million members in the US. Cumulative rates of oseltamivir prescriptions per 100,000 enrolled members (member rate) and per 1000 prescribing physicians (MD rate) were calculated by member and physician characteristics for weeks 36–44 (October and November) of 2004 and 2005 when influenza activity was very low. Rate ratios (RR) and 95% confidence intervals (CI) were calculated. Results: Member rates of filled oseltamivir prescriptions increased significantly from 27.3 in 2004 to 133.6 in 2005. Women were significantly more likely to receive oseltamivir than men in both 2004 (RR 1.2; CI 1.16, 1.24) and 2005 (RR 1.07; CI 1.05, 1.09). Among age groups, the highest prescription rates in 2004 were to persons ≥65 years (member rate 69.0), and the highest rates in 2005 were to persons 50–64 years (member rate 211.3). Members <18 years had greater than a 7-fold increase in prescription rates from 2004 to 2005. General internists had the highest rate of oseltamivir prescribing in 2004 and 2005 (MD rate 4.7 and 19.7, respectively). The highest significant rate increases from 2004 to 2005 were seen in pediatric subspecialists, pediatricians, and infectious disease specialists (RR 11.5, 10.5, and 7.1 respectively). MD rates were highest among MDs with ≥ 20 years since medical school graduation. Conclusions: In late fall 2005 a significant increase in filling of oseltamivir prescriptions occurred in the US during a period of little influenza disease

activity and suggests personal stockpiling. Knowledge of who is prescribing and receiving oseltamivir can be used to target guidance about the use of oseltamivir in inter-pandemic periods.

Abstract P1314

Reliability of Reports of Current-Season Influenza Vaccinations Among Caregivers of Children Aged 6-23 Months

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Background: Validity of caregiver recall of recommended childhood vaccine administration has been studied, but few data are available concerning same-season influenza vaccinations. To assess reliability, we compared caregiver recall of children's influenza vaccine histories with provider records. Methods: Data were obtained from an assessment of trivalent influenza vaccine effectiveness in 6 localities, conducted by the Emerging Infections Program among children aged 6-23 months hospitalized with laboratory-confirmed influenza during the 2005-2006 influenza season and their age- and residence-matched controls. Vaccination history data were collected from caregivers via telephone interview and from primary care providers by mail or fax. Results: Seventy-two percent of 207 caregivers provided an accurate count of doses their children had received in the current season as compared with provider records (sensitivity: 1 dose=75%, 2 dose=65%; specificity: 1 dose=78%, 2 doses=88%). Of 43 caregivers who recalled that their children had 2 doses, only 51% (22) were verified based on provider records. Sixty-one percent (49) of caregivers accurately recalled that their child received a single dose, and 95% (70) reported correctly that their child was unvaccinated that season. Caregivers with inaccurate recall were more likely to overestimate (21%) than underestimate (7%) doses received. Caregiver accuracy decreased with time between vaccination and interview: caregiver dose counts were accurate 83% of the time when the last dose was administered <150 days before interview, 70% when doses were given 150-249 days prior, and 67% when doses when administered ≥250 days ago. Many caretakers recalled doses being administered but could not recall dates. Providers were more consistent at supplying dose dates; providers supplied dates for 95% (126) of the doses given, compared with only 5% (6) supplied by caregivers. Conclusions: Overall, caregivers had a high level of recall. Accuracy of caregiver reports of same-season influenza vaccinations of young children decreased with time and number of doses, with caregivers more likely to overreport the number of doses.

Abstract P1315

Yearly Influenza Vaccination of Elderly: When do the French GP Vaccinate?

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Context: In France, in at risk people, large influenza vaccination (IV) rate is linked to the vaccination policy driven by the national health insurance service (CNAMTS). Each year, at the beginning of October, a voucher is sent to all the at risk people (around 8,6 million), allowing them to get a free IV during the last term of the year. General Practitioners (GP) are very involved in this vaccination campaign. During this period, the timing of the influenza circulation cannot be predicted as the virus can appear at every moment; moreover, around 15 days are necessary to obtain an effective protection after IV which must therefore not be delayed. However, the timing of this IV is still not well known as current epidemiological information and level of knowledge about length of vaccine protection seems to influence it. Objective: To describe, in general practice, the timing of prescription and use of IV in people over 64 years in France. Methodology: Retrospective descriptive study (2003-2006) of information on IV available in the French OMG data base, a computerized network of GP coordinated by the SFMG. The data analysis is interpreted taking account of available epidemiological and media information collected by the French influenza surveillance network (GROG). Final analysis is still in process. Results: Data from 121 GP and 7.900 consultations for IV (65 IV /GP/year) are available. As a first analysis (2003-2005), in people over 64 years, median of IV seems to take place between weeks 43 and 44 (end of October), with no difference between the years. The early beginning of the influenza circulation (2003) or the wide communication campaign about avian flu (2005), if they could have had a quantitative effect, don't seem to have modified the timing of IV in this age group.

Abstract P1316

Clinical Symptoms and the Reliability of Symptomatic Criteria in the Diagnosis of Influenza A and B Infection: A Japanese Multi-center Study Using Commercial Antigen Detection Kits

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Background: Commercial antigen detection kits have made it possible to easily differentiate influenza A and B infection. However, clinical symptoms or the reliability of symptomatic criteria has not been fully compared between influenza A and B infection. Method: The highest body temperature during influenza infection was compared between 1,408 influenza A and 737 influenza B patients in the 2002-2003 season. The reliability of the symptomatic criteria of the Ministry of Health, Labor and Welfare in Japan (J-criteria: presence of sudden onset, fever >38 ℃, upper respiratory infection and general symptoms) was evaluated in the 2005-2006 season (1,611 patients with influenza A and 310 controls), and criteria by Monto, et al (M-criteria) was compared between influenza A (2005-2006) and B (2004-2005 season; 2,479 patients with influenza B and 862 controls). M-criteria includes the presence of cough and fever ≥ 37.8 ℃ (or ≥37.2 ℃ in patients over 64 years). Capilia FluA,B or Capilia Flu A+ B was mainly used as commercial antigen detection kits. Results: Fever: The percentage of patients with fever >38℃ in the 0-6, 7-15, 16-64 and over 64 years age groups were 97.2%, 95.9%, 85.5% and 65.9% for influenza A, and 97.6%, 92.1%, 74.3% and 61.5% for influenza B, respectively. The percentage of patients with >38 ℃ was significantly lower for influenza B than A in adults ($p<0.001$). The percentage of patients with fever <37.6 ℃ was significantly higher for influenza B (8.3%) than for A (4.4%) in adults ($p<0.05$), and were 23.1% and 13.6%% in the elderly, respectively. Reliability of the symptomatic criteria: For influenza A, the accuracy was 65.9% by the J-criteria, and 75.6% by the M-criteria. The accuracy of the J-criteria was 62.5-69.8% for <65 years and 51.9% for > 64 years. The accuracy of M-criteria was 72.3-81.4% in each age group. For influenza B, the accuracy of M-criteria was 64.1% (60.2-67.0% in each age group). Conclusion: The reliability of J-criteria was lower than M-criteria especially in elders. And an accurate symptomatic diagnosis is rather difficult, and commercial antigen detection kits seemed to be rather necessary in influenza B compared with influenza A infection.

Abstract P1317

Mortality Benefits of Influenza Vaccination in Seniors: An Ongoing Controversy

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Influenza vaccination policy in most industrialized countries has attempted to reduce the mortality burden of influenza by targeting seniors ≥65 years for vaccination. However, the effectiveness of this strategy is under debate. Placebo-controlled randomized clinical trials (RCTs) in seniors are few and did not study populations ≥80 years, which suffer the lion's share of influenza-related deaths. One Dutch RCT found 57% vaccine efficacy in seniors 60-69 years for laboratory-confirmed influenza, but suggested a reduced response after age 70 years, consistent with immune senescence. Recent excess mortality studies set in US and Italian seniors were unable to confirm a decline in influenza-related mortality over time periods where vaccination coverage rose substantially. Paradoxically, while excess mortality studies attribute only ~5% of all US winter deaths to influenza (=~32,000 excess deaths/~600,000 total winter deaths among seniors), many cohort studies set in large managed care databases have consistently reported an implausible 50% reduction in the total risk of dying in winter in seniors—a benefit that far exceeds the influenza mortality burden. New studies, however, have demonstrated substantial unadjusted frailty selection bias in many cohort studies, by showing that the reduction in risk of all cause mortality in vaccinated versus unvaccinated seniors is actually greatest in early winter, before the onset of influenza season. Furthermore, adjustment approaches used in most cohort studies (including variables defined by ICD codes in analytic models) are not sufficient. We conclude that frailty selection bias and the use of highly non-specific endpoints (e.g. all-cause mortality) may explain the exaggerated vaccine effectiveness (VE) estimates consistently reported in cohort studies. To build a stronger evidence base, we propose an analytical framework to detect residual bias in cohort studies and suggest greater use of laboratory-confirmed endpoints, despite higher cost and complexity. Finally, we suggest revisiting the possibility of conducting RCTs in seniors, including trials comparing different doses and vaccine formulations. At this point, the evidence left standing points to benefits that are modest at best after age 70 years, leaving ample room for more immunogenic vaccines for seniors. Augmenting current strategies by vaccinating transmission populations might indirectly reduce senior influenza mortality.

Abstract P1318

Italian Guidance on Antiviral Use for Management and Prevention of Seasonal Influenza

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No international consensus exists on the recommendation for the antiviral use during seasonal influenza. The increasing incidence of Adamantine-resistant strains recently observed in the United States and in other Countries and recent data on Neuraminadese Inhibitors (NI) constitute new elements to consider for formulation of guidelines. Italian Society of General Medicine (SIMG), in conjunction with the Italian Societies of Hygiene, Preventive Medicine and Public Health (SITI) and Infectious and Tropical Disease (SIMIT) propose an update guidance on the use of these drugs to improve the positive effect of annual preventive vaccination in at risk subjects. Treatment of influenza and prophylaxis in subjects exposed to infection should exclusively consider NI, since Adamantanes are inappropriate for the considerable incidence of adverse effects and of resistant strains. As regard as the therapeutic indications, their use can be advised with three recommendations: (i) use should be confined to seasonal epidemic peak, defined by Influnet influenza surveillance system, when there is a probable match between clinical and etiological diagnosis, (ii) reorganisation is required to ensure that patients take the drug as early as possible, (iii) NI should be taken on medical advice after appropriate consultation. Antiviral treatment should be prioritised to patients at risk of complications, i.e. subjects≥65 years, patients with diabetes, COAD, cardio-vascular disease, renal failure, immunosuppression. The above categories should all be vaccinated before the epidemic season. However, the protective efficacy of vaccination is not 100% and a sub-optimal matching between the circulating and vaccine strain, usually recorded by the virological surveillance network, increases the risk of vaccine failure. In introducing antiviral prophylaxis in Italy, as an additional instrument of influenza control, their use should be limited to the seasonal peak period (treatment over the entire seasonal period appears currently inappropriate) and prioritised to high-risk subjects who have not been vaccinated (due to contraindications to immunisation or negligence) and to vaccinated subjects in whom there is reason to suspect a poor response to vaccination, especially if there is a documented mismatch between the circulating and vaccine strain. Chemoprophylaxis of healthy carers of high-risk subjects must be evaluated on a case by case basis.

Abstract P1319

Etiotropic Treatment of Influenza: 7 1/2 Years of Clinical Experience Using Neuraminidase Inhibitors

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"If you understand Influenza you understand virology completely". We have followed this guiding principle from the 1950s consistently in particular since Mark von Itzstein's discovery of neuraminidase inhibitors (Nature, 1993). During the past 7½ years we have diagnosed influenza in 188 patients using a rapid test (with subsequent confirmation by PCR) and started treating them antivirally immediately. Since January 1999 this approach spared all of the patient's severe complications and an admission to the hospital. According to N. HEIMBURGER's Leitmotiv that every infection acts via the vessels (the endothelium) and hence via coagulation, we objectivized our diagnosis through the humoral inflammation status. This led to the realization that the sudden onset is caused by the cytokine increase resulting from the virus load. These mediators are released upon the viral destruction of the host cell and cause the typical clinical picture with a physiognomy reflecting the fear of an imminent threat. More important than the fact that neuraminidase inhibitors only help within 48 hours is the realization that their use as early as possible prevents the threatening "cytokine storm". Compared to similar cases prior to January 1999, our group treated with neuraminidase inhibitors showed a reduction in the humoral inflammation status of 50%. The frequent assessment of viral infections and "colds" as banal and self-limiting leads to the sometimes fatal consequences of the resulting treatment. Moreover, the inadequate differentiation between colds and influenza results in an excessive and often harmful use of antibiotics. A Canadian study reported that antibiotics were prescribed for 84% of outpatients diagnosed with influenza (McGeer I., ICAAC 2006). The evaluation of our 188 influenza patients showed a reduction in the necessity of antibiotic administration to below 50%. Following an antibiotic treatment we prescribe probiotics with Semen psylii, in order to bring the mucosa of the small intestine back to normal. Our diagnostic objectivization has to be extended to toddlers and children because they are the fuel for all influenza epidemics.

Compared to the era prior to 1999, when we could only observe the viral damage and wait, today we are in a position to launch an offensive against it.

Abstract P1320

Influenza and *Staphylococcus aureus*: A Possibly Lethal Combination

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Mark von Itzstein's discovery of neuraminidase inhibitors (Nature, 1993) also advanced our understanding of the interaction between influenza viruses and bacteria on the cell surface. Hemagglutinin und neuraminidase in the viral coat ensure effective replication, and in this process hemagglutinin is important for entry into the host cell. A protease secreted by Staphylococcus aureus (S. aureus) increases the adhesion of hemagglutinin to the host cell's receptor. We have often observed foudroyant and aggressive course with the simultaneous occurrence of influenza A virus und S. aureus. The rapid virus replication leads to an intense cytokine reaction ("cytokine storm") and thus to the dramatic clinical course. Since our first compassionate use of neuraminidase inhibitors (January 1999) our experience with 188 proven influenza patients to date has yielded an important insight: Viral diagnosis as early as possible followed by immediate antiviral therapy. The latest studies have confirmed the efficacy of our approach. In addition to using a rapid test, we also determined the humoral inflammation status – the sum of CRP + fibrinogen. We thus won a crucial two days' treatment time before the antibiogram of the bacterial swab became available. CRP levels above 3.5 mg/l are an advance warning of complications such as a bacterial superinfection, for example, and are an indication for antibiotic administration – decisive for the patient's fate. In addition, we were able to show that the humoral inflammation status could be reduced by 50% in patients treated antivirally. In 15% of our 188 patients we observed that the two main bacteria S. aureus and Haemophilius influenzae showed discrepant reactions to an antibiotic (ciprofloxacin und moxifloxacin). This aspect is even more serious with the always imminent problem of MRSA patients with an influenza infection. An MRSA carrier was identified in our patient group. These bacterial risks have to be confronted with a consistent prophylactic strategy – vaccination is vitally important. We are now using a virosomal vaccine for the second year running with clearly positive results. Together with prophylactic vaccination the use of rapid tests and reserved antibiotics has to be guaranteed. Caution is especially recommended when S. aureus is involved.

Abstract P1321

Influenza Vaccination Coverage Rates in Germany During the Winter of 2006/2007

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Objectives: The objectives of the survey were to identify the level of influenza vaccination coverage in Germany in 2006/2007, to understand the drivers and barriers to vaccination, to find out vaccination intentions for next winter and major encouraging factors to vaccination. Methodology: A telephone omnibus survey was conducted among 2007 non-institutionalised individuals representative of the population aged 14 and over. The research was carried out in December 2006. Results: In 2006/2007, influenza vaccination coverage rate (VCR) in the surveyed population decreased by 5 points compared to 2005/2006, down to 28% and close to 2004/2005 figures (27%). In 2006/2007, 45% of the population had never been vaccinated. In the elderly age-recommended population (people aged 60 and over), VCR was 49% (from 59% in 2005/2006), below 2004/2005 figures (52%). The principal motivation for being vaccinated was considering flu as a serious illness (mentioned by 90% of the vaccinated people). Following advice from the family doctor/nurse (71%) and avoiding transmission to family/friends (69%) were respectively the second and third main motivations. The main reasons for non-vaccination among people never previously vaccinated were feeling unlikely to catch the flu (48%), thinking about vaccination without getting it (47%), considering flu as a non serious illness (40%) and absence of family doctor's recommendation (39%). Among the people previously vaccinated but dropping out this year, not feeling concerned (39%), forgetfulness (36%), catching flu despite vaccination (32%) and disbelief in vaccine effectiveness (31%) were the major reasons for this behaviour. For the 2007/2008 winter, 45% of the surveyed population intended to get a flu jab. 68% of the population mentioned that the major encouragement to vaccination was a recommendation from the family doctor. Conclusion: During winter 2006/2007, VCR

was 28% in the population over 14 years of age, undergoing a significant drop compared to last season, suggesting that the high media attention on avian flu in 2005 had had a strong impact on influenza vaccination. Our findings suggest that perception of the seriousness of flu illness is a key decision factor for vaccination and that the family doctor plays a major role in improving vaccine uptake.

Abstract P1322

Clinical Symptoms and the Reliability of Symptomatic Criteria in the Diagnosis of Influenza A and B Infection: A Japanese Multi-center Study Using Commercial Antigen Detection Kits

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Objectives: The objectives of the survey were to identify the level of influenza vaccination coverage in Spain in 2006/2007, to understand the drivers and barriers to vaccination, to find out vaccination intentions for next winter as well as the major encouraging factor to vaccination. Methodology: A telephone omnibus survey (using CATI system) was conducted among 2000 non-institutionalised individuals representative of the population aged between 14 and 74. The research was carried out in December 2006 and January 2007. Results: In 2006/2007, influenza vaccination coverage rate (VCR) in the population decreased by 2 points in comparison with 2005/2006 to 22%. This decrease was due to a 1 point loss in both repeat vaccinations and new vaccinations but was non-statistically significant. In the elderly age-recommended population (defined as the people aged 65 and over), VCR increased to 71% (from 70% in 2005/2006), with 8% people being vaccinated for the first time. In 2006/2007, 64%% of the population had never been vaccinated. Advice from the family doctor/ nurse (44%), considering flu as a serious illness (37%) and older age (30%) were the principal motivations for being vaccinated. Considering flu as a serious illness was a reason mentioned by notably more people than in 2005/2006 (+9%), together with preventing flu from interrupting professional activities (+8%). The main reasons for non-vaccination among people never previously vaccinated were feeling unlikely to catch the flu (63%), not having considered vaccination (43%) and absence of family doctor's recommendation (25%). Among the people previously vaccinated, the major reasons for dropping out this year were forgetfulness (32%), absence of family doctor's recommendation (22%) and not feeling concerned (22%). For the 2007/2008 winter, 30% of the surveyed population intended to get a flu jab. 73% of the surveyed population mentioned that the major encouragement to vaccination was a recommendation from the family doctor. Conclusion: During winter 2006/2007 in Spain, VCR in both the population aged between 14 and 74 and the age-recommended elderly population remained quite stable compared to 2005/2006. Our findings strongly suggest that the family doctor plays a major encouraging role in improving vaccine uptake.

Abstract P1323

Influenza Vaccination Coverage Rates in the United Kingdom During the Winter of 2006/2007

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Objectives: The objectives of the survey were to identify the level of influenza vaccination coverage in the UK in 2006/2007, to understand the drivers and barriers to vaccination, to find out vaccination intentions for next winter as well as the major encouraging factor to vaccination. Methodology: A telephone omnibus survey (using CATI system) was conducted among 2037 non-institutionalised individuals representative of the population aged 16 years old and over. The research was carried out in December 2006 and January 2007. Results: In 2006/2007, influenza vaccination coverage rate (VCR) in the population decreased by 1 point in comparison with 2005/2006, to 25% due to a decrease in repeat vaccination (22% vs. 23% in 2005/2006); first time vaccination was stable (3%). In the elderly age-recommended population (defined as the people aged 65 and over), VCR dropped significantly to 70% (from 79% in 2005/2006), back to 2004/2005 figures (71%). In 2006/2007, 58% of the population had never been vaccinated. Advice from family doctor/nurse (60%), considering flu as a serious illness (50%) and older age (42%) were the top 3 UK motivations for being vaccinated. The main reasons for non-vaccination among people never previously vaccinated were absence of family doctor's recommendation (38%), not having considered vaccination (35%), feeling unlikely to catch the flu (33%) and younger age (32%). Among previously vaccinated people dropping out this year, forgetfulness (28%), feeling unconcerned (24%) and absence of recommendation from the family doctor (19%) were the major reasons for not being vaccinated. For winter 2006/2007, 38% of the surveyed

population intended to get a flu jab, which was lower than last season (41%) as a possible consequence of a late and weak influenza epidemic this year combined with less media coverage on avian flu. 72% of the surveyed population mentioned that the major encouragement to vaccination was a recommendation from their doctor/ nurse. Conclusion: During winter 2006/2007 in the UK, VCR remained stable in the population aged 16 and over compared to 2005/2006 but dropped significantly in the age-recommended elderly population. Our findings strongly suggest that the family doctor plays a major encouraging role in improving vaccine uptake.

Abstract P1324

Influenza Vaccination Coverage Rates in Italy During the Winter of 2006/2007

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Objectives: The objectives of the survey were to identify the level of influenza vaccination coverage in Italy in 2006/2007, to understand the drivers and barriers to vaccination, to find out vaccination intentions for next winter as well as the major encouraging factors to vaccination. Methodology: A telephone omnibus survey (using CATI system) was conducted among 2001 non-institutionalised individuals representative of the population aged 14 and over. The research was carried out in December 2006 and January 2007. Results: In 2006/2007, influenza vaccination coverage rate (VCR) in the population was 24%, the same figure as in 2005/2006. In the elderly age-recommended population (defined as the people aged 65 and over), VCR increased to 66% (from 64% in 2005/2006), with 6% people being vaccinated for the first time. In 2006/2007, 67% of the population had never been vaccinated. The principal motivations for getting vaccinated were advice from the family doctor/nurse (43%), considering flu as a serious illness (24%) and not being in very good health (23%). Not having considered vaccination (38%), feeling unlikely to catch the flu (22%), considering flu as a non serious illness (17%) and absence of family doctor's recommendation (14%) were the main reasons for non-vaccination among the population never previously vaccinated. Among the people previously vaccinated but not this year, lack of interest/motivation (forgetfulness 26%, feeling unconcerned 18%) was the major reason for dropping out, followed by lack of trust in the vaccine (having caught the flu despite vaccination 15% and vaccine not effective enough 14%). Intentions for getting vaccinated in the 2007/2008 winter dropped (from 30% to 28%) as a possible consequence of a late and weak influenza epidemic this year combined with less media coverage on avian flu. 55% of the surveyed population mentioned recommendation by the family doctor as the major encouragement to vaccination. Conclusion: During winter 2006/2007 in Italy, VCR was 24% in the population aged 14 and over, stable compared to 2005/2006. The VCR in the age-recommended elderly population increased to 66%. Our findings suggest that the family doctor plays a major encouraging role in improving vaccine uptake.

Abstract P1325

Vaccination of Children Reduces Hospitalization Rates Due to Influenza in the Elderly on the National and Regional Levels

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A growing body of research suggests that children may play an integral role in the transmission of influenza, not only to other children, but also older members of the community. The elderly are especially vulnerable to the effects of influenza in terms of hospitalizations, medical complications, and deaths. We hypothesize that, in the absence of universal vaccination for all age groups, vaccinating children would have a beneficial effect on reducing pneumonia and influenza-related (P&I) hospitalizations in the elderly population (≥65). We abstracted 6.2 million hospitalization records from the database of medical claims maintained by the Center for Medicare and Medicaid Services for four consecutive influenza seasons (1998-2002). State-level influenza annual vaccination coverage in children and the elderly were obtained from the Behavioral Risk Factor Surveillance System for the same period. We estimated a single-year age distribution of rates of P&I hospitalization by state for each influenza season, defined as July 1 through June 30 of the following year. We observed an acceleration in the P&I rates with age for each state and influenza season. State- and season-specific accelerations were regressed against the percentage of vaccinated children or elderly. State-level vaccination coverage in children was significantly associated with acceleration of the P&I hospitalization in the elderly in the influenza seasons of 1998-99 (β=-0.393, p=0.044) and 2000-01 (β=-0.395, p=0.036). State-level vaccination coverage of the elderly was significantly association with the decline in P&I rates in 2000-01 (β=-0.239,

p=0.040). The association between influenza vaccination coverage and P&I rates was also examined longitudinally, contrasting vaccination rate in four consecutive influenza seasons with corresponding P&I rates by state. For example, in Connecticut, there was a consistent and significant association between P&I acceleration and child vaccination coverage: when vaccination coverage declined by 6 percentage points in four years, the acceleration in P&I hospitalization rates in the elderly increased by approximately 10% (p=0.002). No significant associations were found between elderly vaccination coverage and P&I rate. These results suggest that, on the national and regional levels, vaccination of children reduces hospitalization rates due to influenza in the elderly.

Abstract P1326

Implementing the Influenza Pandemic Plan in Denmark at Local Level

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Background: According to the recommendations from WHO, the National Board of Health in Denmark published a national pandemic plan in April 2006. The plan followed the guidelines from WHO and consisted of 57 pages describing the national strategy and health preparedness planning and also included an addendum of 91 pages with more detailed information on influenza, case definitions, risk groups, how to handle contacts, vaccines, antivirals, etc. A further job then had to be done to implement the plan at local level. Material and Methods: The National Board of Health established a working party in September 2006 with representatives from primary and secondary health care sector, the Danish Medicines Agency, the Emergency Management and the municipalities. The following subgroups were organized for preparing guidelines for handling a pandemic at local level: GPs; Ambulance Services; The regional Emergency Management Office; Acute emergency wards; Medical wards and Pediatrics wards; The regional Clinical Microbiology Laboratories; Pharmacies; Medical Officers of Health; The Municipality Health Service. In each subgroup 3-5 members very included, mostly professionals with specific knowledge on the topic. Results: A working plan for each of the above mentioned groups was prepared and each subgroup composed a paper according to a specific outline of their special tasks during the 6 phases of a pandemic: Interpandemic period (phases 1 and 2), the prepandemic alert period (phases 3-5), and the pandemic (phase 6). All subgroups described the handling of a patient with a potential pandemic flu by their specialty according to the different phases. During their work a number of practical issues were raised primarily concerning the pre-hospital phases of the emergency; logistics, e.g. transportation of laboratory specimens, where to see the patient, should relatives be evacuated from the household, how to provide subjects with possible infection with their daily needs, protective covering of the health care persons, payment, requisition of antiviral drugs. The results of all subgroups were distributed for comments to the rest of the working party and discussed during plenary sessions. The resulting product was published in the Danish National Board's official recommendations for preparedness and will be distributed to the regional level. Conclusion: Although a pandemic has not yet occurred, emergency plans should be prepared involving local actors at all levels. During this process, many practical issues arose, which should be taken into account to prevent insufficient handling of infected subjects. The process described above will also be useful for other future preparedness activities.

Workshop Topic #14

Preclinical Vaccines and Other Intervention Strategies

Poster Abstracts

Abstract P1401

Development of Candidate H7N3 Live Attenuated Influenza Vaccine

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The outbreak of highly pathogenic avian influenza H7N1 emerged in Netherlands during 2003 resulted to more than 80 human cases of conjunctivitis and mild respiratory illnesses and 1 fatal case. To prepare candidate live influenza vaccines for potential future pandemic we use a classic genetic reassortment with non-pathogenic avian viruses and the cold-adapted (ca) H2N2 master strain (MS) A/Leningrad/134/17/57 (Len/17). Len/17 is currently used in Russia for preparing licensed live attenuated vaccines for adults and children. Previously it was shown that a reassortant between Len/17 and non-pathogenic H5 influenza virus is attenuated for chickens and mice. In the present study we evaluated a 6:2 reassortant between Len/17 and A/Mallard/Netherlands/12/00(H7N3). The reassortant A/17/Mallard/ Netherlands/00/84(H7N3) (Len17/H7) demonstrated cold-adapted (ca) and temperature-sensitive (ts) phenotypes similar to those of Len/17. The HA gene sequence of Len17/H7 was identical to that of the parent H7N3-wt virus. According to the hemagglutination-inhibition (HI) test with a panel of ferret antisera to different avian and human H7 viruses, the antigenic profile of the reassortant was similar to that of the H7N3-wt parent strain as well as of human H7 isolates from the Netherlands including the virus isolated from the fatal case. The reassortant demonstrated high growth capacity in embryonated hens eggs (CE) at optimal (34^{0}C) temperature, comparable to that of the parent Len/17 MS. Also, Len17/H7 was shown to be attenuated for chickens similar to the Len/17 parent whereas H7N3-wt virus caused 60% mortality. Like the Len/17 MS, Len17/H7 was completely attenuated for mice. After intranasal inoculation with 10^5-10^6 EID_{50} Len17/H7 replicated well in nasal turbinates of mice but did not replicate in the mouse lungs. Despite the lack of replication in mouse lungs, Len17/H7 induced serum virus-specific IgG as high as 3,7±0,8 log_{10}.

Abstract P1402

Immunostimulated Nanoparticles Incorporated Viral Glycoprotein Antigens and Plant Saponins as Highly Immunogenic Influenza Subunit Vaccine for Mucosal Immunization

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One of the most attractive delivery systems for elaboration of low toxicity and highly immunogenic influenza subunit vaccine for needle free mucosal immunization is immunostimulating complex (ISCOM) - a unique nanoparticle structure about 40-60 nm size formed by encapsulating antigens, lipids and triterpene saponins of plant origin. In the research presented hemagglutinin and neuraminidase antigens were isolated from purified influenza virus A/FPV/Rostok/34 (H7N1) by non-ionic MESC detergent treatment, separated from internal viral proteins by centrifugation and from detergent by dialysis. Highly purified low toxicity saponins GG6, GP7 and SO6 were isolated from Glycyrhiza glabra, Gipsophila paniculata and Saponaria officinalis plants indigenous to Kazakhstan by HPLC fractionation. ISCOM nanoparticles have been assembled from HA+NA antigens, lipids and plant saponins by dialysis technique. Two-weeks old chickens were immunized intranasally (6 µg of HA+NA per bird) and two weeks after single immunization chickens sera were tested in ELISA and birds challenged live A/FPV/Rostock/34 virus in dose 100 EID_{50}/chicken. The results of study have shown that ISCOM subunit vaccine administered intranasally induced high levels of serum antibody and protected chickens against challenge after single immunization. ISCOMs containing GP7 saponin prevented infection of 75% of chickens, ISCOMs incorporated SO6 saponin protected 85% of chickens and ISCOMs possessed GG6 saponin protected 90% of chickens. In same vaccination experiments intranasal inoculation of pure HA+NA subunit vaccine protected 30% of chickens and immunization by HA+NA vaccine mixed with alum hydroxide adjuvant protected 50% of chickens.

Abstract P1403

Development of 'Pandemic Vaccines'; Can the Ferret Predict Efficacious HA Dose Levels in H5N1 Vaccines for Use Man?

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A significant hurdle in the development of 'pandemic influenza vaccines', is the fact that in the pre-pandemic phase, efficacy evaluation of 'pandemic vaccines' in man is based on surrogate endpoints like HAI titers for which correlation with protection cannot be validated in the absence of disease. Nevertheless, based on HAI titers it has been concluded that non-adjuvated H5N1 influenza vaccines require two doses of vaccine at HaemAgglutin (HA) dose levels up to six-fold higher than for the seasonal vaccines (90 mcg/dose instead of 15 mcg/dose). However, without true efficacy data showing protection from challenge exposure in man, it remains unclear whether this apparent low immunogenicity of H5N1 influenza virus is true or an HAI assay artifact. We have designed a preclinical program to show proof of concept (POC) for a novel influenza vaccine in ferret and to support dose determination for such vaccine in man. Ferrets were chosen as test animals because their susceptibility to influenza viruses is comparable to man and influenza virus infections (seasonal as well as high pathogenic avian strains) in man and ferrets develop similarly. Reduction of viral replication after challenge exposure was chosen as the primary endpoint for vaccine efficacy in our ferret studies because reduction of viral replication is the relevant parameter for reduced disease transmission, which should be the primary goal for a pandemic vaccine rather than protection from a lethal challenge. High mortality (in individual cases) by highly virulent strains but with limited virus shedding poses a much lower risk to the population than highly virulent strains that are able to replicate and shed efficiently from infected individuals. HAI titers and virus neutralization titers were also measured, as were clinical symptoms, body temperatures and body weights. First, we established a dose response curve for seasonal H1N1 influenza vaccine strain in naive ferrets that were previously exposed to an H3N2 influenza virus. This H3N2 pre-exposure was conducted to mimic the situation in man. The objective of this phase was to establish a reference dose level for non-adjuvated seasonal vaccine in the ferret model. Second, we showed that equal or higher levels of protection (reduction of viral replication) can be reached with adjuvanted H1N1 vaccines at much lower HA levels than the reference dose. Third, we will test H5N1, adjuvanted and non-adjuvanted, vaccines in ferrets in a similar design to confirm the predictive value of the H1N1 studies. By establishing the ratio between the lowest efficacious dose level for an H5N1 vaccine in H5N1-naive ferrets and the lowest efficacious dose level for an H1N1 vaccine in H1N1-naive ferrets we aim to provide data that will support dose determination for an H5N1 vaccine in man. A summary of the results of the various studies will be presented.

Abstract P1404

MDCK Cells as Substrate for Influenza Vaccine Production: Results of In Vivo Testing of Cell Properties (Tumorigenicity and Oncogenicity) and Qualification as a Safe Substrate for Vaccine Production

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The results of an extended testing program of cell properties of an MDCK cell line that was adapted to serum free growth conditions, in particular the results of tests for tumorigenicity of the intact cells (the phenotypic growth characteristics) and the tests for oncogenicty of cellular components such as DNA will be presented. The results are used in a risk-assessment for the use of this MDCK cell line as substrate for influenza vaccine production. Conventional influenza vaccine production is based on influenza seed virus propagation in embryonated chicken eggs. Timely supply of the required number of embryonated eggs is at risk in case of outbreaks of, or veterinary counter measures to control, avian diseases. The egg-based production process is also inherently vulnerable to contaminations and consequently inactivation and removal of contaminants during production are essential to render a safe product. The use of continuous cell lines (CCLs) has been recognized by vaccine producers and health authorities as an attractive alternative to embryonated eggs. Vaccine production on continuous cells allows continuing or expanding influenza vaccine production independently of the supply of embryonated eggs. In contrast to eggs, a cell bank is extensively tested for absence of infectious microorganisms and potential oncogenic agents and the use of closed bioreactors should allow a sterile production process in contrast to the virus propagation in embryonated eggs. Various CCLs are considered as substrates for influenza vaccine production e.g. Vero, MDCK and PerC6. Independent of the CCL chosen as substrate, the qualification of cells for use in the production of a vaccine includes assessment of the history and general characteristics of the cells and testing and characterization of the cell bank used for the manufacturing of a specific product. The cell bank is comprehensively tested for absence of infectious microorganisms, cell properties such as the in-vivo phenotypic growth characteristics of the

cells, potential oncogenicity of cellular components, identity of the cells and their genetic stability. In addition to testing for absence of oncogenicity of cellular components, the risk of residual cell-substrate DNA in the vaccine is reduced by decreasing its biological activity. This is accomplished by reducing the size of residual cellular DNA (e.g. by DNAase treatment or other methods) to far below the size of a functional gene and by limitation of residual DNA to less than 10 ng/dose (as recommended by WHO). The risk assessment based on the cell properties that will be presented and general requirements for purification of the product (e.g. DNA digestion and limitations to residual cellular DNA) shows that this substrate is as safe as other continuous cell lines that are or will be qualified as the result of a similar testing program and comparable final product specifications.

Abstract P1405

Emergency Production of Avian Flu Vaccines

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Everyone knows that vaccines are the best prevention method against diseases and "prevention is the best weapons to reduce health care cost". In the past, vaccines were evaluated as low margin products, so most pharmaceutical companies ignored and/or reduced R&D spending in the vaccine development. Potential pandemic influenza caused by the high pathogenic avian influenza virus (HPAIV) is now around corner and the current flu vaccine production facilities would not be able to produce sufficient vaccines for the demand. The countries currently do not have vaccine manufacturing facilities will be the most vulnerable. The Vaccine R&D Center of Taiwan National Health Research Institutes was assigned to develop strategic plans for emergency production of avian flu vaccines. In this presentation, we described how to renovate a P2+ facility containing a positive pressure cell culture room, and two negative pressure rooms for virus growth and downstream purification processes within two months and minimum cost. Within 6 months from the ground zero, we have successfully (1) developed the production processes for growing the WHO vaccine strain NIBRG-14 in MDCK cell at 60 liters scale; (2) established all basic QC tests; (3) performed mouse immunogenicity studies and found out that the novel adjuvant formulated inactivated virus at 0.5 µg of HA antigen could elicit both high virus neutralization titer and strong cellular immune responses; (4) demonstrated the vaccine candidate formulated in alum could protect mice from the wild type H5N1 challenges. To enhance the virus growth and yield, we have screened different serum-free media in the presence of different culture media supplements and found some novel combinations to be capable significantly increasing the virus yield at least by 4 fold. With the current P2+ facility we can produce about 7,500 doses of avian flu vaccines containing 15 µg of HA antigen in every month. The production processes in the micro-carriers bio-reactor technology are now being optimized. Vero cell adapted NIBRG-14 virus strain has also been generated as the back-up production plan. Our strategies could be generally applicable to those countries currently do not have flu vaccine manufacturing facilities. Financial Supported by Taiwan Pandemic Influenza Vaccine R&D Program and NHRI.

Abstract P1406

The Quality and Kinetics of the Antibody Response in Mice After Three Different Low Dose Influenza Vaccination Strategies

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Influenza vaccines are the most cost-effective intervention in reducing the burden of disease in both influenza epidemics and pandemics. Unfortunately, in a pandemic scenario shortages in vaccine supplies will occur, and dose sparing vaccination strategies such as the use of adjuvants or new administration routes need therefore to be investigated. In this study we compared the quality and kinetics of the serum antibody response elicited in mice after three different low dose vaccination strategies using an influenza A/Panama/2007/99 (H3N2) split virus vaccine. Ninety-two mice were divided into four groups, and vaccinated with one or two doses three weeks apart. One group was vaccinated intradermally with low dose (3 µg) hemagglutinin (HA), whereas the other three groups were vaccinated intramuscularly with either low dose, low dose adjuvanted with aluminum hydroxide (60 µg), or the standard human dose of 15 µg HA. Sera were collected weekly, and the mice were sacrificed three weeks after the first or second vaccination. The serum antibody responses and cross reactivity against drifted H3N2 strains were tested in the hemagglutination inhibition, virus neutralization and enzyme-

linked immunosorbent assays. The antibody responses detected after intradermal or intramuscular low dose vaccination were similar in all assays, and lower than observed after the 15 µg vaccine dose. The low dose adjuvanted vaccine elicited a serum antibody response comparable to the human dose. However, the IgG1/IgG2a ratio in this group indicated a more pronounced Th2 profile. Furthermore, the second immunization with the low dose adjuvanted vaccine did not result in any increase in cross-reactive HI antibodies, and gave a peak serum antibody response one week later compared to the other vaccination groups. Our murine data suggest that the intradermal route of administration does not show any obvious advantage over the intramuscular route in inducing a serum antibody response, and that none of the low dose vaccination strategies is as effective as intramuscular vaccination with the normal human dose. However, the low dose aluminum adjuvanted vaccine may present a feasible alternative in case of limited vaccine supply.

Abstract P1407

An Improved Method for the Quantitation of Haemagglutinin in Influenza Virus Vaccine Preparations

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The assessment of potential influenza virus vaccine strains includes a number of factors. Apart from antigenicity and immunogenicity, growth properties of the virus and yield of antigen, specifically the haemagglutinin (HA), are of key importance. The recently developed H5N1 vaccine reference strain NIBRG-14 (with HA and NA genes derived from the clade 1 virus A/Viet Nam/1194/2004) has been reported to yield low amounts of HA. Currently, the method for the measurement of HA yield of influenza virus vaccine strains involves SDS PAGE analysis of denatured and reduced samples followed by quantitation of the viral protein bands and calculation of the amount of haemagglutinin as a percentage of total viral protein. However, with some H5N1 vaccine reference strains, such as NIBRG-14, this measurement is not reliable due to the migration characteristics of viral proteins in SDS PAGE. In these strains, the glycosylated haemagglutinin HA1 and HA2 bands comigrate to varying extents with the nucleoprotein and matrix protein bands, respectively. We have found that the introduction of a deglycosylation step prior to gel electrophoresis removes the problem of comigration completely. We used mass spectrometry to confirm that the bands used for quantitation using the deglycosylation method were indeed single identifiable proteins with no cross contamination of other virus or egg proteins to interfere with subsequent analysis. The improved method for quantitation allowed us to reproducibly assess various preparations of H5N1 vaccine reference strains for haemagglutinin content. This quick and reproducible method will facilitate research into the reasons for the low yield properties of clade 1 H5N1 vaccine reference strains such as NIBRG-14 which will hopefully result in the development of improved vaccine strains for potential pandemic influenza strains.

Abstract P1408

A Novel Approach to H5N1 Influenza A Virus Vaccine Development: The Use of M2 Cytoplasmic Tail Mutants

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Background: Highly pathogenic H5N1 avian influenza A viruses are enzootic in Asia and have spread to Europe and Africa. The persistent circulation of these viruses in birds provides ample opportunity for them to infect humans. Indeed, H5N1 viruses have overcome host species barriers and infected over 250 individuals, more than half of whom died from their infections. Concern regarding the pandemic potential of H5N1 viruses is thus clearly warranted as is the need to develop effective vaccines against these viruses. Methods: We and others have previously demonstrated that deletions of the M2 cytoplasmic tail of A/WSN/33 (H1N1) and A/Udorn/307/72 (H3N2) influenza A viruses causes growth defects in cell culture. We, therefore, tested the feasibility of using M2 tail mutants as live attenuated vaccines against H5N1 virus. We generated a series of highly pathogenic H5N1 (A/Vietnam/1203/04) M2 tail deletion mutants by reverse genetics and compared their growth properties with those of wild-type virus in MDCK cells and mice. Results: We found that mutants in which more than 22 amino acids were deleted from the C-terminus of the M2 tail were viable but replicated less efficiently than did the wild-type virus in MDCK cells (1,000~10,000-fold lower). Conversely, a mutant containing an 11-amino-acid deletion from the C-terminus (M2del11) grew as well as the wild-type virus. To validate these in vitro results in an animal model, we infected mice with each of the mutant viruses and examined virus titers in several organs. As expected, the mutants possessing deletions of more than 22 amino acids were not recovered from any of the infected mice. Interestingly, replication of the M2del11 virus was more than one log lower in the lungs and nasal turbinates, and 4 logs lower in the spleens and kidneys of infected mice relative to the wild-type virus. These data indicate that the M2del11 virus is highly attenuated in mice,

even though no growth defect was detectable in MDCK cells. Conclusions: Our results suggest that the M2del11 virus has potential as a candidate vaccine for H5N1 virus infection. Experiments to evaluate the protective efficacy of M2 tail mutants against H5N1 virus infection are underway.

Abstract P1409

MDCK Cells as a Tool For Screening Temperature Sensitive Live Influenza Vaccine Candidate Reassortants

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Background: Live cold-adapted trivalent Russian influenza vaccine is regularly prepared in embryonated hens' eggs. The determination of temperature sensitive (ts) and cold-adapted (ca) phenotype of reassortants for a live influenza vaccine has usually been conducted using eggs. As attempts are made to move away from eggs, it is important to develop an equivalent cell based assay. It has previously been shown that the Russian ca, ts and attenuated master donor virus (MDV) A/Leningrad/134/17/57(H2N2) showed a significantly more temperature sensitive phenotype in MDCK cells than in eggs. The cut off temperature for type A influenza viruses in MDCK cells was determined to be 37°C since at this temperature there is a clear and definite difference in growth between MDV and currently circulating non-ts influenza viruses. In this study we selected current influenza A and B viruses that appear to be naturally temperature sensitive in eggs and have evaluated the use of MDCK cells as a tool for determining their ts phenotype. Also, we compared this assay with the same performed in eggs. Methods: Naturally occurring temperature sensitive current influenza A(H1N1), A(H3N2) and B viruses were grown both in eggs and MDCK cells at the following temperatures: 32, 37, 38, 39 and 40°C and were compared with A and B MDVs. Viral titers were determined by either EID50 or TCID50. Results: It was shown that current influenza A and B viruses appear to be naturally temperature sensitive to reproduction in eggs and demonstrated strong non-ts phenotype in MDCK cells at 37 and 38°C respectively. Conclusion: These findings can be used to support live influenza vaccine production. It is important for evaluation of the ts phenotype of currently circulating influenza viruses as potential candidates for a reassortant influenza vaccine. Determination of ts phenotype in MDCK cells provides a new universal attenuation marker for primary screening of ts candidate reassortants for live influenza vaccine prior to their evaluation for genome composition.

Abstract P1410

Efficacy of a DNA-Based Multi-valent Vaccine Against Influenza A (H3)

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Annual influenza vaccines are targeted against the specific influenza virus isolates that best represent the strains currently circulating in humans. The effectiveness of this vaccine is improved when the circulating strain closely matches the vaccine strain. However, viruses that escape vaccine-induced immunity can create an environment where the vaccine no longer prevents laboratory-confirmed influenza infection. Current influenza vaccines are limited in their inclusion of a single isolate for each circulating strain of influenza (H1N1, H3N2, and B). We hypothesized that simultaneous vaccination against multiple hemagglutinins (HAs) within a given subtype (H3) would yield broad, cross-protective immunity, thus increasing vaccine effectiveness. To directly address this issue, we developed a multi-valent vaccine against influenza A (H3) that focuses on the HA, which we deliver in a plasmid DNA vector via the gene gun. This vaccination strategy is known to predominantly stimulate antibodies of the IgG1 isotype in BALB/c mice. We found that simultaneous vaccination with three H3 HAs (A/Hong Kong/1/68 (HK68), A/Victoria/3/75 (VI75), and A/Leningrad/360/86 (LE86)) provided immunity against viruses expressing homologous H3 HAs without evidence of immunologic interference within the H3 subtype. Additionally, immunity against viruses expressing heterologous H3 HAs (A/Port Chalmers/1/73, A/Texas/1/77, A/Memphis/6/86, A/Memphis/7/90, and A/Beijing/353/89 (BE89)) was observed. This immunity was quantitated in the form of vaccine-induced HA-specific IgG1 antibodies, the expression of which correlated with a reduction in virus detected in lungs of mice after sub-lethal challenge. Importantly, the strength of the immune responses against the three H3 HAs delivered simultaneously was similar to that seen when the HAs were delivered individually. Vaccination with HK68, VI75, and LE86 simultaneously did not stimulate immunity against viruses expressing HA from heterologous H3 viruses after BE89 (A/Beijing/32/92, A/Wuhan/359/95, and A/Sydney/5/97). Our findings demonstrate that a broad, multi-valent vaccine targeting an influenza HA subtype can be developed. These data have implications for design of both seasonal and pandemic influenza vaccines.

Abstract P1411

Synthetic Multiepitope Peptides Identified In Silico Induce Protective Immunity Against Multiple Influenza Serotypes

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Influenza causes yearly epidemics of mild disease and, occasionally, pandemics with millions of fatalities. Currently, no vaccine is effective against all Influenza strains. Analysis of Influenza sequences from animal and human isolates using CLUSTALW and a novel proprietary epitope prediction algorithm identified six conserved T-cell reactive regions in several proteins. Immunization of transgenic mice with a preparation of these six regions as chemically-synthesized peptides (FLU-v) induced a specific HLA-A*0201-mediated CD8+ T-cell response. This T-cell population also reacted specifically against human cells infected with several non-related Influenza strains, confirming that the identified regions contain epitopes naturally presented by infected human cells and conserved amongst non-related viruses. Moreover, FLU-v immunization significantly increased survival of transgenic mice against lethal challenge with Influenza. Overall, FLU-v represents a promising Influenza vaccine candidate, obviating the need for yearly vaccinations and allowing the stockpiling and initiation of a worldwide vaccination program in advance of a pandemic outbreak.

Abstract P1412

Immunization With Low Dose Adjuvanted Split H5N1 Pandemic Vaccine Protects Ferrets Against Homologous Challenge

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Background: In the event of an influenza pandemic there is insufficient world-wide capacity to deliver the billions of vaccine doses necessary to protect the world's population. In addition, high doses of non-adjuvanted H5N1 vaccine have been shown to be very poorly immunogenic in healthy adult subjects. In the present study, adjuvantation has been selected as the antigen-sparing strategy of choice because it is readily available and it has been shown to improve substantially immune response against various antigens. Aims: To evaluate the potency of adjuvantation to reduce amount of H5N1 monovalent split antigen needed to confer protection of ferret against homologous H5N1 pandemic virus challenge. Methods: Four groups of 6 ferrets (Mustela putorius furo) were immunized intramuscularly with 15, 5, 1.7 or 0.6 µg HA of H5N1/A/Vietnam/1194/2004 (NIBRG-14) vaccine containing a fixed amount of a novel proprietary adjuvant system. Two control groups consisted of PBS- or adjuvant-treated animals. Ferrets were vaccinated on days 0 and 21 and challenged intratracheally on day 49 with 10^5 $TCID_{50}$ (50% Tissue Culture Infective Dose) of the H5N1/A/Vietnam virus. Throat swabs and lung tissues were collected after the challenge to assess virus shedding by quantitative virus titration culture on MDCK cells. Serological responses to the vaccine were measured using the hemagglutination inhibition assay (HI) on day 42. Gross pathology and histopathology analysis was performed on day 5 post-challenge. A complete macroscopic post-mortem [gross-pathology] examination was performed and all abnormalities were recorded. This included examination of the external surfaces and all orifices, the thoracic, abdominal and pelvic cavities with their associated organs and tissues and the neck with its associated organs and tissues. In addition, brain and liver were collected and fixed for histology in 10% neutral buffered formalin. Results: Challenge with H5N1/A/Vietnam/1194/2004 in ferrets vaccinated with adjuvanted H5N1 vaccines showed an antigen dose-dependent protection. All animals immunized with 15 µg and 5 µg HA of the adjuvanted vaccine were protected against the lethal challenge. For the viral load in lungs, all ferrets immunized with PBS or the adjuvant alone exhibited viral loads higher than 10^5 $TCID_{50}$/g of lung tissue. Conversely, administration of adjuvanted vaccines reduced the virus load below the threshold of 10^2 $TCID_{50}$ per gram of lung tissue or per ml fluid from pharyngeal swabs in 65% and 75% of animals, respectively. Little difference could be seen between ferrets immunized with 1.7, 5 or 15 µg HA of the adjuvanted vaccine. Importantly, it should be noted that most animals from placebo (PBS) and adjuvant only groups died or were euthanized on days 2 and 3, while most animals in the vaccinated groups survived through to euthanasia on day 5. Consequently viral loads were not measured on the same day post challenge for all animals. Finally, mean HI antibody titers against homologous H5N1/A/Vietnam/1194/2004 virus were higher than the threshold value of 40 in all groups of ferrets immunized with adjuvanted vaccines. All animals showed inflammatory changes in the lungs characterized by sub-acute bronchioalveolar inflammation and/or primary atypical pneumonia. Pulmonary oedema was not observed macroscopically in animals immunized with the highest doses (1.7 µg, 5 µg or 15 µg HA) of the adjuvanted vaccine. Similarly, hepatic necrosis was not observed microscopically at these same vaccine doses, while animals in the 0.6 µg vaccine group and in the control groups showed multifocal necrosis in liver tissues. Together, these findings suggest vaccine

mediated protection from influenza virus induced pathology. Conclusions: Upon challenge with wild-type virus, only animals vaccinated with adjuvanted vaccines showed a high rate of survival, with reduced viral load in lung tissue and pharyngeal swabs, as well as reduced pathological symptoms. These results strongly support the use of adjuvantation coupled with antigen sparing as the most effective and efficient approach to pandemic vaccine development.

Abstract P1413

Immunogenicity of Adjuvanted Split H5N1 Pandemic Vaccine in Mice

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Background: In an influenza pandemic, most people will be naïve for the pandemic virus strain and will likely require two vaccine doses to achieve a protective response. Non-adjuvanted H5N1 candidate vaccines elicit a substantial immune response only if large doses of antigen are used. Adjuvantation could improve the immunogenicity of an H5N1 vaccine and therefore allows the use of a reduced amount of antigen. This antigen-sparing approach would be beneficial for increasing vaccine supply. Aims: To evaluate, in mice, the effect on vaccine immunogenicity of reducing the dose of antigen in an adjuvanted H5N1 monovalent split vaccine. Methods: C57Bl/6 mice received two (28 days interval) administrations of different doses (3.0, 1.5, 0.75, 0.38 µg HA) of H5N1 A/Vietnam/1194/2004 split vaccine with a novel proprietary adjuvant. Immune responses were compared to the administration of two doses of the non-adjuvanted split H5N1 vaccine. Spleens were collected on day 6 post-booster to evaluate the cellular immune response by intracellular cytokine staining (ICS) technique. Sera were collected 21 days post-booster to evaluate the humoral response by antibody ELISA and by hemagglutination inhibition (HI) assay. Results: In mice, the adjuvanted H5N1 split vaccines induced significantly higher anti-H5N1 antibody responses measured by ELISA than the non-adjuvanted H5N1 ones (GMT = 30,444 [95% CI: 37,905;24,452] with 0.38 µg split adjuvanted H5N1 vaccine versus 49 [95% CI: 122;20] with the 3 µg HA of non-adjuvanted split H5N1 vaccine formulation). No antigen dose/response effect was observed between mice immunized with different doses of adjuvanted H5N1 vaccines (p>0.5). Adjuvanted H5N1 split vaccines also induced significantly higher anti-H5N1 HI titers to the homologous strain than the non-adjuvanted H5N1 vaccine (GMT = 1,810 [95% CI: 2,351;1,394] with 0.38 µg adjuvanted split H5N1 vaccine versus 11 [95%CI: 21;6] with the 3 µg non-adjuvanted split H5N1 vaccine). No antigen dose response effect was observed between mice immunized with different doses of the adjuvanted H5N1 split vaccine (p>0.5). Nevertheless, a trend for lower anti-H5N1 HI titers (2-fold reduction) was observed between the highest antigen dose of 3 µg (GMT = 3,620 [95% CI: 9,558;1,371]) and the lowest dose of 0.38 µg (GMT = 1,810 [95% CI: 2,351;1395]). Spleens were collected 6 days post-booster to evaluate the cellular response (H5N1-specific CD4+-T cell responses) by ICS. Whatever the dose (0.38 or 1.5 µg HA), significant difference was observed between CD4 T cell responses induced by non-adjuvanted H5N1 vaccine (0.15 or 0.21% H5N1-specific CD4 T cell responses after immunization with 0.38 or 1.5 µg, respectively) and adjuvanted H5N1 vaccine (0.77 or 0.60% H5N1-specific CD4 T cell responses after immunization with 0.38 or 1.5 µg, respectively). No antigen dose response effect was observed between mice immunized with two different doses (1.5 or 0.38 µg) of adjuvanted H5N1 vaccines. Conclusions: Immunogenicity studies in mice showed that adjuvanted H5N1 split vaccine induced significantly higher humoral (anti-H5N1 ELISA and HI titers) and cellular (CD4+ T cells) responses than the non-adjuvanted H5N1 split vaccine. No antigen dose response effect was observed for humoral and cellular immune responses in mice immunized with adjuvanted H5N1 split vaccine suggesting that even lower doses of HA than evaluated here may be sufficient to observe a dose response effect in this model.

Abstract P1414

The Immunogenicity of a PER.C6® Cell Grown Influenza H7N1 Virus Vaccine in a Murine Model

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In recent years, influenza H5, H7 and H9 subtypes have crossed the species barrier and transmitted directly from birds to humans resulting in illness and mortality. In order to increase pandemic preparedness, it is important that vaccines candidates are produced from all of these avian subtypes and tested for immunogenicity in preclinical models. In this study we investigated the kinetics and quality of the immune response induced in mice after vaccination with aluminium adjuvanted or non-adjuvanted formulations of a PER.C6® cell grown split influenza H7N1 vaccine. The vaccine strain was produced by reverse genetics. Eight groups of 16 mice were

immunized subcutaneously with 12, 6, 3, or 0.2 µg HA with or without aluminium hydroxide adjuvant (60µg Al). The adjuvanted vaccine was extemporaneously mixed prior to administration and the mice were vaccinated with one or two doses three weeks apart. Blood samples were collected weekly, and the animals were sacrificed three weeks after the first or second vaccination. The serum antibody responses were tested by hemagglutination inhibition (using both horse and turkey erythrocytes), micro neutralization and Luminex antibody bead immunoassays. The antibody levels detected after vaccination were generally low, but a dose response effect was observed. The aluminium adjuvant enhanced the immunogenicity of the vaccine in the group receiving the highest vaccine dose. The second immunization boosted the immune response in all the groups, with the highest levels of antibodies found in the mice vaccinated with the 12 µg aluminium adjuvanted vaccine. We found higher influenza-specific serum IgG1 than IgG2a levels after vaccination, suggesting a Th-2 like profile. Our murine data showed that this H7N1 vaccine was weakly immunogenic in mice, as judged by the low serum antibody levels elicited after vaccination.

Abstract P1415

The Use of Mannan to Enhance the Immunogenicity of Influenza Vaccines

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While a vaccine for human influenza (H1N1) does exist, it is not 100% effective, particularly in the elderly and young children, and a 'booster' shot is required every year to keep pace with strain-drift. Recently a number of experimental vaccines have been developed for the less prevalent but much more deadly 'bird flu' (H5N1) strains of influenza. Due to the lower immunogenicity of these strains as compared to H1N1 however, larger doses and multiple administrations are required to elicit immune responses, and it is not known whether these responses are protective. Further, the only effective method of culturing influenza viruses to date is via embryonated chicken eggs, a labour intensive process that requires considerable resources, which would limit the speed at which large amounts of H5N1 vaccine could be produced should a pandemic occur. This study investigated the potential of an experimental adjuvant, mannan, to enhance the immunogenicity of H1N1 and H5N1 vaccines. When administered via the intranasal route, mannan-adjuvanted immunisation induced levels of respiratory-tract IgA that were clearly above those induced by un-adjuvanted immunisation, which were negligible or absent. When administered intra-muscularly, a single mannan-adjuvanted immunization induced serum IgG far exceeding that induced by unadjuvanted immunization.

Abstract P1416

Baculovirus-Insect Cell Expression of Avian Flu Hemagglutinin and Chimeric Virus-Like Particles

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Influenza viruses are enveloped viruses of the Orthomyxoviridae family with a single-stranded, eight-segmented RNA genome of negative polarity. The influenza hemagglutinin (HA) glycoprotein is the major envelope protein accounting for ~80% to form the spikes. The M1 matrix protein is the most abundant protein in the viral particles that are the key driving force for forming recombinant influenza virus-like particles (rVLPs). Here we used baculovirus-insect cell expression system to express the full-lengh (H5HA (A/Tailand/1(KAN-1)/2004), the ectodomain with or without cleavage site mutation, the ectodomain hybrided with the transmembrane domain of a coronavirus. rVLPs were obtained by baculovirus-insect cell expression of M1 gene of H1HA (A/WSN/33), and fabricated with this series of H5HAs and fluorescence-tagged M2. This approach can be used for vaccine evaluation and screening drug candidates against avian influenza virus infection.

Abstract P1417

Influenza Viruses Lacking M2 Protein as Potential Live Attenuated Influenza Vaccines

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It has been demonstrated that the M2 protein of influenza viruses is important not only for uncoating, but also for packaging of the viral genome. Further, an influenza virus

lacking the transmembrane and cytoplasmic domains of M2 (M2 knock out; M2KO) was shown to be attenuated in both cell culture and mice as compared with its wild-type counterpart. Here we examined the efficacy of an M2 KO influenza virus as a live attenuated influenza vaccine. The M2 KO virus grew as efficiently as the wild-type virus in cells stably expressing the wild-type M2, indicating the feasibility of production of the vaccine. Mice inoculated with 5×10^3 plaque forming units (PFU) of the wild-type virus exhibited significant weight loss and ultimately died, whereas mice inoculated with even a 500 times higher dose of M2 KO virus than the wild-type did not show any signs of weight loss, indicating that the M2 KO virus is highly attenuated. All mice intranasally vaccinated with ≥ 3×10^4 PFU of M2 KO virus developed a high titer of antibodies against the wild-type virus and survived when challenged with lethal doses of the wild-type virus, substantiating the potential of the M2 KO virus as a live attenuated vaccine.

Abstract P1418

Microcarrier Cell Culture System for the Development of Influenza H5N1 Vaccine

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In the past decades, influenza vaccines have been mainly manufactured by egg-based culture, which is labor-intensive and lack of flexibility. In addition, the current egg-based flu vaccine production capacity would not be able to meet the demand if an influenza pandemic occurs. Therefore, vaccine production using mammalian cell technology is becoming viable and attractive. In this study, an H5N1 vaccine strain (NIBRG-14) obtained from the UK National Institute for Biological Standards and Control was successfully grown in Madin-Darby canine kidney cells using a microcarrier culture system. Various process parameters such as medium content, virus infection titer and cell metabolism were optimized in 100-ml spinner flasks. In a 1-L bioreactor system, peak virus titer could reach 2048 HA units/50 µL and $10^{8.6}$ $TCID_{50}$/ml using serum-contain medium. After purification and inactivation, HA protein content reached 31.56 µg/ml. In mice immunogenicity study, two doses of the purified vaccine antigen adjuvanted with alum phosphate induced good immune responses in low (0.2 µg HA) and high (1.0 µg HA) dosages (HI antibody geometric mean titers: 113 and 243, respectively). In conclusion, this study demonstrated an attractive strategy for scaling-up and provided valuable information on the development of a large-scale microcarrier culture process for producing inactivated influenza H5 vaccine. (Financial Supported by Taiwan Pandemic Influenza Vaccine R&D Program and NHRI).

Abstract P1419

Conjugation of Immunostimlatory DNA to Conserved Viral Antigens as a Universal Influenza Vaccine

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Standard influenza vaccines rely on generating strain-specific neutralizing antibody responses to prevent viral infection. Frequent viral mutations of the envelope hemagglutinin proteins result in a yearly race to select appropriate strains and produce vaccine to meet public health needs. These annual efforts to produce standard flu vaccine do not address the threat of emerging pandemic strains. To address the deficiencies of standard vaccines, we have conjugated the conserved influenza antigen nucleoprotein (NP) to immunostimulatory DNA sequences (ISS) to generate a highly immunogenic antigen (NP-ISS) that can be used alone or in combination with standard vaccine to induce potent, broadly reactive immunity. Strong NP-specific cell mediated immune responses that kill virus infected cells may provide heterosubtypic immunity that can limit virus replication, speed viral clearance, reduce morbidity and transmission of influenza without the need to create a new vaccine every year. We have tested the immunogenicity of NP-ISS alone and in combination with standard inactivated vaccine in various animal models. NP-ISS induces strong Th1 and CTL responses that reduce viral titers and provide protection against shift and drift strains in mouse challenge systems. Co-delivery of NP-ISS with standard vaccine enhances the antibody responses to HA in both mouse and primate models, and allows greater than a 30-fold dose sparing of HA in the mouse model. NP-ISS represents a unique vaccine component that can provide strong, cross-strain protective immunity and may be used in conjunction with standard vaccine to enhance immunogenicity and provide dose sparing of the HA components. We are testing the activity of other conserved influenza antigens conjugated to ISS. The use of ISS conjugated conserved antigens represents a promising approach to creating a broadly reactive, universal influenza vaccine. Research funding supported by NIAID grant 5U01 AI56447-4.

Abstract P1420

A Live H5N1 Influenza Vaccine That Lacks the NA Segment

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Highly pathogenic avian H5N1 influenza A viruses have been spreading throughout Asia, Europe, and Africa since 2003. Although human-to-human transmission is very rare, more than 250 people have been infected by these viruses, with high mortality, raising serious concerns about their pandemic potential. The development of an effective vaccine for H5N1 influenza is, therefore, of great and urgent import. Here, we generated a mutant A/Vietnam/1194/04 virus that lacks the NA segment (VN1194NA-) by reverse genetics and tested its efficacy as a vaccine in a mouse model. The VN1194NA- virus was viable in cell culture and replicated in only the nasal turbinates of infected mice. Three weeks after being immunized with $1x10^5$ pfu of VN1194NA- virus, mice were challenged with 100 LD_{50} of highly pathogenic H5N1 VN1194, A/Vietnam/1203/2004, and A/Indonesia/7/2005 viruses. All of the immunized mice survived, whereas all of the unimmunized mice died of systemic infection. Viruses were isolated on day 6 post-challenge from lungs but not from the nasal turbinates, brain, spleen, or kidneys of immunized mice, indicating that the VN1194NA- lacking its NA segment protected mice from lethal and systemic infections caused by highly pathogenic H5N1 viruses. These results demonstrate the viability of this strategy for the development of vaccines against H5N1 virus infection.

Abstract P1421

Enhanced Antibody Induction With a DNA Vaccine Expressing a Severely Truncated Influenza HA

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Whilst good immune responses and protective data have been derived for DNA vaccines in animal models, a stumbling block to vaccine development has been mimicking this response in humans and large animals, and a variety of methods are being assessed to improve their immune response. We report an enhanced antibody response to an unusual influenza HA DNA construct. During construction, a fragment of bacterial genomic DNA spontaneously inserted itself into the HA gene causing premature termination of translation approximately one-third the way into the HA2 region. Curiously, antibodies against this truncated HA protein recognise intact virus and demonstrate HI and VN activity. In addition, the truncated HA induces an approximate 10 fold increase in anti-HA antibody titre compared with full length HA. The presence of the bacterial DNA is not a factor in the increased antibody response whilst the secreted nature of the truncated HA is a contributory factor. The structure of the HA responsible for the enhanced immune response will be described along with other forms of immunologically active truncated HA.

Abstract P1422

Mucosal or Systemic Delivery of VLPs Induces Protective Immunity Against Homologous and Heterologous Strains of Influenza Virus

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Recurrent outbreaks of highly pathogenic avian influenza virus pose the threat of pandemic spread of lethal disease and make it a priority to develop safe and effective vaccines. Influenza virus-like particles (VLPs) have been suggested to be a promising vaccine approach. In this study, we developed VLPs containing influenza A/PR8/34 (H1N1) hemagglutinin (HA) and matrix (M1) proteins, and investigated their immunogenicity, long-term cross-protective efficacy, and effects on lung pro-inflammatory cytokines in mice. Intranasal or systemic immunizations with VLPs containing HA induced high serum antibody titers and neutralizing activity against PR8 as well as A/WSN/33 (H1N1) viruses, and protected mice from a lethal challenge with mouse-adapted PR8 or WSN viruses even 5 months post-immunization. Influenza VLPs induced mucosal IgG and cellular immune responses, which were reactivated rapidly upon virus challenge. Long-lived antibody secreting cells were detected in the bone marrow of immunized mice. Immune sera when administered intranasally were able to confer 100% protection from a lethal challenge with PR8 or WSN, which provides further evidence that anti-HA antibodies are primarily responsible for preventing infection. Taken together, these results indicate that non-replicating influenza VLPs represent a promising strategy for the development of a safe and effective vaccine to control the spread of lethal influenza viruses.

Abstract P1423

A Study on the Immunogenicity of a H5N1 Vaccine Based on the Hemagglutinin of A/Indonesia/CDC625/2006

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Since 1997, highly pathogenic avian influenza A (H5N1) viruses have infected humans with lethal outcomes. Originated from Asia, the viruses have spread to other continents with increasing number of human cases been recorded in recent years. These events have raised concerns that the viruses will eventually acquire the ability to be transmitted directly from human-to-human, leading to a pandemic. The availability of a pandemic vaccine thwarts this threat and many innovative methods have been developed to address this need. Using reverse genetics, our laboratory have generated an inactivated H5N1 vaccine containing a modified hemagglutinin (HA) gene from the A/Indonesia/CDC625/2006 (H5N1) and the rest of the seven genes from the A/Puerto Rico/8/34 (H1N1). The size and the appearance of the recombinant virus were similar to the parental strain which was tiny with a diffused edge. The growth of the recombinant virus was well-supported by 10-day old embryonated chicken eggs with up to 5 x 10^8 plaque forming unit (pfu) per mL, although this was still about 4-fold less than the parental strain. The hemagglutinin activity could be readily detected by both turkey and horse red blood cells. Mice injected subcutaneously with formalin-inactivated whole virion elicited good humoral neutralizing activity 3 weeks after immunization. This response could be further enhanced by the addition of an external adjuvant (p value < 0.05). Using a murine immature dendritic cell (DC) line, D1 cells, we show that the virus is capable of activating DC, including up-regulating the mRNA expression of certain chemokines. However, the responses were substantially weaker than those induced by other immunostimulants, such as lipopolysaccharides. No significant difference was found between the DC responses to the recombinant virus against the parental strain. Inactivation of the viruses using ultraviolet light did not affect their ability to activate DC. Based on these findings, including a human-compatible adjuvant into the pandemic vaccine might be an essential step to potentate the immunogenicity of the vaccine. Currently, several experimental adjuvants are in trial to examine their adjuvanting property on our H5 vaccine.

Abstract P1424

Induction of Heterosubtypic Immunity to Influenza Virus by Immunization With Inactivated Virus

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Vaccines against avian influenza viruses, that are considered highly potential pandemic threats to humans, are not currently available. Although recovery from live influenza virus infection is known to induce heterosubtypic immunity, the protective mechanisms particularly by inactivated virus are not well understood. In this study, we investigated the heterosubtypic protective immunity induced by inactivated influenza virus. Intranasal immunization of mice with inactivated influenza virus A/PR8 (H1N1) provided complete protection against the homologous virus and a drift virus within the same subtype, A/WSN (H1N1), but only a low level of partial protection against the heterosubtypic virus, A/Philippines (H3N2). However, co-administration with cholera toxin subunit B as adjuvant significantly increased this heterosubtypic protection to 100%. Analysis of immune correlates indicated that humoral immune responses with cross-reactive neutralizing activity in respiratory mucosal sites and in blood played a major role in conferring the protective immunity against heterosubtypic A/Philippines infection. Inactivated influenza virus was found to bind to dendritic and monocyte/macrophage cells suggesting a potential benefit for mucosal delivery of influenza vaccine. This study has a significant implication for developing vaccines against pathogenic avian influenza virus.

Abstract P1425

A Chimeric Virus-Like Particle Vaccine Containing Influenza Hemagglutinin and Neuraminidase Induces Complete Protection Against Both Homotypic and Heterosubtypic Challenge

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We have designed an alternative influenza virus-like particle (VLP) vaccine strategy based on the simultaneous expression of the murine leukemia virus (MLV) Gag protein

along with influenza HA and NA. The high yields of MLV Gag and the natural targeting of Gag, HA and NA to lipid raft domains result in significant yields of chimeric influenza VLPs that are consistently 100 nm in diameter, strongly immunogenic, and easily purified. Infection of Sf9 cells with a single baculovirus vector encoding MLV Gag, and the HA and NA of A/PR/8/34 (H1N1) results in VLP accumulation in medium supernatants. Purification of VLPs from serum-free medium by sedimentation at 100,000 x g followed by banding on 20-60% sucrose step gradients results in the appearance of a tight visible band associated with a sharp peak of hemagglutination activity. SDS-Page and Western blot analysis of these fractions show the presence of Gag, HA and NA with minimal contaminating proteins. In two separate experiments, immunization of mice at 0 and 4 weeks with 5-7 µg of H1N1-VLPs (containing approximately 0.5 µg HA) formulated with a TLR-4 agonist by I.M. or I.P. inoculation resulted in mean hemagglutination-inhibiting (HAI) titers against A/PR/8/34 (H1N1) of approximately 1:1000, but no detectable HAI activity against A/HK/68 (H3N2). However, challenge of H1N1-VLP-immunized animals 4 weeks following the second immunization resulted in 100% protection against both A/PR/8/34 (H1N1) and A/HK/68 (H3N2) challenge. While H1N1-challenged mice showed no evidence of weight loss and morbidity, H3N2-challenged mice exhibited only minor weight loss, remained fully active, and rapidly regained weight. In contrast to vaccinated animals, naïve control mice challenged with either virus became morbid and died. These results demonstrate that MLV Gag-based chimeric VLPs represent a potent vaccine strategy with the potential for protection across subtype boundaries. H3N2 and H5N1 VLPs are now being produced and are being evaluated for vaccine efficacy.

Abstract P1426

Development and Characterization of Novel Live Attenuated Influenza B Virus Vaccine Candidate

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B/Lee/40 virus was cold adapted by serial passages in progressively low temperatures for use as a vaccine donor strain. Temperature sensitive and cold-adapted phenotypes were achieved during adaptation. Cold-adapted B/Lee/40 was attenuated in mice and replicated in upper and lower respiratory tract at very low level. The induction of virus specific immune response was confirmed by enzyme-linked immunosorbent assay, hemagglutination inhibition and neutralization assay. We generated reassortant virus carrying two major surface proteins, hemagglutinin and neuraminidase, from virulent B/Shangdong/7/97 and other internal genes from cold-adapted B/Lee/40. Reassortant virus also appeared to be attenuated and protected mice from lethal challenge with wild type B/Shangdong/7/97. Vaccination with reassortant B/Lee/40 virus also resulted in specific antibody response and inhibited wild type virus replication in mice. The results suggest that the live influenza vaccine could provide effective prevention of influenza B virus epidemics.

Abstract P1427

Combining the Use of Whole and Split Virus Vaccine Formulations in Mouse Model as an Alternative Prime-boost Vaccination Strategy

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Vaccination against influenza remains the best available method of limiting the impact of influenza. However, the pandemic threat of influenza presents a need for novel vaccination strategies. An ideal pandemic vaccine candidate should elicit a rapid immune response and should be able to mount both cell-mediated and humoral immunity. Split virus vaccines elicit antibody-mediated protection against homologous or closely related strains whilst whole virus vaccines may also induce a cell-mediated immune response with broad cross-reactivity. The present study investigated the systemic humoral and cellular immune responses in mice after vaccination utilizing a prime-boost regime which combines the use of split and whole influenza vaccine formulations. Mice were vaccinated intramuscularly with one or two doses of inactivated influenza split and whole virus vaccines containing 15 µg HA of A/Panama/2007/99 (H3N2) strain per dose. Sera were collected at various time points after vaccination and used in ELISA and HI assays to examine antibody response. Splenocytes were isolated at days 6 and 4 after the first and second dose of vaccine, respectively, and used in analyses of T-cell response by flow cytometry and ELISPOT assays. Additionally, splenocytes were activated in vitro and supernatants were used to determine the concentrations of IFN-γ, Interleukin (IL)-2, IL-4, IL-5 and IL-10. We showed that whole virus vaccine induced a rapid antibody response and a dominant Th1 profile (IFN-γ and IgG2a), which may suggest a stimulation of cell-mediated immunity. In contrast, vaccination with split virus vaccine induced a more Th2 skewed immune

response indicating the enhancement of humoral immunity. However, mice vaccinated with a priming dose of whole virus vaccine and a booster dose of split virus vaccine were able to elicit a rapid primary and secondary immune response and a balanced Th1/Th2 profile suggesting an effective stimulation of both humoral- and cell-mediated immunity. In contrast, using split virus vaccine as a priming dose and whole virus vaccine as a boosting dose did not change the dominance of the Th2 response observed after the first dose. Therefore, utilizing a prime-boost regime with whole and then split virus vaccine most effectively stimulated both arms of the adaptive immune system.

Abstract P1428

Cross-protective Efficacy of Reverse-Genetics-Based H5N1/PR8 Reassortant Vaccines Against Challenge Infection With Homologous and Heterologous Clade Viruses in Mice

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Since 2003, highly pathogenic H5N1 influenza A viruses have caused outbreaks in poultry which resulted in confirmed human infections in 11 countries. There is concern that H5N1 may cause a pandemic if it acquired the ability to transmit among humans. A major component of preparedness plans to mitigate the impact of an H5N1 pandemic is vaccination. CDC has developed several reverse genetics-derived pre-pandemic vaccine candidate viruses. H5N1 influenza viruses isolated during the past three years are categorized into several different clades based on genetic and antigenic characteristics of the hemagglutinin (HA) gene. Most currently circulating H5N1 viruses belong to clade 2 and this group can be further divided into sublcades. Pandemic vaccines we developed include strains from the most highly prevalent clades in poultry that were also causing human infections. As countries prepare for a possible H5N1 pandemic by stockpiling vaccine, the choice of an appropriate vaccine strain becomes paramount. To elucidate this selection, we analyzed the protective efficacy of vaccines from each of the clades against infection with viruses from a heterologous clade. Reassortant viruses containing attenuated HA and intact neuraminidase (NA) genes from A/Vietnam/1203/04 (clade 1), A/Vietnam/Hanoi30408/04 (clade 1) and A/Indonesia/05/05 (clade 2) and 6 internal genes from A/Puerto Rico/8/34 (PR8) viruses were produced and designated as VN1203/PR8, VN30408/PR8 and Indo05/PR8, respectively. Mice were immunized with 10 micrograms of each formalin-inactivated vaccine with or without Alum twice, two weeks apart and were challenged with two clade 1 viruses 4 weeks after the second immunization. All mice immunized with reassortant vaccines were completely protected from lethal challenge infection. Immunized mice showed no sign of illness and all survived. Hemagglutination inhibition (HI) titers in sera from immunized mice revealed protective level of HI antibody (>40HI) against the homologous vaccine strain but not the heterologous strain after the second immunization. Preliminary results from microneutrarization test showed a similar pattern of antibody reactivity. The results indicate that mice vaccinated with inactivated H5N1 vaccine against one clade can develop cross-protective immune responses against a different clade.

Abstract P1429

Use of BALB/c Mouse Model Measuring Antigenic Variation and Degrees of Cross-protection Between Pre-pandemic Vaccines and a H5N1 Antigenic Variant

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Highly pathogenic avian influenza A H5N1 viruses may acquire the ability to cause the next pandemic. Since these viruses first emerged in humans in 1997, multiple pre-pandemic vaccine strains have been generated and stockpiling of egg-based vaccines has begun in several countries. Optimal influenza vaccines are those that are antigenically closely matched with the circulating disease-causing strain. Unfortunately, because of continuing evolution of influenza viruses, pre-pandemic vaccines may not be an exact antigenic match with the eventual pandemic strain. We have used the BALB/c mouse model in an effort to better understand the potential of two H5 pre-pandemic vaccines to provide cross-reactive antibody and cross-protection against an antigenic variant H5N1 virus, A/Vietnam/1203/2004 (VN/1203). Formalin inactivated purified whole virus vaccines were prepared from either A/Duck/Singapore-Q/F119-3/97 (Dk/Sing, H5N3) or A/Hong Kong/213/03 (HK/213) virus. Mice were immunized intramuscularly with one dose of vaccine equivalent to 3 µg of hemagglutinin (HA) protein. And post-vaccination sera were collected to assess the extent of antibody cross-reactivity between these two H5 vaccines and VN/1203 virus by Horse hemagglutination-inhibition (HHI), micro-neutralization, and ELISA. Both H5 vaccines elicited HHI and neutralizing antibody responses to homologous viruses and also induced substantial anti-VN/1203 HA-specific IgG in all immunized

mice. Compared with Dk/Sing, HK/213 vaccine induced higher cross-reactive HHI, neutralizing, and HA-specific IgG antibody response to VN/1203 virus. To compare the relative cross-protective efficacy induced by the two H5 vaccines, mice were challenged intranasally with $10^{4.5}EID_{50}$ (=200 LD_{50}) of the antigenic distinct VN/1203 virus. Both H5 vaccines provided cross-protection from death and severe illness, but fail to protect animals from lung infection and mild illness. Mice that received HK/213 vaccine, which was antigenically closer to VN/1203 virus, exhibited significantly less weight loss and lower lung viral titers compared with that detected in mice immunized with the Dk/Sing vaccine. These data suggest that the BALB/c mouse model could be used to monitor the likely degree of cross-protection afforded by pre-pandemic vaccine candidates for newly emerging variants with pandemic potential.

Abstract P1430

Improved Immunogenicity of a Live, Attenuated Influenza A H5N1 Candidate Vaccine Based on A/HONG KONG/213/2003 (H5N1) is Associated With Strain-specific Efficacy Following a Single Dose of Vaccine in Mice and Ferrets

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We have previously evaluated live attenuated influenza virus (LAIV) vaccines against H5N1 viruses that were generated through reverse genetics where the modified hemagglutinin (HA) and neuraminidase (NA) genes came from either A/VietNam/1203/04 (H5N1) (2004 wt) or A/HK/491/97 (H5N1) (1997 wt) while the six internal protein genes were derived from the cold-adapted influenza A/AnnArbor/6/60 (H2N2) (AA ca) master donor vaccine strain. Results from preclinical studies in mice and ferrets revealed that two doses of vaccine were required to induce detectable serum neutralizing (Nt) and hemagglutination-inhibiting antibodies (HAI Ab) and complete protection from replication of homologous and heterologous H5N1 wt challenge viruses in mice and ferrets. A specific amino acid residue in the H5 HA [N223 of A/HK/213/2003 (H5N1) (2003 wt)] has been identified to enhance the immunogenicity of the HA protein (PNAS 102:12915, 2005). We also generated a vaccine with the HA and NA genes derived from 2003 wt to determine whether an LAIV vaccine with a more immunogenic H5 HA would also be more efficacious. Unlike the 1997 ca and 2004 ca vaccines, a single dose of the 2003 ca vaccine induced serum Nt and HAI Ab titers against the homologous wt virus in mice and ferrets and a single dose of this vaccine provided complete protection from challenge with the homologous, but only partial protection from heterologous, H5N1 wt viruses in both models. Two doses of the 2003 ca vaccine elicited a boost in Nt Ab titer and broadly cross-protective serum Nt Ab response and conferred complete protection against pulmonary viral replication and extrapulmonary viral dissemination of homologous and heterologous H5N1 wt challenge viruses in mice and ferrets. Similar results were obtained in animals that received two doses of either the 1997 ca or 2004 ca vaccine. Thus, the enhanced immunogenicity of the 2003 ca vaccine correlated with efficacy against challenge with homologous wt virus after a single dose, but did not extend to cross-protection from challenge with variant H5N1 wt viruses. As with the other H5N1 ca vaccine candidates, two doses of the 2003 ca vaccine were required for protection against heterologous H5N1 viruses.

Abstract P1431

A Novel Dry Powder Vaccine Formulation (GelVac) for Influenza Vaccines

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A novel dry powder vaccine formulation (GelVac) suitable for nasal delivery as well as reconstitution for injection is being developed for influenza vaccines. It is based on a distinct ionic polysaccharide (GelSite polymer) that provides an in-situ gelling property for sustained antigen release and also an antigen stabilization effect. Both inactivated whole virion and split antigens have been successfully tested with the formulation. The powder vaccine has been stable at ambient room temperature for over 2 years (ongoing). When delivered intranasally, GelVac powder vaccine was highly immunogenic and protective in rats, an animal model suitable for powder delivery. A distinctive protective effect in the upper respiratory tract was observed compared to immunization by injection. A phase I clinical safety study with GelVac powder without an antigen demonstrated consistent nasal delivery, no lung deposition or sneezing, and good acceptance. This same powder or equivalent dried vaccine formulation could be readily reconstituted for injection. A much higher immune response based on serum HAI titer was obtained following one or two intramuscular immunizations in animal models. A strong adjuvant-like or immunoenhancing effect of the GelSite

polymer (≥ 2 fold higher by HAI) was demonstrated at a very low polymer concentration. These results from preclinical and clinical studies clearly indicate the potential advantages of this innovative dry powder vaccine formulation for influenza vaccines – room temperature stability, prolonged shelf life, and cold chain free distribution that are critical for pandemic preparedness and epidemic control.

Abstract P1432

Live Influenza Vaccine Provides Broad Spectrum and Immediate Protection Against Heterologous and Heterotypic Lethal Challenge in Mice

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A live attenuated influenza vaccine has been evaluated as fast-acting prophylactic against acute influenza infection. In a mouse infection model, concurrent vaccination with the cold-adapted influenza virus (X-31 ca, A/H3N2) and virulent challenge resulted in reduction of pathogenic symptoms of acute infection. Moreover, vaccination one to four days prior to lethal challenge by heterologous (A/H1N1) or heterotypic (influenza B) virus provided enhanced protection. Vaccine specific antibody was not detected within five days following vaccination and did not cross-react with influenza B virus antigen. Vaccination immediately prior to challenge resulted in the generation of significant pool of reassortant virus between the vaccine and virulent strains suggesting that the acquisition of attenuating gene(s) from the live vaccine contributes to the attenuation of virulence. Our results suggest that immediate protection from lethal challenge is mediated in part through genetic interference by live vaccine without the involvement of a specific antibody response. The results of this study demonstrate that immediate and broad spectrum protection is conferred by live influenza virus vaccine and could be further extended to various influenza strains. This vaccination strategy may offer new options for the control of influenza outbreaks and could be used to stem a potential influenza pandemic.

Abstract P1433

New Influenza Vaccine Grippol TC: Cell-derived Viral Antigens in Combination With Novel Adjuvant

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A wide spectrum of modern vaccines is available today for infectious diseases immune prophylaxis and control. The situation regarding influenza vaccines is one of the most favorable. However there are some problems still to be resolved with a view to future perfection. Most of the existing vaccines contain viral antigens obtained from the egg-allantoises-derived virus. The manufacture of the vaccines is therefore directly dependent on the availability of eggs. Furthermore this restricts the use of vaccine in persons with a known allergy to egg-proteins. On the other hand, there are difficulties concerning the vaccination efficacy of the individuals with low immune response (including elderly and children under 3 years of age). There is one more field of vaccine development management of great importance today: discovery of new safe vaccine adjuvants that on the one hand allow the antigen content to decrease and on the other hand provide increased vaccination efficacy even in persons with low immune response. Another prospective investigation field is the application of cell-derived viral antigens as an alternative to conventional egg allantoises technology for vaccine production. This approach offers several undoubted advantages. On WHO experts opinion, in view of avian influenza situation, the use of cell-derived viral antigens for vaccine production makes manufacturing independent and stable, with the opportunity to significantly increase production capacity. Five years ago two companies Solvay Pharmaceuticals BV in the Netherlands and Petrovax Pharm company in Russia combined their efforts, scientific potential and own patented technologies in order to present the world with new influenza vaccine that combines the state-of-the-art technological features in the field of influenza vaccine development. New vaccine Grippol TC contains immune modulator Polyoxidonium and antigens of cell-derived influenza virus (cell culture MDCK). This principally new technology was developed and implemented by Solvay Pharmaceuticals B.V. for the influenza vaccines production process. Cell technology provides following advantages: 1) a virus growing on the well known controlled cell-culture substrate enabling the process to became a standardized closed procedure excluding any type of contamination; 2) manufacturing stability making vaccine production independent of an external substrate supplier; 3) the possibility of rapid increase in production volume; 4) an increase in the number of vaccinated people due to the inclusion of

individuals with egg allergy. Polyoxidonium was synthesized by Professor Nekrasov A.V. and is already patented in Russia, Europe and USA. PO is biogenic biodegradable water-soluble compound that is metabolized in the organism by natural way. PO is absolutely safe and cause neither immediate nor delayed side effects. This has been confirmed by many years of PO application in different fields of clinical practice as an immune modulator and detoxicant. The new influenza vaccine presented therefore combines two modern technologies: viral antigens obtained from sterile cell-derived influenza virus and safe immune adjuvant Polyoxidonium. New influenza vaccine Grippol TC is the modified analogue of influenza trivalent polymer-subunit vaccine Grippol (Russia) that has been used in Russia for mass influenza prophylaxis more than ten years. The unique property of Grippol is that it contains three times less of antigen content in comparison with other existing inactivated split and subunit vaccines. Extensive experience of Grippol application over many years shows an excellent safety and high prophylactic effectiveness, even in persons with low immune reactivity. Now Grippol TC successfully passed non-clinical trials, permission for clinical trials is already received. Grippol TC registration is in process and Clinical Trials will begin soon.

Abstract P1434

Antibodies Against Hemagglutinin of a H5N1 Strain of Avian Influenza A Virus Block Cell-cell Membrane Fusion Induced by Defective Vaccinia-HA5 Recombinant

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The hemagglutinin (HA) gene of an avian influenza A virus (H5N1, Vietnam strain) was incorporated into a vaccinia virus (based on the New York City Board of Health strain). The recombinant virus dVV-HA was defective and could propagate only in RK44 cells stably expressing an essential vaccinia virus protein. The dVV-HA was purified by ultracentrifugation through sucrose cushion and gradient. It was found that the HA of the H5N1 strain was assembled into the vaccinia virion by Western blot assay. FACS assay showed that the HA was expressed on the surface of dVV-HA infected cells, indicating that the HA was properly glycosylated, oligomerized, matured and transported to the cell membrane. The cell-cell membrane fusion was observed when infected-RK44 or 293T-cells were treated by low pH buffer (pH 5.0) and trypsin (0.1µg/ml), indicating that the HA expressed in RK44 and 293T cells was functional. Interestingly, rabbit antibodies, raised using the same HA protein purified from a baculovirus expression system, dramatically inhibited the cell-cell fusion compared to pre-immunization sera and non-related antibodies. The preliminary results suggest that the antibodies against the H5 protein prevent the spread of viruses from cell to cell. Safe nonreplicating poxviral vectors expressing H5-HA including the defective vaccinia recombinant or vectors based on modified vaccinia Ankara may be developed into vaccine candidates and used to prime against H5N1 avian strains.

Abstract P1435

Grippol TC: Non-clinical Studies Summary

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A new influenza vaccine was developed using the latest modern technologies in the field of vaccine manufacture. This new vaccine, Grippol TC, (a modified analogue of Russian influenza vaccine Grippol®) includes MDCK-derived influenza virus antigens and biogenic water-soluble synthetic immune adjuvant Polyoxidonium. Preclinical studies were performed to evaluate immunogenicity, safety and toxicity of Grippol TC. Mice were vaccinated twice with half a dose of Grippol TC. An increase in hemagglutinin inhibition (HI) titers was found for all three virus strains included in the vaccine. Furthermore, comparative studies between the eggs-derived vaccine Grippol and MDCK-derived vaccine Grippol TC demonstrate comparable immunogenicity between the vaccines. Studies of acute toxicity, local tolerability, sensibilising properties, and pyrogenicity and safety in different animals (mice, rats and guinea pigs, rabbits) demonstrated Grippol TC and Grippol® equivalence. Thus, appearance, clinical examination results, body mass, food and water consumption, CNS activity indexes, patho-morphological study results (necropsy, organs weight, marrow smears, histological data) in animals immunized with Grippol TC were similar to that for Grippol-immunized animals demonstrating new vaccine safety and non-toxicity. Bone marrow picture as well as hematological indexes studied reflected natural development of post-vaccination response. The incidence of allergic reactions was very low and the probability of allergic reactions occurrence was considered to be minimal. Considering adjuvant presence it was important to conduct a broadened investigation on Grippol TC immune safety. The capability of Grippol TC to influence the humoral, cellular, and macrophagal immune response chains in mice at the different post-vaccination periods was assessed. It was found that the post-vaccination specific immune response formation had no negative impact on native immune reaction development to the vaccine-heterologic antigens, neither at

early nor at delayed post-vaccination intervals. In addition, a stimulating effect on humoral immune response and increase of phagocytic cells activity was registered. The results indicated the absence of polyclonal non-specific activation testifying Grippol TC safety for host immune system. These data indicate that in preclinical studies Grippol TC was immunigenic and safe. Clinical trials Phase I are planned in spring 2007.

Abstract P1436

Immune Response to Live Attenuated H5N1 Influenza Vaccines in Ferrets

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A number of avian influenza virus subtypes have been reported in humans and the avian H5N1 subtype has caused over 160 deaths over the past three years. Live, attenuated viral vaccines are being developed with the goal of preventing severe disease and death caused by pandemic influenza. To produce live attenuated H5 pandemic influenza vaccine candidates, the multibasic amino acids at the proteolytic cleavage site between HA1 and HA2 were removed from H5N1 viruses isolated from humans in 1997, 2003 and 2004. Modified HA and NA gene segments from these H5 viruses were combined with the six internal gene segments of the cold adapted A/Ann Arbor/6/60, the master donor virus for FluMist® vaccines, using reverse genetics. The resultant candidate H5 vaccine strains all have the characteristic cold adapted (ca), temperature sensitive (ts) and attenuation (att) phenotypes. Ferrets immunized intranasally with the live, attenuated vaccine candidate, ca A/Viet Nam/1203/04, were completely protected from homologous and heterologous wt H5N1 challenge. Unlike most seasonal live, attenuated vaccine strains, ca A/VN/1203/04 replicated poorly in the upper airways of ferrets. Consistent with this observation, no serum HAI antibodies were detected after a single dose of vaccine; two doses were required to elicit a detectable serum HAI antibody response. Despite this fact, H5 influenza-specific IgG secreting B cells were detected using an ELISpot assay in the blood and paratracheal lymph nodes of ferrets vaccinated with a single dose of ca A/VN/1203/04 and the number of these cells increased following a second dose. Typical of most avian influenza viruses, the HA of ca A/VN/1203/04 binds preferentially to a2'3 sialic acid receptors. In order to evaluate the impact of this receptor preference on vaccine replication in the upper respiratory tract of ferrets and consequently on immunogenicity, a number of different ca A/VN/1203/04 HA variants containing single amino acid changes in the receptor binding region were obtained. Replication of these HA receptor variants in the upper respiratory tract of ferrets and their immunogenicity will be presented.

Abstract P1437

Intradermal Delivery as an Effective Alternative Route for Influenza Vaccination–Preclinical Evaluation in Mouse and Rabbit Models

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Influenza vaccine is administered mainly intramuscularly or subcutaneously. Alternative and potentially more efficient delivery routes have been explored. Cutaneous immunization has received much attention as the skin is highly accessible, is drained by a large array of lymphatic vessels and contains powerful antigen presenting cells at much higher levels than in deeper tissues. These cells, which include Langerhans dendritic cells in the epidermis and interstitial dendritic cells in the dermis, can induce an efficient immune response due to their capacity to sample and process antigens in situ before migrating to draining lymph nodes where they stimulate naïve resting T cells. Current influenza vaccines need to be improved, especially for the elderly who respond less vigorously to vaccination than younger adults and are more prone to influenza infection and its complications. Influenza vaccination via the intradermal (ID) route could provide enhanced protection for this population. We used mouse and rabbit models to document the safety and immunogenicity of trivalent inactivated influenza vaccine delivered ID. Different dosages were administered ID to young adult (6 week-old) BALB/c mice and compared with IM. The humoral response induced by ID vaccination (specific ELISA IgG and hemagglutination inhibition, HI) was significantly higher for all three vaccine strains than that induced by the IM control. ELISA and HI antibody persistence after 5 months was comparable with ID and IM: similar titre drop observed in the two groups. Immune responses induced by ID vaccination were also compared between aged (17 month-old) and young (6 week-old) C57BL/6 mice. Titers were lower in aged mice than in young mice. However, by increasing the ID vaccine dosage in the aged animals, we were able to restore the influenza-specific response. Finally, repeated dose toxicology studies in rabbits revealed no systemic toxicity and transient local erythema and edema. Conclusion: the intradermal delivery of a trivalent inactivated influenza vaccine was shown to be safe in animal models and able to induce a higher immune response

than intramuscular vaccination. These encouraging results led to the ongoing clinical evaluation of an intradermal influenza vaccine using an easy-to-use and reliable microinjection system.

Abstract P1438

Evaluation of a PER.C6® Cell Grown Influenza H7N1 Virus Vaccine in Balb/c Mice

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The ongoing threat posed by avian influenza highlights the need for global preparedness. Influenza H5 and H7 subtypes cause pathogenic outbreaks in domestic poultry, and both these subtypes have caused human fatalities. There is a need for a better understanding and increased knowledge of these subtypes as vaccine candidates to ensure an appropriate vaccine could be rapidly produced in the event of a pandemic. The FLUPAN consortium has developed the first human vaccine against avian influenza H7N1 virus. The vaccine strain (RD-3) was produced by reverse genetics from a highly pathogenic avian influenza virus (A/chicken/Italy/13474/99 (H7N1). An inactivated split virus vaccine was produced on the PER.C6® human cell line and the vaccine used in a phase 1 clinical trial. A challenge study using a similar immunisation protocol to that used for the clinical trial was performed in mice to evaluate the protective efficacy of the vaccine. Groups of 20 Balb/c mice were immunised subcutaneously with two doses of vaccine containing 12µg or 20µg haemagglutinin per dose with or without aluminium hydroxide adjuvant (60µg aluminium). Fourteen days after the second immunisation animals were challenged with the live pathogenic influenza virus A/chicken/Italy/13474/99. Following challenge animals were weighed and observed for clinical signs of disease for fourteen days and nasal washings were collected daily for seven days to monitor virus replication. Antibody titres measured by haemagglutination inhibition were very low. However, survival rates in all the treatment groups were significantly improved compared to those in un-immunised controls. Mild usually transient clinical signs of disease were observed in all of the animals which received 12µg of non-adjuvanted vaccine and some animals in the 20µg adjuvanted and non-adjuvanted groups. All animals which received 12µg of adjuvanted vaccine were symptom free. Virus was recovered from fifteen and sixteen animals respectively in the low and high dose non-adjuvanted vaccine groups and twelve and eleven animals respectively in the low and high dose adjuvanted groups. The duration of shedding and titre of virus was significantly less than that observed in un-immunised controls. The study demonstrates that the PER.C6® H7N1 vaccine induces significant levels of protection in mice.

Abstract P1439

Initial Cellular Immunity and Cross Protection Studies in Balb/C Mice Immunized With an H5N1 Vietnam 1203 (Clade 1) and Indonesia/05 (Clade 2) Inactivated Whole Virus Pandemic Flu Vaccines

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Influenza specific T cell responses play a role in diminishing the lethal effects of highly pathogenic strains of Influenza and in speeding up recovery from influenza infection. To evaluate the extent to which H5N1 whole virus vaccines induce T cell responses, groups of BalbC mice were immunized with non-adjuvanted and alum adjuvanted H5N1 Vietnam (VN) 1203 and Indonesia (IN) /05 inactivated whole viral vaccines, a B/Jiangsu strain whole viral vaccine, or a recombinant H5-HA antigen using a 2 dose regime (day 1 and 21). Mice were sacrificed on days 7, 28 and 42 and their spleenocytes, in vitro stimulated with inactivated whole virus preparations (H5N1 VN1203; H5N1 IN/05: H1N1 NC; H3N2 NY and B JS) or r H5-HA antigen were analysed by ELISPOT. Other groups of mice, similarly immunized with either of the two H5N1 vaccines (with and without alum) were challenged on day 42 with either live VN1203 (clade 1), IN/05 (clade 2) or Hong Kong /156/1997 (clade 3) viruses and monitored for disease symptoms and death. The ELISPOT analysis indicated that large numbers of INF-γ secreting T cells (a marker, although not exclusively, for TH1 cells) were induced after antigen stimulation on day 8 and 28, subsequently declining slightly on day 42. In contrast, the number of IL-4 secreting T cells (a maker for TH2 cells) were low on day 8, increased markedly by day 28 and subsequently declined slightly on day 42. Thus it appears that a mixed INF-γ and IL-4 secreting T cell response was induced following the booster injection. Good cross stimulation could not only be observed among the H5N1 and rH5-HA antigens but also with the H1N1 and H3N2 whole viral preparations. The fact that no cross stimulation with the H1N1 and H3N2 whole viral preparations was observed for the rH5-HA immunized mice suggested that common antigens to Influenza type A strain,

such as the nucleoprotein or matrix protein are responsible for the Il-4 secreting T cell cross stimulation response. The challenge experiment demonstrated that immunization with the Vietnam 1203 or the Indoneasia/05 whole virus vaccines gave good homologous and cross clade protection against all 3 clades.

Abstract P1440

Investigations on Correlates of Immunity and Protection in Mice Immunized With Vero Cell-Derived H5N1 Whole Virus Candidate Vaccines

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Over the last decades HI (hemagglutination inhibition) antibody titers have been considered to be the best measurement for definition of correlates of immunity and protection of human influenza virus infections and vaccinations. With the emergence of avian influenza virus infections in humans, especially H5N1 in 1997 and, to a lesser extent, H7N7 and H9N2, it became more apparent that the HI assay may not the appropriate measurement for characterization of infection of humans with avian influenza A viruses or determining the efficacy of influenza vaccines with hemagglutinin A subtypes other than H1N1 or H3N2. It has been therefore generally accepted that additional serological assays such as neutralizations assays should be included the serological analysis of sera of pre-clinical studies in animals and clinical studies in humans. Baxter has produced H5N1 whole virus candidate vaccines against H5N1 clade 1 (A/Vietnam/1203/2004) and clade 2 (A/Indonesia/05/2005) strains at an industrial scale using its serum protein free Vero cell fermenter technology. These candidate vaccines have been shown to be highly immunogenic in mice and guinea pigs by inducing cross-neutralizing antibodies (which has been confirmed in a clinicical phase I/II study in humans) and showing cross-protectivity against the homologuous strain of the same clade and the heterologuous strains of the other clades in mice. The analysis of sera of dose-finding studies of these vaccines in mice has shown correlations between antigen dose, induction of binding antibodies (measured by ELISA) and neutralizing antibodies, and protection against disease symptoms or death after homologuous or heterologuous challenge with infectious H5N1 viruses of clade 1, clade 2, and clade 3. The suitability of the obtained results for determination of correlates of immunity and protection in a mouse model and their possible application for determination of the efficacy of H5N1 vaccines in humans will be discussed.

Abstract P1441

A Reverse-Genetics-Based Replication-Deficient DelNS1 Virus (H1N1) Influenza Vaccine Protects Ferrets Against Influenza After One Single Intranasal Immunization

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A genetically modified replication-deficient influenza IVR-116/delNS1 (H1N1) virus lacking the NS1 gene was evaluated for safety, immunogenicity and protection efficacy in ferrets. Except for marginal nasal serous discharge, no clinical findings, virus shedding or any significant deviations from the usual hematological and biochemical parameters were found in animals immunized intranasally with the IVR-116/delNS1 vaccine virus. Serum IgG titers and a marked increase in virus-specific mucosal IgA could be determined in immunized animals. Moreover, immunized ferrets were protected from such disease symptoms as fever, the occurrence of mucopurulent nasal discharge and decreased levels of animal activity after a wild-type virus challenge. In naïve animals, shedding of the challenge virus was detected in 50% of the animals one week after the wild-type virus challenge. In contrast, immunized ferrets cleared the challenge virus by day 3 after the challenge. Our findings strongly support the potential of replication-deficient influenza viruses lacking the NS1 gene as mucosal vaccine candidates with an optimal balance of safety and immunogenicity.

Abstract P1442

Development of Cross-Protective Vaccine Candidates Against H5N1 Influenza Viruses

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The highly pathogenic avian influenza (HPAI) H5N1viruses have caused devastating outbreaks in poultry and hundreds of fatal infections in humans. These viruses have also generated great concerns about a potential pandemic influenza. Vaccination is thought to be the most effective way for reducing illness and death in the event of influenza pandemic. The hemagglutinin (HA) protein is the primary target for neutralizing antibodies and the major effective component of current inactivated influenza vaccines. Since the viral HA genes are changing continuously, and whether the current H5N1 or other strains will be the cause of the next pandemic remain unknown, it is important to develop cross-protective vaccine candidates that may induce protective immunity against different influenza strains. In this study, we have synthesized a codon-optimized HA gene of A/Vietnam/1203/04 H5N1 strain (a reference strain for vaccine studies recommended by WHO), and then cloned the ectodomain of the HA into a mammalian protein expression vector. We examined the immunogenicity of the plasmid encoding the HA, the purified HA proteins, and the inactivated H5N1 viruses. Immunization of BALB/c mice with the HA-expressing plasmid DNA or HA proteins induced high titers of anti-HA antibodies, which was comparable to the titers generated by the inactivated H5N1 vaccine. We also found that the anti-HA antibodies were cross-reactive to the HA proteins of several other H5N1 strains isolated in China. To further examine the cross-protective immunity of these vaccine candidates against H5N1 strains in vivo, we challenged the control and HA-immunized mice with a highly pathogenic H5N1 strain A/Goose/HLJ/2003 (H5N1). This virus could kill 100% of infected chickens and ducks within 24h post infection, and cause 100% morbidity and about 80% mortality in mice within 2 weeks post infection. We observed the morbidity and mortality after the lethal challenge, and found that all the mice vaccinated with the HA-expressing plasmid DNA or HA proteins remained healthy and survived, whereas100% of the control mice (unvaccinated or vaccinated with control DNA or proteins) became sick and 80% of them died within 14 days post challenge. Our results indicate that it is possible to develop cross-protective vaccine candidates against potential pandemic H5N1 influenza viruses.

Abstract P1443

Preclinical Testing of an Inactivated Split-Virion Influenza A H5N1 Vaccine Administered Alone or in Combination With Aluminum Hydroxide Adjuvant or a New Adjuvant System

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Rationale: In the context of developing new adjuvants for pandemic vaccines, studies in mice and monkeys evaluated the immunogenicity and efficacy of an inactivated split-virion influenza A/H5N1 vaccine, given alone or in combination with aluminum hydroxide adjuvant (Al) or a new adjuvant system (AdjA). Methods: Mice received two IM injections of either non-adjuvanted, Al-adjuvanted or AdjA-adjuvanted vaccine. All 3 vaccines were formulated with an identical, sub-optimal dosage of 0.3µg of hemagglutinin. Cellular and hemagglutination inhibition (HI) responses were evaluated 3 weeks after the second injection. To evaluate efficacy, 4 groups of 8 cynomolgus macaques received 2 IM injections, 4 weeks apart, of either: non-adjuvanted, Al-or AdjA-adjuvanted vaccine (30µg HA), or a saline control. Three months after the second injection, monkeys were challenged intratracheally with parental wild-type influenza A/Vietnam/1194/2004 (H5N1) and sacrificed 5 or 15 days later. Immune responses were analyzed by ELISA and HI assays. Viremia was assessed in the upper and lower respiratory tract by RT-PCR and viral titration on MDCK cells. Histopathology of lungs examined the severity and extent of pneumonia. Results: In both species, functional antibodies (HI) were only detected after the second injection in animals that received adjuvanted vaccines. However, after the challenge an HI response was also detected in monkeys that received non-adjuvanted vaccine, but not in control animals, indicating a priming effect of the non-adjuvanted vaccine. Viral titration showed that monkeys vaccinated with adjuvanted vaccines were fully protected against challenge, whereas monkeys vaccinated with non-adjuvanted vaccine were partially protected in both lower and upper respiratory tracts. Consistent with this, broncho-interstitial pneumonia was reduced in extent and severity in vaccinated monkeys, especially in those vaccinated with the AdjA-adjuvanted vaccine. Conclusions: An inactivated split-virion influenza A H5N1 vaccine adjuvanted with aluminum hydroxide or the new adjuvant system protected monkeys against challenge with the parental wild-type virus with the most marked reduction of pneumonia observed with the new adjuvant. The antigen-sparing potential of this new formulation and it ability to induce cross-protection against other H5N1 strains are currently in clinical and preclinical evaluation.

Abstract P1444

New Paradigm in Influenza Transmission Prevention: Modulating Respiratory Secretions to Contain Cough Bioaerosol

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Rationale: Influenza virus is transmitted to non-infected subjects when an infected patient expels droplets loaded with pathogenic microorganisms to the surrounding environment as an aerosol when coughing or sneezing. Droplets concentration and size distribution may each play an important role in disease transmission. To control the spread of influenza virus, the path from the infected person (transmissor) to a non-infected person (recipient) must be interrupted. Increasing crosslinking-binding sites in the airway mucus has the potential to break the chain of transmission of influenza infection while enhancing mucociliary clearance, since more cohesive mucus will be more resistant to break-up during high-speed airflow interaction; hence fewer particles will be released as aerosol. Methods: To assess the primary mechanism of our mucomodulators (XLs), we have built our evidence moving from basic laboratory studies to an ex-vivo epithelial model and then to an in-vivo large animal model. We exposed anesthetized dogs to different dose concentrations of aerosolized placebo or mucomodulators. We assessed: cardio-respiratory pattern, tracheal mucus clearance, airway patency, and mucus viscoelastic changes. We used a mathematical modeling to evaluate the effect of viscoelasticity on the onset of instabilities that lead to droplet formation in the trachea. Results: We have acquired evidence of acceptable mucociliary clearance in an ex-vivo epithelial model, reduction in the number of particles from the aerosol pattern and increased "expectoration" in a system model. Dogs maintained normal cardio-respiratory pattern with XL administration. Tracheal mucociliary clearance in dogs showed a sustained 40% mean increase, and there was no mucus retention in the airways. Conclusions: The ex-vivo frog palate and the in-vivo mammalian models, appear to be appropriate and complement each other to better assess the effects that our mucomodulators exert on the mucociliary clearance defense mechanism. We achieved a desired target: particles were reduced to almost disappear from the aerosolization pattern, plus an unexpected increased "expectoration". Airway mucus cross-linked by mucomodulators is better cleared from a normally functioning respiratory system, either due to enhanced interaction with cilia or airflow-dependent mechanisms. Mathematical modeling also supports the concept that it is feasible to achieve reduction of the aerosol formed during coughing.

Abstract P1445

Evolution of a New Paradigm to Complement Influenza Pandemic Preparedness Plan in Resource-Limited Countries

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Our research group has developed a comprehensive program to control bioaerosol for pandemic preparedness that includes innovative products, interventions and strategies suitable to integrate in a health system. The proposed program main strategy evolved from a novel approach (mucomodulators) to reduce the number and/or size of infectious droplets contained in the aerosol when an infected person coughs or sneezes. We have been granted conditional ethical approval to test the safety use of one of our mucomodulators in human subjects. Rationale: A majority of countries have been working in partnership with the World Health Organization to implement measures that will help them be prepared whenever the feared Influenza Pandemic arrives. Vaccination is the current main strategy to control the flu pandemic. Resource-limited countries, though, are extremely concerned that they might face the Influenza Pandemic largely unprotected because of foreseeable extreme difficulties in accessing vaccines against the new strain of influenza virus. Hence, other control measures such as containment are now required. The mucomodulators would most likely represent containment for transmission of the influenza virus during outbreaks, allowing strategic sectors of society to be protected and remain functional, and allowing more time to make adjustments in the strategies to reduce casualties. Methods: Our proposed program addresses organizational, environmental and individual key factors that might lead to and suggest new avenues in Pandemic Preparedness. The program is tailored to prevent transmission of the flu virus and other respiratory pathogens regardless of the route of transmission: direct contact, droplets or airborne in a safe, simple and consistent fashion. We plan to characterize the aerosol pattern in human volunteers during coughing and to assess current public health practices and physical barriers to protect first responders and frontline health care workers. We plan to assess how patients can be transported and transferred safely within and between institutions and between different levels of care. Lessons Learned under this initiative could be applicable to several diseases transmitted by bioaerosol. Although we do not expect risk to participants, and knowing that something out of the ordinary might happen, the benefits gained through this approach far surpasses those risks.

Abstract P1446

Combination Vaccine Virus Candidates Against Influenza and NDV are Attenuated by Alteration of the Stalk Length of the Chimeric Neuraminidase Protein

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Highly pathogenic avian influenza A (HPAI) (H5N1) and Newcastle disease virus (NDV) are avian pathogens of major economic importance. Vaccination is recognised as a key element in the protection of poultry from these viruses, and the use of multivalent vaccines is considered desirable in order to lower the burden of vaccination. It has been proposed that a balance between the hemagglutination and neuraminidase activities of influenza virus is critical for its efficient replication, and that this balance is affected by the stalk length of the neuraminidase (NA) protein. Therefore, modulation of neuraminidase stalk length may offer a means for the development of live attenuated vaccine viruses. We tested whether this strategy could be applied to bivalent influenza viruses which incorporate a chimeric neuraminidase protein derived from both influenza virus and Newcastle disease virus. We generated recombinant influenza viruses expressing (i) the hemagglutinin (HA) protein of influenza A/Vietnam/1203/04 (H5N1), modified by the removal of the polybasic cleavage peptide; (ii) the ectodomain of the NDV hemagglutinin – neuraminidase (HN) protein in the place of the ectodomain of the influenza NA; and (iii) the influenza PB2 protein modified at residue 627 (K to E). A set of three bivalent viruses was generated, incorporating short, intermediate or long stalked chimeric NA-HN proteins. All three viruses were efficiently propagated in 10 day old embryonated chicken eggs, and gel electrophoresis of viral RNA confirmed the presence of expected gene constellations. The PB2, NA-HN and HA encoding segments were also identified by RT-PCR and sequencing. Growth curves were performed in 10 day old eggs. Short-stalked virus grew to 2*10^7 PFU/ml, intermediate-stalked virus grew to 2*10^8 PFU/ml and long-stalked virus grew to 1*10^6 PFU/ml, demonstrating the impact of changes to the stalk region on growth phenotype. Short and intermediate-stalked chimeric viruses were further characterised: the viruses failed to grow in human airway epithelial cell cultures and demonstrated low pathogenicity in mice, providing evidence of a high level of attenuation in model mammalian systems. We propose that these viruses have potential as live bivalent avian influenza and Newcastle disease virus vaccines.

Abstract P1447

Integrase-defective Lentiviral Vectors: A Novel Approach to Influenza Virus Vaccines

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Influenza virus is a major public health threat due both to the yearly epidemic and to the threat of a new pandemic. Trials of vaccines in preparation for a pandemic have been only partially successful. Lentiviral vectors are powerful inducers of cell-mediated and humoral immune responses, however they carry the inherent risk of nuclear integration of genetic material. Integration-defective lentiviral vectors (ID-LV) do not integrate or replicate, and remain in the nucleus of the transduced cells as episomal circular DNA forms (E-DNA). We have previously demonstrated that E-DNA is stable, and can express functional proteins. To evaluate the feasibility of using ID-LV as a delivery system in the development of an influenza vaccine, we studied the immune responses directed against the conserved influenza A nucleoprotein (NP) expressed from an ID-LV in cell culture, and in the influenza mouse model. ID-LV-NP was produced by co-transfection of three-plasmid (including packaging vector, an Env vector, expressing VSV. G protein and the HIV-1-based transfer vector expressing NP) in 293 T cells. NP-expression was confirmed by FACS analysis of infected cells. To evaluate the ability of T cells to recognize proteins expressed by lentiviral vectors, mice dendritic cells (DC) were transduced with ID-LV-NP, or control and co-cultivated with PBMC derived from mice previously exposed to influenza. DC transduced with ID-LV expressing an influenza virus protein, but not ID-LV control, activated proliferation and cytolytic activity of syngenic T cells. As a preliminary in vivo study, we investigated stability and cellular responses to GFP in mice inoculated intramuscularly with GFP-expressing ID-LV. We found that ID-LV persists with sustained gene expression after a single intramuscular injection in vivo. Three months after inoculation, splenocytes from immunized and naïve mice were stimulated with H2Dd-restricted GFP and control peptides. A specific response to the H2Dd-restricted GFP peptide measured by ELISPOT was evident in the mice treated with ID-LV-GFP, while responses were absent in controls suggesting that ID-LV can be used as a delivery system in vivo. No integrated vector was found in muscle samples by PCR amplification. Results from mice immunized with ID-LV-NP, and after viral challenge, will be presented.

Abstract P1448

Development of a Clade 2.3 H5N1 Avian Influenza Virus for Pre-pandemic Vaccine Clinical Trials

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Highly pathogenic avian influenza, subtype H5N1, caused outbreaks in poultry in Asia in 1996 and caused fatal human infections for the first time in 1997. As a result of continued circulation of H5N1 in poultry and wildlife since its emergence, the H5 hemagglutinin has evolved into multiple distinct antigenic groups. Viruses from four of these antigenic groups have caused multiple human infections since 2004. The most recently identified group, termed clade 2.3, caused human infections in Anhui province of China and Lao People's Democratic Republic. Inactivated vaccines are a major component of preparedness for the possible emergence of a pandemic H5N1 influenza virus. Industrial manufacturing of influenza vaccines in eggs requires development of an antigenically appropriate virus with the highest possible safety characteristics and high yield. Wild type highly pathogenic avian influenza viruses do not meet these requirements for vaccine production. To meet this need, we developed a new H5N1 pre-pandemic candidate vaccine by reverse genetics and evaluated its safety and yield. Reassortant virus Anhui/01/2005(H5N1)-PR8-IBCDC-RG5 (Anhui/PR8) was produced in compliance with WHO guidelines and under Good Laboratory Practice (GLP) specifications. The virus contains 6 internal genes from A/Puerto Rico/8/34 (PR8) and the NA and modified HA genes from A/Anhui/01/2005(H5N1). The reassortant virus was rescued by transfection of plasmid DNAs into certified Vero cells. The HA and NA genes of rescued virus are identical to those of the parental virus except for the modified HA cleavage site where 4 basic amino acid residues were removed. Anhui/PR8 reassortant virus grew to very high titers in embryonated eggs and was trypsin-dependent for the formation of plaques in chicken embryo fibroblast (CEF) cells. The reassortant virus was evaluated in mammalian (ferrets) and avian (chickens) animal models and was confirmed to be non-pathogenic. Anhui/PR8 is suitable as a pre-pandemic inactivated vaccine candidate for subsequent clinical trials in humans.

Late-Breaking Abstracts

Oral Presentations

ANTIVIRALS AND RESISTANCE
Abstract O113

Targeting Influenza H5N1: A Broad-Spectrum Antiviral Strategy Focusing on the Host

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Viral infections represent a major threat to human health. Vaccine development is one strategy to protect from specific virus infections. Pandemic viral strains exhibit genetic drift or reassortment of genes, so that although there is benefit in developing a vaccine against an H5 influenza virus, the likelihood of a good match with a pandemic strain may be low. Antiviral drugs that are also pathogen-specific have utility in pandemic situations, yet concerns relating to development of drug resistant influenza virus strains and evidence of limited reduction in the duration of viral shedding in children with influenza treated with oseltamivir, necessitates consideration of complementary antiviral strategies. A complementary strategy that focuses on the host rather than the pathogen is the basis for the development of broad-spectrum antivirals– to identify the targets of virus infection that are disabled by the infecting virus or co-opted to facilitate viral replication. We provide evidence that an interferon (IFN) response to viral infection, and/or IFN treatment, induce an activated phenotype in target cells that results in an antiviral state and an optimized innate immune response, regardless of the virus. For influenza virus infection, we provide evidence for critical determinants of the innate immune response, which prescribe susceptibility to an aggressive infection or a resistant host. Moreover, data are provided that demonstrate the protective effects of IFN-alpha and IFN-ß treatment against influenza virus infection, both in vitro and in vivo. We report on the development of non-peptide IFN mimetics, with enhanced receptor affinity and bioactivity, specifically in the context of antiviral activity against H5N1. Additionally, data are presented that confirm the utility of broad-spectrum antivirals, focusing on the development and bioactivity of small compound orotidine monophosphate decarboxylase (ODCase) inhibitors as antivirals. ODCase is an important enzyme in the de novo biosynthetic pathway of pyrimidines, a cellular enzyme co-opted by RNA viruses. In mammalian cells, the salvage pathway for pyrimidine biosynthesis may be invoked, but no such redundancy exists for viruses. A panel of ODCase inhibitors has been designed and compounds synthesized specifically as antivirals. Evidence is provided for their antiviral activities against influenza virus infection.

PRECLINICAL VACCINES AND OTHER INTERVENTION STRATEGIES
Abstract O114

New Effective Nasal Immunizations of Influenza Vaccine by a Natural Mucosal Adjuvant From the Lungs and its Synthetic Compound

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Vaccines administered i.m. or s.c., induce IgG-mediated protection in the systemic immune compartment but insufficiently induce mucosal sIgA. To improve protective mucosal immunity, intranasal vaccination has been studied. Many of the mucosal adjuvants reported up to now are Toll-like receptors-mediated dendritic cell activators, such as poly (I:C), CpG, LPS and bacterial lipids, and toxin based adjuvants, such as cholera toxin and Escherichia coli heat-labile toxin. However, these adjuvants induce not only local and systemic immunities but also induce inflammatory cytokines through the innate immune system. We recently found a natural mucosal adjuvant, pulmonary surfactant, in the lungs which efficiently delivers influenza virus hemagglutinin (HA) to the dendritic cells and markedly stimulates both local and systemic immunities. Intranasal administration of influenza HA vaccine combined with Surfacten, a modified pulmonary surfactant free of antigenic c-type lectins, or a synthetic adjuvant vehicle mimicking the constituents of pulmonary surfactant induced antigen-specific mucosal sIgA in the airway and IgG in the blood without significant inflammatory side effects in mice and mini-pigs. The adjuvanticities of Surfacten and its synthetic compound were almost equivalent to that of cholera toxin. The intranasal administration of HA-Surfacten stimulated the expression of MHC class II, CD40, and CD86 molecules in the CD11c-positive cells isolated from the nasal mucosa of mice. In this paper, the effects of Surfacten and a synthetic surfactant vehicle on mucosal and systemic immunities and trial for their practical use as new nasal vaccine adjuvants will be presented.

CLINICAL VACCINE EVALUATION
Abstract O115

Effects of Influenza and Pneumococcal Conjugate Vaccination in Preventing Respiratory Infections in Children: A Randomised, Double-Blind, Controlled Trial

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Objective: To evaluate the effects of combined influenza and pneumococcal conjugate vaccination, or influenza vaccination alone on respiratory tract infections (RTI) in children. Methods: Randomized double-blind controlled trial of 579 children aged 18 to 72 months recruited in primary care centers in three cohorts from 2003 to 2005. Participants were randomly assigned to receive two doses of parenteral inactivated trivalent sub-unit influenza as well as heptavalent pneumococcal conjugate vaccinations (IV+PV), influenza and placebo vaccinations (IV+plac), or control recombinant hepatitis B and placebo vaccinations (HepB+plac). The first two cohorts were followed for 18 months and the third cohort for six months. Outcomes were PCR-confirmed influenza and all-cause febrile RTI, and related primary care consultations, antibiotic prescriptions and acute otitis media (AOM) episodes. Results: During influenza seasons, the occurrence of confirmed influenza was reduced by 52% (95% confidence interval [95%CI]:7-75%) and 51% (95%CI: 3-75%) in the IV+PV and IV+plac groups compared with the control group. All-cause febrile RTI were reported with a rate of 5.2 in the IV+PV group, 6.1 in the IV+plac group and 6.9 in the control group per 1,000 child days which equals reductions of 24% (95%CI: 2-48%) and 13% (95%CI: -13-33%), respectively. Corresponding figures for AOM episodes were 0.5 in the IV+PV group, 0.4 in the IV+plac group and 1.2 in the control group per 1,000 child days which equals reductions of 57% (95%CI: 10-80%) and 71% (95%CI: 29-88%), respectively. Both vaccines did not have a statistically significant effect on these outcomes outside the influenza seasons. Conclusion: In this study covering three influenza seasons, parenteral influenza vaccination with or without conjugate pneumococcal vaccination substantially reduced confirmed influenza and physician-attended AOM episodes. Both vaccination strategies had a modest effect on all-cause febrile RTI episodes during influenza seasons and no such effect outside these seasons.

PRECLINICAL VACCINES AND OTHER INTERVENTION STRATEGIES
Abstract O116

Non-Pharmaceutical Interventions for Influenza Prevention in Households–Pilot Findings of a Large Cluster Randomized Controlled Trial in Hong Kong

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Background: There is little data on the efficacy of non-pharmacological interventions including face masks and hand hygiene for prevention of influenza virus transmission. Additionally, limited data are available on the transmission dynamics of influenza among household members. We implemented a pilot study of the feasibility and efficacy of masks and hand hygiene to reduce influenza virus transmission in Hong Kong households (February to June 2007) as part of a larger community trial planned for 2008. Design and subjects: We conducted a cluster randomized controlled trial of about 120 households (composed of at least 3 members) in Hong Kong where an index patient reported influenza-like-illness with symptom onset <48 hours and sought care from one of 26 outpatient clinics. After confirmation of influenza virus infection in an index patient using the QuickVue rapid test, a study nurse visited the index patient's household to administer the intervention within 12-24 hours, and revisited after 3, 6 and 9 days. Interventions: 1, control; 2, distribution of surgical face masks and education on their use; 3, distribution of alcohol and soap dispensers and education on hand hygiene. Measurements: Clinical influenza in secondary contacts as assessed by self-reported symptoms. Nose and throat swabs are collected from index patients and secondary contacts at each home visit for laboratory confirmation. Primary outcome: The proportion of secondary contacts with clinical and laboratory-confirmed influenza. Relevance: We will discuss our study design, the implications of early findings (including the difficult accrual of an adequate sample size due to various experimental and practical constraints), and lessons we are learning from the ongoing pilot study. While the current influenza season has been 'mild' (likely due to lack of recent drift in subtypes) our subtropical location in Hong Kong is ideal due to the long and relatively indistinct seasonality usually marked by both winter and summer peaks. While our study design may not allow us to capture pre- or a-symptomatic community transmission, the alternative design of following a large healthy cohort would require considerably greater resources given the low annual attack rate of influenza.

Late-Breaking Abstracts

Poster Presentations

Abstract P1501

High Titer Growth of Human and Avian Influenza Viruses in an Immortalized Chick Embryo Cell Line Without the Need for Exogenous Proteases

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Current influenza vaccine production technology is largely restricted to growth in embryonated chick eggs and often requires one to two eggs per dose. This system requires that hundreds of millions of "clean" embryonated eggs are available each year to meet the demand for influenza vaccines. In addition, this system requires significant downstream processing to purify virus away from egg components and is subject to significant loss of product if the eggs used are found to be contaminated with exogenous agents, such as Salmonella spp. While several major manufacturers are moving toward a cell culture-based vaccine production system, this process and resolution of the ensuing regulatory issues has been slow. In part, this process has been hampered because of concern over the existing cell lines that are known to grow influenza virus, such as Madin-Darby Canine Kidney (MDCK) cells and African green monkey cells (Vero) and a commercially available human retinal cell line. In all cases, existing cell lines require the addition of exogenous proteases, such as TCPK-trypsin, to release sufficient virus from the cells. Viruses grown in this manner and in embryonated eggs are also typically modified through reassortment and/or reverse genetics to allow high-titer growth. We now report use of an immortalized chick embryo cell line, termed PBS-1, that is capable of growing unmodified recent isolates of human and avian influenza A and B viruses to extremely high titers (>107 PFU/ml). Strains demonstrated to grow on PBS-1 cells thus far include: A/NewCaledonia/20/1999 (H1N1), A/HongKong/2652/2006 (H1N1), A/Hiroshima/52/2005 (H3N2), B/Florida/07/2004, B/Malaysia/2506/2004, and the high pathogenicity avian A/Whooper Swan/Mongolia/244/05 (H5N1). Influenza viruses grown in PBS-1 cells are released into the culture fluid without the need for exogenous proteases, thus simplifying downstream processing. In many cases, PBS-1 cells out perform primary chick kidney cells in growth of recent influenza isolates. PBS-1 cells are free of any exogenous agents, are non-tumorigenic, and are readily adaptable to a variety of culture conditions, including growth on microcarrier beads. In addition to offering a significant improvement in vaccine production, PBS-1 cells should prove valuable in diagnostics and as a cell line of choice for influenza virus research.

Abstract P1502

Serological, Clinical and Epidemiological Evaluation of Influenza Vaccine in Elderly at Nursing Homes in Taiwan, 2004-2006

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This study was to evaluate influenza vaccine in elderly at different nursing homes by measuring the levels of vaccine-induced antibody, their effectiveness against different circulating wild-type virus strains, and investigating the factors affecting the protection. One nursing home in Taipei County during 2004-05 flu epidemic season, and two more in Hsinchu and Nantou County were selected during the following 2005-06 flu season. Two sets of serum samples were collected: (1) 90 paired sera (3 weeks and 8 months after the vaccination) from the elderly during 2004-05 (males: females =50:40, age mean ± SD = 75.33±11.94), and (2) 178 tripled sera (within 3 weeks before vaccination, 1 month and 6 months after vaccination) from 163 elderly (males: females =97:66, age mean ± SD =75.33±11.94, 155 vaccinated in 2005) and 15 nursing home employees (males: females =2:13, age mean ± SD =41.47±12.83, 14 vaccinated in 2005) during 2005-06. Our results showed the geometric mean titers (GMT) of vaccine-induced HI antibody in nursing home elderly against A/Kinmen/618/2003 (H3N2)[vaccine-like strain], Taipei/NTU6/2004(H3N2) and Taipei/00017/2005(H3N2)[two wild-type strains] at 3 weeks after vaccination in 2004-05 flu season were 97.74, 17.55, and 16.25. The GMT at 6 months after vaccination were 71.27, 28.28, and 28.28, respectively. Moreover, their seroprotection rates (HI antibody titer ≥1:40) against these 3 viruses at 3 months post-vaccination increased were from 90.2%, 21.1%, and 28.9% to 90.0%, 44.4%, and 44.4% at 6 months post-vaccination. During the following flu epidemic season in 2005-06, the vaccinated elderly had higher HI antibody serotiters, seroprotection rates, and the proportions of HI antibody with 4-fold rise against A/Taiwan/CDC00702/2004(H3N2) at one month post-vaccination than unvaccinated ones (GMT:69.99 vs 15.87, p=0.001; seroprotection rate:83.1% vs. 44.1%, p=0.013; 4-fold rise:51.1% vs. 11.1%, p=0.013). Interestingly, GMT of the elderly was much lower than that of nursing home employee before the vaccination (20.76 vs. 25.62) but they increased dramatically at one month after the vaccination with even higher serotiters than the employee (70.17 vs. 63.63). Therefore, this study showed the trends of GMT declined against the influenza vaccine-like strain but increased against the circulating strains. In addition, vaccination is important for elderly to booster immune responses.

Abstract P1503

Re-evaluation of Usefulness of Throat Swab Specimens for the Influenza Rapid Diagnostic Test

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The rapid antigen-detection kit is a good tool for diagnosis of influenza in clinical settings. As to the specimen for the kit test, nasal swabs, nasal aspirates and throat swabs have been used so far, and the former two have been considered superior to throat swabs in terms of sensitivity, and recommended as the best specimens for this kind of test. However, many clinicians still want to continue to use the throat swab if possible, because of some difficulties they often meet at the bedside, in collecting nasal specimens including rejection by patients. We, therefore, tried to examine whether throat swab specimens are truly useless for diagnosis of influenza using the kit, or not. Throat swab specimens collected from 439 patients who attended a clinic with influenza-like symptoms in the 2004-2005 season, were subjected to both virus isolation using cell culture system and a rapid diagnostic kit, QuickVue Rapid SP influ (DS Pharma Biomedical), and the results of the latter test were analyzed in terms of the sensitivity and specificity by comparing with those of virus isolation, the golden standard for the diagnosis. The sensitivity and specificity of the kit for all 439 specimens were 87.8 % (72/82) and 90.2% (322/357) for type A influenza, and 80.4% (176/219) and 95.0% (209/220) for type B, respectively. These results seemed appreciable as almost comparable levels with those reported so far, in the same kind of studies using nasal swabs and/or nasal aspirates. Furthermore the sensitivity and specificity were calculated separately for various age groups set at every 10 years from 0 to 89 years old, in order to investigate whether there is any difference among them or not, and there found no significant difference. Thus, it was suggested that the throat swab would still be competent specimen useful enough in the clinical setting for diagnosing influenza, using the rapid antigen-detection kit regardless of the age of the patient, as far as it could be collected properly.

Abstract P1504

First Human Case of Highly Pathogenic Avian Influenza A (H5N1) in Sub-Saharan Africa

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Outbreaks of an unprecedented epizootic avian influenza A (H5N1) virus that is highly pathogenic to poultry in February 2006 in Nigeria was a warning signal that the virus could cross the species barrier and cause many human fatalities and also increase pandemic threat. We describe the features of the first human infection with influenza A (H5N1) in sub-Saharan Africa. An H5-specific reverse-transcription PCR assay (RT-PCR) and RT-PCR for NA gene were used for the detection of virus directly in clinical specimens. Samples from a 22-year old lady with influenza-like illness from Lagos state of Nigeria were confirmed to be positive for H5N1 virus and the isolated virus A/Nigeria/641/6/2007) was shown to be identical to a highly pathogenic H5N1 virus (A/chicken/Nigeria/641/2006) previously isolated from chickens in Nigeria during the 2006 outbreak. The complete genome sequence of the virus has been completed and details will be described. Owing to the pandemic potential of the virus and the strategic position of Nigeria and peculiar public health situation of the Africa continent, urgent and active measures must be taken to stop further spread of the virus.

Abstract P1505

Seroepidemiology of Influenza A/H5N1 Virus in the Italian Population Since From 1992 Until to 2007

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The first documented outbreak of human respiratory disease caused by avian influenza A/H5N1 viruses occurred in Hong Kong in 1997. Avian influenza A viruses, including those that are highly pathogenic in poultry, had not previously been associated with respiratory disease in humans. One thousand three hundred and sixty-four samples of sera gathered from subjects in Italy from 1992 to 2007 were studied. A minimum of 70 serum samples were collected during any year. The age of patients from which the samples were taken and analysed varied from 6 months to 92 years. Serum antibodies for influenza A/H5N1 were measured using modified Single Radial Haemolysis assay, using turkey erythrocytes instead of sheep erythrocytes. The whole A/H5N1/Vietnam/1194/2004 inactivated virus was used as the antigen for the test. The specificity of the assay was established through the titration of 20 duplicates of sera from positive controls and 20 duplicates of sera from negative controls. Hyperimmune sheep sera produced by the NIBSC, were used as a positive control. A Pool of 10 sera from 1 year old children, gathered by laboratory analyses in 1965 were used as negative controls. The accuracy of the assay was evaluated by calculating the percentage of difference between the geometrical average of the haemolysis area expressed in mm^2 of the undiluted positive control sera evaluated in triplicate, and the area of haemolysis in mm^2 of the same sera evaluated in triplicate and diluted at 1:2 and 1:4 in Phosphate Buffered Saline (PBS). Samples with an area of haemolysis of more than 25 mm^2 were considered to be positive and seraprotected. Of patients in the range of age between 0 and 64 years, 5.98 % were seroprotected against the A/H5N1/Vietnam/1194/2004 virus, and this was also true for 12.99 % of subjects of over 65 years of age. The seroprotected subjects were analysed again in order to remove non-specific antibodies, and these samples were adsorbed in a 1:1 vol mixture of A/H1N1/New Caledonia/20/99 and A/H3N2/California/7/2004 viruses (2000 UE/ml). Of these, 53.57 % of the patients passed from seroprotected to negative after this test. No evident difference was found between the percentage of seroprotected subjects in the different years that were analysed. The study has demonstrated that SRH test appears to be sensitive to detect influenza H5 antibodies, but caution must be exercised in interpreting these data.

Abstract P1506

Integrating Meteorological Considerations Into the Global Surveillance of Novel Influenza

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Influenza surveillance involves clinical, epidemiological, virological and serological surveillance. For non-specific symptoms/signs of influenza, clinical surveillance becomes quite difficult. The effectiveness of epidemiological surveillance for formulating better public health policies relies on timely investigation, efficient coordination and transparent exchanges of valuable information. Furthermore, virological and serological surveillance require more resources and technical support that might not be available in developing countries/areas where the highly pathogenic avian influenza (HPAI) H5N1 viruses are endemic. Since H5N1 viruses have caused many outbreaks among poultries and wild birds from Asia to Europe and Africa, it has become a crucial public health mission to timely monitor an emerging reassortant virus derived from avian/swine and human hosts with pandemic potential in a more cost-effective approach. Therefore, we initiated a multi-disciplinary study to improve influenza surveillance by integrating meteorological factors into epidemic or epizootic surveillance. Analyzing synoptic and local meteorological data in regions north of the 25°N latitude where the first outbreaks of HPAI H5N1 originated, we found that a temperature drop shortly preceded the HPAI outbreaks among birds in each of the Eurasian regions stricken in 2005 and 2006. We also observed meteorological differences in Japan and Korea between the years when H5N1 outbreaks (2003-2004, 2006-2007) occurred and those in which they did not. Beyond HPAI, the arrival of a cold air mass preceded the 2003-2004 outbreaks of low pathogenic avian influenza (LPAI) H5N2 in Taiwan. Through active virological surveillance in live-bird markets during 2005-2006, we found that the peak months for AI viruses in Taiwan were similar to those in Hong Kong and China in recent years. In addition, multivariate modeling on our hospital emergency department-based syndromic surveillance system (ED-SSS), which monitors daily human influenza-like illness (ILI) cases in Taiwan, also showed the relationship between low temperature and increasing ILI cases for several days. In conclusion, regional meteorological monitoring may serve as an integral part of a global avian

influenza surveillance system network for enhancing virological/serological surveillance besides using GPS to trace the migration routes of wild birds. More international collaboration is needed to help predict potential H5N1 outbreaks and identify where they might occur.

Abstract P1507

Multiplex PCR for Typing Influenza and Human Respiratory Syncytial Viruses

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Introduction: Human Influenza (Flu) is a major cause of respiratory disease in children with clinical symptoms similar to human Respiratory Syncytial Virus (hRSV). The frequency of co-infection with hRSV and influenza virus is not well documented in the literature and is essentially unknown in both developed and developing countries. In this report we describe the development and application of a multiplex PCR (mPCR) capable of detecting both Flu A and B and hRSV A and B in a single tube. Materials and Methods: Four sets of primers were designed for Matrix gene (M) of Flu A and B viruses and Nucleocapsid gene (N) of hRSV A and B respectively. mPCR was standardized with Influenza A and B and hRSV A and B in one tube. Standardized mPCR was applied on nasopharyngeal aspirates (NPAs) from children <5 years visiting Pediatrics OPD with ALRI over a period of one year. RNA was extracted by RNeasy kit and cDNA was synthesized using random hexamers. Specificity was tested by amplification of RNA from heterologus viruses and sensitivity by comparing with known $TCID_{50}$ of standard strains of each virus. Results: Sensitivity of the mPCR assay was $0.1TCID_{50}$ for Flu A and B and $1TCID_{50}$ for hRSV A and B respectively. Of 100 NPAs, hRSV B was detected in 7(7%), Flu A in 4(4%) hRSV A and Flu B in 1 each (1%). Influenza and RS viruses were found in 13 of 100 clinical samples from ALRI. Mixed infection with hRSV B with Flu A was detected in 2 (2%) samples. Conclusions: Our mPCR can be used for the detection and subtyping of Influenza A and B along with hRSV A and B in clinical respiratory samples. The assay is rapid, specific, sensitive and comparatively economical and capable of detecting co-infections in both surveillance and diagnostic settings.

Abstract P1508

Human Influenza Virus's Strains Surveillance in New Delhi, India

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Background: Influenza virus is known to cause, global epidemics and pandemics. Surveillance will be enhancing the prevention and control activities for influenza virus. The aims of this study are to report the preliminary data on circulating strains of influenza viruses from national capital territory, India. Material and Method: Nasal swabs were collected in viral transport medium from patients of influenza like illness (ILI) Samples were collected from two sites (a) Comprehensive Rural Health Service Project (CRHSP) of AIIMS, Ballabhgarh. (b) Employees Health Service Clinic of AIIMS, New Delhi. Each sample was passage at least twice in MDCK cell line. The presence of influenza virus was detected by Hemagglutinin assay (HA). If HA was positive, further sub typing was done by Hemagglutinin Inhibition assay (HI) using anti-sera provided by WHO. Result: One thousand seven hundred thirty one (1731) samples have been collected. Influenza viruses were isolated in 79 (4.56 %) of 1731 samples. HI has been done on 72 samples, 35 (49%) have been identified as subtype H3N2, 19(26%) as H1N1 and 18 (25%) as influenza B. All the influenza positive samples were conformed by reference centre at National Institute of Virology, Pune. Maximum number of influenza positive cases was seen during winter months of December-March and rainy months of July-August during the entire course of the study. During the year 2004-2005, H3N2 was the predominating strain followed by H1N1 in 2005-2006. There has been an increase in the number of influenza B positive samples this year. Conclusions: The pattern of circulating influenza strains needs to be closely monitored as with new influenza strains emerging each year, identification of circulating strains by coordinated global surveillance is crucial to vaccine development. Our data shows that influenza virus circulation pattern is not type cast and comprehensive surveillance is important especially in Asia, since new influenza strains often originate here.

Abstract P1509

The Role of the Asia-Pacific Advisory Committee on Influenza (APACI) in Influenza Awareness

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A coordinated approach to the promotion of influenza awareness, with the intent to improve the prevention and control of influenza, continues to be needed in the Asia-Pacific Region. This renewed focus on seasonal influenza is occurring as a result of the ongoing threat from the Highly Pathogenic Avian Influenza A (H5N1) virus. The APACI was established in early 2002 to address epidemiological issues relating to influenza and the impact of the disease in Asia. Its members include influenza researchers, and public health and infectious disease experts from countries in the Asia-Pacific region. The committee is a joint initiative of key opinion leaders in the region, supported by, GlaxoSmithKline, Novartis, Roche, Sanofi Pasteur and Solvay Pharmaceuticals. APACI's educational activities are aligned with, and supplemental to, those of the World Health Organization (WHO). The APACI is working in cooperation with the WHO to complement its work on influenza surveillance and promote influenza awareness throughout Asia. A newsletter (Influenza – Asian Focus) is published twice annually and is accessible on APACI's website www.apaci-flu.org along with educational materials which include influenza slide sets. APACI has assisted with the establishment of country-specific awareness programmes: the Influenza Foundation Thailand in 2004 and the Influenza Foundation India in 2005. Malaysia is proposing the formation of a group in 2007. These groups assist with the review and development of country-specific recommendations for influenza prevention and control.

Abstract P1510

Serological and Virological Surveillance of Swine Influenza in China

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Swine influenza (SI) is an acute respiratory disease caused by influenza A viruses within the Orthomyxoviridae family. Pigs may serve as intermediate hosts for the introduction of new influenza viruses into the human population. This study reports a recent serological and virological surveillance in China. A total of 7203 serum samples were collected for antibody detection from 14 provinces. The sera were tested by haemagglutination inhibition (HI) assay, for the presence of antibodies against H1N1, H3N2, H5N1 and H9N2 subtype influenza viruses, were performed according to standard methods. 6752 nasal swabs and organs collected for virus isolation were inoculated into amnionic and allantoic cavities of in 9- to 11-day old specific-pathogen-free embryonated chicken eggs or MDCK cells. The presence of SI virus was determined by haemagglutination (HA) assay. And subtype identification was determined by HI assay and RT-PCR. Serological examinations of pigs between 2005 and 2006 revealed high seroprevalences for both H1N1 (22.9%) and H3N2 (55.9%) from a total of 7203 serum samples. And seropositive pigs for H9N2 and H5N1 subtype avian influenza virus were also detected in some areas with very low percent (0.02% and 0.03%, respectively). Twenty-one strains of SI viruses, including H9N2, H3N2, H1N1 and H1N2, were isolated and identified. Phylogenetic analyses of eight gene fragments of all these viruses showed that H9N2 viruses were directly transmitted from poultry, H1N1 isolates were classical SI viruses, H3N2 viruses were human influenza virus and the H1N2 viruses were triple reassortant viruses with PB2, PA, M, NS and NP gene of swine origin, HA and NA gene of avian origin and PB1 gene of human origin.

Abstract P1511

Reconstruction of Influenza Epidemics in Mexico

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Introduction: This study is aimed to understand the spread routes, duration and impact of seasonal and pandemic influenza in different regions of Mexico. Material and Methods: This project is based in information obtained from historical archives and from the monthly morbidity and mortality reports from the Secetaria de Salud (Ministry of Health) of Mexico for the period between 1922 and 2006. Census data were acquired from INEGI (Mexico). The geographical information system was IRIS II from INEGI (Mexico). Results: A total of 11 large epidemics of influenza were detected in the historical record for the central Valley of Mexico. Nine of them (1806, 1826,1837, 1891, 1899, 1918, 1920, 1957, 1969) occurred as single disease and in two cases (1805 and 1846), epidemics were associated to typhus. The 1918 epidemic of influenza occurred in three waves, the first, and smaller, occurred in

April-May 1918; the second, with the highest mortality, in October-November 1918 and the third one in February-March 1920. The pattern of seasonal influenza had a heterogeneous distribution that remained constant throughout the period of study. Some states (Nayarit, Q. Roo) mantained the lowest rates in the country and deferred the entrance of the pandemic flu for two or three months. In contrast, other states (Coahuila and Baja California Sur) always displayed the highest rates of influenza and were among the first states to peak during the flu pandemics. Conclusions: The spread and impact of influenza in Mexico has a defined and relatively constant pattern throughout history. This information will be very useful in the future to better understand the dynamics of influenza epidemics in Mexico.

Abstract P1512

Influenza Immunization Rates for Health Care Workers in Ontario

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Background: In Ontario, immunization of health care workers is not mandated by legislation. However, under the Influenza Surveillance Protocol for Ontario Hospitals (July 2000) and the Influenza Surveillance Protocol for Long-Term Care Facilities policy directive (November 1999), influenza immunization coverage rates for residents and staff of LTCHs and staff of public hospitals are to be reported to the local medical officer of health (MOHs) by December 1st of each year. Influenza immunization rates for staff of long-term care homes (LTCHs) and hospitals have been submitted to the Ontario Ministry of Health and Long-Term Care (MOHLTC) by local public health units since the 1999/00 influenza season. Methods: LTCHs and hospitals in Ontario submit staff influenza immunization rates as of November 15th each year to MOHs. Health units submit annual immunization rates to the MOHLTC through a web-based system. Provincial immunization rates derived from anonymized aggregated data are reported annually by the MOHLTC in the Ontario Influenza Bulletin. Results: In the year following the development of the hospital and LTCH protocols, health care worker immunization rates significantly increased, but have remained relatively stable since that time. Since the 1999/2000 influenza season, influenza immunization rates for staff at LTCHs ranged from a high of 90% in the 2000/2001 season to a low of 82% in 2002/2003. Prior to the release of the surveillance protocol, staff influenza immunization rates were approximately 40%. Influenza immunization rates among hospital staff have fluctuated from a high of 63% in the 2000/2001 season to a low of 44% in 2002/2003. Prior to the protocol, staff immunization rates were approximately 20%. Post Severe Acute Respiratory Syndrome (SARS), immunization rates increased 11% among hospital staff only, and have subsequently maintained this level. Conclusions: Influenza immunization rates have remained constant for the last four years among hospital and LTCH staff. New strategies are being developed in order to increase immunization rates among these populations.

Abstract P1513

Comparison of Hemagglutinin Inhibition Assay and Reverse Transcription PCR for Typing Human Influenza Virus Isolates

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Introduction: Human Influenza viruses hemagglutinin (HA) subtypes can be determined by hemagglutinin inhibition (HI) test, but neuraminidase (NA) subtyping is difficult as NA subtype-specific antisera are not easily available. However by RT-PCR using HA and NA specific primers both HA and NA subtyping can be performed. In this study we report the results of comparison of HI and RT-PCR for typing influenza isolates. Material and Method: RNA was extracted from viral isolates in MDCK cells using RNeasy kit. cDNA was synthesised using AMV-RT with random hexamer primer. In the first set, primers from matrix (M) and non structural (NS) gene were used to type influenza A and B respectively. In the second set primers for H1, H3, N1 and N2 (Poddar, 2002 and self designed for H3) were used to subtype influenza A viruses. HI was performed as per WHO protocol using reference anti-sera provided by in WHO kit. In addition RT-PCR for H5 was standardized with WHO primers using cloned H5 gene provided by L. Poon (Univ. of Hong Kong). Results: HI done on 72 viral isolates identified, 35 (49%) as subtype H3, 19 (26%) as H1 and 18 (25%) as influenza B. RT-PCR was done on 43 of the 72 isolates and typed 17 as H1N1, 16 as H3N2, 10 as Influenza B. There was 100% correlation between typing done with RT-PCR and HI. Conclusion: To perform HI on viral isolates at least an HA titre 64/128 is required which may require 2-3 passages in MDCK cells. RT-PCR however can be performed on low titre virus and is a rapid and sensitive technique for typing influenza viruses. Thus RT-PCR is a useful tool for typing of influenza viruses and in the current scenario of spread of H5N1 viruses to humans, can provide rapid epidemiological information on circulating influenza viruses.

Abstract P1514

A High-Yielding and Robust Plant Transient Expression System for Vaccine Production

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Medicago has developed a rapid, robust and high yielding vaccine production system based on transient expression in Nicotiana benthamiana. This manufacturing technology delivers a fast response and critical surge capacity in an efficient cost-effective manner. The technology can deliver a flu vaccine for testing in five weeks after the identification and reception of hemagglutinin and neuraminidase plasmids form the pandemic strain. Using this system, recombinant hemagglutinin and neuraminidase from different influenza strains were produced within one week after infiltration of bacterial vectors into leaves. When tested in a mouse model, recombinant neuraminidase made in plants was superior at preventing viral replication in the lungs and upper respiratory tract of the animals than the baculovirus-derived neuraminidase. Animal testing with recombinant hemagglutinin is ongoing. Medicago anticipates to initiate full preclinical studies with an H5N1 in 2007 and to launch a phase I in 2008. The simplicity and the robustness of this system also allows modulation of glycosylation through co-expression of helper enzymes. As a result, recombinant proteins bear glycans without plant-specific sugar moities. This talk will highlight Medicago's manufacturing capacity in the case of a pandemic, describe how recombinant proteins are purified from plant extracts, and review efficacy studies conducted with recombinant influenza antigens.

Abstract P1515

Characterization of Neuraminidase Gene of Human Influenza A/H3N2 Viruses Isolated in Northern Taiwan, 2000-2004

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In order to understand the variation of NA gene of influenza A (H3N2) virus in northern Taiwan, 43 strains of clinical isolates in Taipei during 2000-2004 were collected. The result indicated that the deduced amino acid substitution rate of NA was about 0.5% per year. As compared with the A/Moscow/10/99 vaccine strain, amino acid changes within at least one of the seven NA antigenic determinants (I-VII) were found in approximate half of the isolates (20/43) and the most common changes were at position 332, 401, 431 and 432. Only one amino acid change (D151G) was observed in the catalytic site of NA. All isolates contained the seven conserved asparagine-linked glycosylation sites found in the NA of the progenitor A/Hong Kong/8/68 strain. Variation of plaque size was observed in the plaque assay of clinical isolates. After purification of one clinical isolates, one small-plaque virus strain (NA-) was obtained and a 586 nucleotide deletion (303-888nt) of NA gene was found. Although the deficiency of NA enzyme activity, it still can grow in MDCK cell line. However, the virus yield was 45-fold less than wild-type when low MOI (10^{-5}) was used. The receptor-binding ability of the defective virus was low but no compensatory substitutions in the HA gene were found. Tamiflu™ did not influence on the NA- virus growth in vitro. Our results suggest that NA activity may not be the essential requirement for human influenza A/H3N2 virus growth.

Abstract P1516

Efficient Production in E. coli of Influenza Vaccines Including M2 and HA Antigens

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We have shown that covalent linkage of a Toll-like Receptor (TLR) ligand to a disease antigen of interest significantly

increases the immunogenicity of the antigen and the efficacy of the resulting vaccine candidate. This approach has been applied to the conserved surface antigen of influenza virus, M2e, and to the variable surface antigen, HA. Each antigen is expressed as a fusion protein with flagellin (a TLR5 ligand) from S. typhimurium. The M2e antigen is presented as four copies of the 24 aa ectodomain, head-to-tail, at the C-terminus of flagellin. Two doses of this soluble protein in aqueous solution provides mice protection from death due to an LD90 challenge. The globular head of HA fused to the C-terminus of flagellin, also produced in E. coli, provides complete protection from death and illness following an LD90 challenge. We have produced flagellin-HA fusions for a variety of A-type (H1, H3, H5) and B-type (B Lee, B Malaysia, B Shanghai) influenza viruses. Robust expression in a prokaryotic system provides a practical option for global immunization against both pandemic avian and seasonal human influenza viruses.

Abstract P1517

Exhaled Nitric Oxide in a Murine H5N1 Influenza Model

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Background: Since H5N1 Influenza is known to increase airway inflammation (AI) and death in mice and because exhaled nitric oxide (ENO) increases with AI, the aim of this study was to determine whether ENO levels could discriminate between normal, H5N1 infected and treated H5N1 infected mice. Methods: Animals were infected with H5N1 (Duck) Influenza virus at day 0 (D0), (1:200, 50ul, i.n.). ENO was measured using a NIOX chemiluminescence analyzer (Aerocrine, Sweden) and adapted to obtain measurements from a 2-liter chamber. ENO levels were allowed to build in the chamber for 20 minutes while nitric oxide (NO)-free air was drawn through the box at a rate of 5ml/min. Initially 10 mice/box were measured but due to death in G4, all subsequent measurements used 7 mice/box. ENO were performed at D5 p.i. Treatment groups. GI-Control. No infection or treatment G2- H5N1/aminoguanidine (100mg/kg/d) G3-H5N1/Ribavirin (37.5 mg/kg/q12h) and G4-H5N1/saline (100ul/d, i.p). Results: Reproducibility: ENO levels over 4 days at 20 minutes remained constant. D4 and D7 p.i. (n=10) were 5.0 and 5.1 ppb, respectively. D4, D5 and D6 p.i. (n=7) were 3.8, 3.3 and 3.4 ppb, respectively. Treatment Groups at D5 p.i.: G1=3.3 ppb, G2=1.9 ppb, G3=2.0 ppb, and G4=1.5 ppb. Discussion: An increase in ENO was anticipated in H5N1 infected mice compared to normal controls. However a decrease in ENO was actually observed in the H5N1 infected mice and this was postulated to be due to oxidative stress (NO binding with superoxide). Therefore we treated G4 mice at D5 with bovine superoxide dismutase (SOD) (7mg/kg, i.p.). ENO at pre-dose, 2h, 4h and 24h post-SOD were 1.5, 2.7, 2.8 and 1.5 ppb respectively and supported our theory. Since Ribavirin has also been shown to inhibit nitric oxide synthetase the reduced ENO was not unexpected. Conclusion: Changes in ENO levels are seen in murine H5N1 Influenza compared to controls and treatment with SOD reversed these changes. ENO may have future research and clinical applications.

Abstract P1518

Influenza Virus Infection in Humans Generates a Broad Range of CD4+ and CD8+ T Cell Responses

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A recent analysis of the current influenza epitope literature revealed that most studies on T cell responses have focused on HA and NP, with relatively smaller numbers of epitopes reported for other influenza-derived proteins. This study aimed at characterizing immunodominance in the T cell response to influenza virus infection in humans. Instead of focusing on a particular strain or subtype, this study was based on a selection of 23 different subtypes of human isolates and pandemic strains, as well as a number of avian strains. A total of 4080 peptides were selected on the basis of supertype motif and predicted binding capacity to 7 common HLA supertypes, and the conservancy level amongst the selected strains. All peptides were tested for immunogenicity in IFNγ ELISPOT assays using PBMC from a total of 46 donors of different ages and ethnicities. As many as 251 distinct immunogenic class I and class II-restricted epitopes were identified in the course of this study. Of these, only 6 were previously identified in the literature. On average, each donor recognized 10 class I and 7 class II epitopes. Epitopes were identified from all influenza proteins and covered all 7 HLA supertypes tested. The set of epitopes identified and the immunodominance pattern was unique for each individual. Only 9% of the epitopes were seen in more than one donor. These results revealed an extreme breath of the T cell response to influenza infection, and suggest that immunodominance exist at the individual level, but much less so at the population level. All in all, the set of epitopes identified herein provides a high coverage for a diverse set of human and avian strains and in most ethnicities. The results obtained in the present study might guide future research aiming at developing epitope-based influenza vaccines and diagnostic tools.

Abstract P1519

Diclofenac Augments Influenza Virus Replication In Vivo and In Vitro

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Influenza-associated encephalopathy and Reye's syndrome treated with nonsteroidal anti-inflammatory drugs, such as diclofenac, often cause severe neurologic complications including brain edema. However, the etiology of the mechanisms of brain edema is little known to date. In the present study, we analyzed the effects of diclofenac on influenza virus replication in vitro and in vivo. We found that the clinical dose of diclofenac (2 mg/kg) increased the mortality of newborn mice infected with influenza A virus and that mice treated with diclofenac exhibited higher virus genome numbers, viral protein expressions, peak titers, cytokine expressions (TNF-α and IL-6), active caspase-3 compared to mock-treated mice. Next, we detected higher virus-genome numbers and viral protein expressions in influenza A virus-infected MDCK and A549 cells treated with diclofenac at doses up to 100 μM. However, in contrast to mouse models, diclofenac caused reduction of virus budding and released virus titers in cell systems.These results suggest that diclofenac augments the viral multiplication in infected cells and cytokine expressions, leading to exacerbation of virus infection.

Abstract P1520

Characterization of H5 Avian Influenza Virus Isolates in Asia

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H5N1 highly pathogenic avian influenza (HPAI) virus has spread in poultry from Southeast Asia to the north, Europe, and Africa. We have been conducting molecular epidemiology of avian influenza and the assessment of pathogenicity of the H5 virus isolates in birds and mammals. Six H5 avian influenza virus strains, Dk/Yokohama/aq-10/03 (H5N1), Ck/Yamaguchi/7/04 (H5N1), Ck/Ibaraki/1/05 (H5N2), Swan/Mongolia/3/05 (H5N1) and Swan/Mongolia/2/06 (H5N1) were characterized. These H5 strains were compared with other 30 isolates from birds in the world by phylogenetic analysis. Pathogenicity of the H5 viruses for chickens, ducks, mice, and miniature pigs was assessed by experimental infection. It was revealed that the H5N1 isolates in Mongolia were closely related to each other and classified into the cluster of "Qinghai Lake" H5N1 viruses including those isolated in European, Mediterranean, African, and Asian countries. Swan/Mongolia/05 (H5N1) and Swan/Mongolia/06 (H5N1) caused severe disease in ducks with 30% mortality. H5 viruses other than Ck/Yamaguchi/04 (H5N1), replicated in upper respiratory tracts of pigs without any clinical signs. It was indicated that Mongolian isolates in 2005 and 2006 were closely related not only to viruses isolated from water birds in Qinghai Lake, China but also to those isolated in Tyva, Iran, Russia, Germany, Nigeria, Sudan and those in Japan in 2007. While they were different from those in Vietnam, Thailand, Indonesia, indicating that the water birds from which those viruses were isolated should have been infected in China or on the way back to Siberia. Since it is concerned that these HPAI viruses may perpetuate in lakes where water birds nest in summer, intensive global surveillance of avian influenza in migratory water birds is important. So far, we have not isolated HPAI virus from migratory ducks and swans that flew from their nesting lakes in Siberia.

Abstract P1521

New HPAI Viruses H5N1 in Russia (2006-2007 Years)

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The outbreak of H5N1 highly pathogenic influenza in Russia was first registered in summer 2005 in Siberia. Next some outbreaks occurred in Russia South, some of European countries, Azerbaijan, Turkey, Egypt and Nigeria. Phylogenetic analysis of HA gene showed viruses isolated in Russia in 2005 and aforenamed countries form a cluster with strains isolated from birds during the spring outbreak on Qinghai Lake in China in 2005. In spring 2006 we isolated two H5N1 influenza viruses which had phylogenetical similarity with viruses isolated on Qinghai Lake in 2005. Analysis indicates viruses were return from Near East or South of Russia. In the middle of June of 2006 we fixed high mortality of wild birds on Ubsu-Nnur lake which borders on Mongolian Republic. Ornitologists evaluated nearly 4000 birds die out, principally Podiceps cristanus. We also isolated H5N1 highly pathogenic influenza virus from dead birds, it was similar with viruses of Qinghai group. It was virus from territory of China. During planned monitoring we isolated some H5N1 viruses from clinical healthy wild birds belonging to different families, and besides all isolates were

highly pathogenic for chickens. Phylogenetical analisis of isolates also indicated they were close to viruses of Qinghai group. There wasn't death of wild and domestic birds on the territory of Russia within a period of autumn migration 2006. There was sporadic death of poultry in winter 2007 in Western part of Russia. It was probably migratory birds which have the winter nesting places in this territory were the cause of birds' death in Southwest regions of Russia. As for Moscow region, death of poultry was probably determined by importation of birds from epizootic regions. We isolated highly pathogenic H5N1 influenza virus from dead domestic birds that had also phylogenetical similarity with Qinghai group viruses. Analysis of samples from people who contacted with sick or dead birds did not elicit cases of H5N1 influenza virus infection. This work was made with financial support of BIO-INDUSTRY INITIATIVE, USA.

Abstract P1522

The Application of Environmental Modeling to the Selection of Avian Influenza Study Locations and Sentinel Flocks

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BioTEMS (Bioagent Transport and Environmental Modeling System) is a robust modeling system capable of integrating biological factors, such as agent survival, host and vector population dynamics, with abiotic factors such as elevation and soil type to predict where pathogens introduced or endemic to an area are likely to survive or amplify. This model has applications to many pathogens, including influenza surveillance. There has for the past several years been heightened monitoring of the spread of the H5N1 strains of influenza in Asia, Europe and Africa. There is also the potential for these strains to cross to the Americas, most likely through Alaska. However, less likely routes are also possible and monitoring of locations as remote as the Central Atlantic coast of the US has merit. Such efforts become more feasible if resources can be focused on sites likely to yield representative isolates of circulating virus strains. When applied to an area in coastal Maryland, the BioTEMS model correctly predicted where diverse flocks of water fowl would be found. The birds at such a sight could be captured for viral isolation, or the site could be used as a location for sentinel flocks. Preliminary results also suggest that soil variables alone (from the US Geologic Survey) can be correlated with coastal locations hosting diverse water fowl flocks.

Abstract P1523

Induction of Cross-Neutralization Against H5N1 Virus After Vaccination With Seasonal Influenza Vaccine in the Elderly

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The purpose of this serological study was to demonstrate the ability of influenza season's vaccine to induce neutralizing antibody to avian influenza A (H5N1) virus in the elderly. A total of 118 elderly people were divided into 58 vaccinees (mean age 68.5 years) and 60 non-vaccinees (mean age 68.4 years). Antibodies to H1N1 and H3N2 viruses were detected by hemagglutination inhibition test (HI), and antibody to H5N1 virus was detected by microneutralization (NT) test. Geometric mean titers (GMT) of H5N1 NT antibodies prior to vaccination in both groups (2.72 vs. 2.98) were not statistically different. At one month after seasonal influenza vaccination, factorial increase in titer in the vaccinated group was higher than that in the non-vaccinated group (0.82 v.s.0.40) with odds ratio 3.22. Three (5.2%) subjects in the vaccinated group showed H5N1 seroconversion, while only one (1.7%) in the non-vaccinated group did. All these three vaccinees had H5N1 NT antibody titers of $\geq$ 40, meanwhile the titer of 1:20 was found in the non-vaccinee. The results indicate that cross neutralization against avian influenza H5N1 virus can be found in the elderly; and vaccination with influenza season' vaccine can increase the frequency of seroconversion and level of protective antibody titer to H5N1 virus.

Abstract P1524

A Cluster-Randomised Household Study of Mask Use in Control of Respiratory Virus Transmission

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Aims: To determine the impact of the use of surgical masks and

P2 masks in households on the interruption of transmission of influenza and other respiratory viruses. Methods: Prospective cluster randomized study comparing surgical masks, P2 masks with no masks in interruption of viral transmission between household members. Families of children presenting to emergency department with influenza like illness (ILI) were randomised to one of the three groups and followed up for development of respiratory illness in other family members. Results: 53 families (104 adults) were recruited in the Australian winter. 15 families were randomised to surgical mask group, 15 to the P2 mask group and 22 to the control group with 30, 30, and 44 adults in each group respectively. Using intention to treat analysis, we found no significant difference in the relative risk of respiratory illness in the mask groups compared to control group. However, compliance with mask use was low. Only 53% of people wore them most of the time, 27% found masks a nuisance, 30% found them physically uncomfortable, and 20% said their child did not like them wearing a mask. In a subgroup analysis of people who wore their masks, a trend in protective effect was seen, but did not reach significance. Conclusions: There are no published RCT's on mask use, yet recommendations on mask use are commonly made in disease control policy. In terms of general use in the community, we found compliance to be low. We did not achieve adequate power to demonstrate a significant benefit from mask use in preventing respiratory virus transmission in families. We are continuing recruitment in 2007 to achieve target numbers and evaluate the efficacy of masks in the community.

Abstract P1525

Routine Influenza Vaccination of Healthy Adults Aged 50-65 Years; Costs, Benefits and Budget Impact

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Objectives: The Dutch Health Council requested data on the balance between costs and effects as well as the budget impact of including healthy adults aged 50-64 years into the influenza vaccination program. Methodology: An available decision model was adapted to assess the cost-effectiveness. Rate difference methods using peri-season and the summer as reference period were applied to estimate the 6-year average (excess) morbidity and mortality during influenza seasons. Direct medical costs as well as productivity loss were accounted for. Productivity was valued using the friction cost method. The discounted incremental cost-effectiveness ratio (iCER) was expressed in costs per life-year gained. Results: Using the peri-seasonal period as reference period, the influenza attack rate was estimated at 1.97%, and the iCER was €28,019. Subgroup analyses for ages 50-54, 55-59 and 60-64 years showed iCERs of €44,558; €37,632 and € 15,810, respectively. Inclusion of 50-64 year-olds into the Netherlands vaccination program would require a yearly investment of €23 million. Savings in terms of direct medical costs and productivity loss were estimated at €1,7 million and €3 million and the net budget impact was estimated at €18 million annually. Using the summer period as reference, the average attack rate was estimated at 3.94% and the discounted iCERs all fell below €20,000. Potential savings were estimated to be higher and the net budget impact would amount to €13,1 million annually. Conclusion: The attack rate and iCER depend on the reference period chosen. Based on the most conservative estimates, the iCER fell below the accepted threshold of €20,000 per life year gained only for healthy adults aged 60-65 and this group should therefore be recommended for influenza vaccination. With the summer as reference period, the iCER fell below €20,000 per life-year gained for all five-year age categories above 50 years. As productivity losses represent the largest potential gain one might question funding this program from public means.

Abstract P1526

Latex Particles Coated With Sialic Acid-Containing Glycoprotein Are Agglutinated By Influenza Viruses

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Influenza virus (FluV) is cause of respiratory illness which is a major public health problem worldwide. In each year, new influenza vaccines are produced based on WHO recommendations. The prediction of viral strains which will spread among the people in next season is critical issue to prevent influenza by vaccination. The antigenic differences among FluV isolates can be detected by hemaggulutination inhibition (HAI) tests with ferret antisera. In previous studies, it was showed that influenza A viruses bound to sialyloligosaccharides on the cell surface with specificities that varied according to the host species of origin. Though the HAI test is a useful tool, the result depending on the kind of the red blood cell used for the examination becomes a problem. To dissolve this problem, we made the latex particles coated with sialic acid-containing glycoprotein that diversity in sialic acid linkage type and length of oligosaccharide. The particle agglutination (PA) titers using 0.05% carboxylate modified latex (CML) particles linking with LSTc-BSA (LSTc-BSA-CML)

containing NeuAc alpha 2-6Gal sequence showed comparable results to hemaggulutination (HA) titers using 0.7% human red blood cells when using viral strains A/Moscow/13/98(H1N1), A/New Caledonia/20/99(H1N1), A/Kumamoto/102/02(H3N2), B/Shangdong/07/97 and B/Johannesburg/5/99 as reference antigens. After the neuraminidase treatment, LSTc-BSA-CML was not agglutinated by any reference antigens. The PAI titers were lower than HAI titers tested all H1 subtype clinical isolates. On the other hand, for type B influenza viruses, the PAI titers were as high as the HAI titers. For H3 subtype isolates, the PAI titers tend to be higher than HAI titers. The difference of both agglutination inhibition titers in tested clinical isolates might reflect a structural difference or density of the sugar chain on the particles. These results suggest that the LSTc-BSA-CML can bind to influenza viruses and their binding is inhibited specifically by viral antigen specific antibody. The comparable sensitivity and specificity of PAI to HAI and its stability on storage are factors in favor of using LSTc-BSA CML to determine antigenicity of influenza viruses.

Abstract P1527

Does Influenza Viral Population Change in a Patient Infected With Influenza?

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Hypothesis: Influenza viruses are very changeable to evade host immunological attacks. It is reported previously that an isolated influenza virus from an individual is heterogeneous genetically. The viral genetic variation must occur in a patient infected with influenza virus. Therefore, the composition of influenza viral population at early infection phase can differ from that at late infection phase. To confirm our hypothesis, we cloned and sequenced the RT-PCR products on HA gene of influenza viruses from the same patient with time and compared the composition of viral population. Methods: We collected nasal washes from patients with influenza-like illness at early and late phase in clinical course during 2001-2004 influenza season. We isolated influenza viruses with MDCK cells by the standard procedure. We extracted viral RNA from isolates and performed the RT-PCR on HA1 region of HA gene in AH3 influenza viruses. The PCR products of 980 base pairs were cloned and sequenced in the commercial laboratory. Result: We analyzed 10 cases of 4 males and 6 females. They were from 1 month to 6 years old. Nucleotide sequenced clones from each isolate were classified into major population (a large number of identical clones), minor population (a small number of identical clones) and no cluster (a group of unique clones). The percent of major population in each isolate was from 54.5 to 90.0. At early infection phase, all isolates consist of one major population and no cluster clones. At late infection phase, all isolates have one major population, a few or no minor populations and no cluster clones. The major population in isolates at early and late infection phase from an individual had the same nucleotide sequences. However, the sequences of major population from each individual were not identical except the cases occurred around the same time. As for the deduced amino acid sequence of major populations, the comparable results were obtained. Conclusion: The major population of influenza virus from an individual dose not change during the clinical course, whereas influenza isolates are proceeding genetically in the natural course.

Abstract P1528

Protection From Influenza by Recombinant Nucleoprotein Requires CD40 Expression

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Influenza nucleoprotein (NP) elicits co-dominant CD8 T cell responses in mice during primary infection and dominant CD8 responses during heterotypic secondary infections. Achieving the full magnitude of these responses requires CD40 expression on non-T cells and CD40L expression on T cells. Because NP is conserved among viral strains, enhancing these responses via immunization is proposed to confer protection to multiple serotypes that vary at other immunogenic epitopes. Therefore, we tested whether vaccination with recombinant (r) NP could enhance NP-specific CD8 responses during infection with influenza PR8. C57BL/6 mice were given intraperitoneal injections of 30 ug rNP and 20 ug LPS at days 0 and 10, then challenged intranasally with influenza PR8 at either day 20 or day 40. Whereas mice immunized with PBS alone lost 10-15% of their body mass by one week after infection, those given rNP/LPS typically lost about 5%. However, the same treatment of CD40-/- mice did not prevent PR8-induced weight loss, indicating that systemic administration of rNP can confer protection from influenza in a CD40-dependent manner. Preliminary results suggest that CD40 expression on hematopoietic cells is necessary and sufficient for this protective response. At 8 days post-infection, the numbers of NP-specific CD8+CD62Llow lymphocytes in the lung were relatively unchanged between PBS and rNP/LPS-treated C57BL/6 mice. In the spleen, however, the numbers of NP-specific CD8 cells were up to 9-fold higher in the immunized C57BL/6, but not in CD40-/- mice. These differences were further enhanced in the C57BL/6 mice if rNP/LPS were co-administered with an agonistic anti-CD40 antibody. Therefore, systemic immunization with rNP enhances the splenic NP-specific CD8 response to PR8 in a CD40-

dependent manner, which correlates with protection from infection-induced weight loss. These observations suggest that vaccination with a conserved intraviral protein can elicit a cytotoxic T cell response that may contribute to protection from variant serotypes of the virus. The results further suggest that designing vaccine strategies that enhance CD40 signaling will be beneficial. Supported by NIH AI061511.

Abstract P1529

Towards a Unified Nomenclature System for the Highly Pathogenic H5N1 Avian Influenza Viruses

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Several recent publications have used differing names for emerging lineages of the highly pathogenic H5N1 avian influenza viruses. This has made discussion and comparison of the various lineages difficult. The H5N1 viruses have now appeared in at least 57 countries on three continents and continue to infect humans as well as to evolve and diversify at an alarming rate. Remarkably, the hemagglutinin protein has not been replaced in the various isolates since 1996 while other genes have undergone reassortment yielding many different genotypes. As such, following the evolution of the HA provides an initial constant by which the strains may be effectively compared. A consistent clade nomenclature system based upon the evolution of the HA is proposed for several reasons: 1. To unify the system so that interpretation of sequence/surveillance data from different labs becomes easier; 2. To remove stigmatizing labelling of clades by geographical reference; 3. To provide for easy future expansion of the phylogenetic tree; 4. To provide a starting point for a more extensive system to follow based upon antigenic variation and reassortment into multiple genotypes. An international group of 8 scientists and their collaborators was convened to initiate this process with the encouragement and approval of three international agencies: World Health Organization (WHO), World Animal Health Organization (OIE) and the Food and Agriculture Organization (FAO). Phylogenetic analysis was performed by a variety of approaches on all of the publicly available H5 HA sequences that have evolved from the A/Goose/Guangdong/96 H5N1 isolate. Initial results support the idea that the currently circulating HPAI H5N1 viruses could be effectively grouped into numerous clades logically designated by a hierarchical numbering system. For example the so-called 'Fujian-like lineage' within the antigenically distinct Clade 2 of H5N1 would be designated Clade 2.3, with distinct branches called 2.3.1 and 2.3.2 etc., while the 'Qinghai lineage' would be designated Clade 2.2 (currently without branches). Such a system, universally accepted, could serve as a model for other influenza lineages and genes. The progress of the international working group and collaborators will be provided in this presentation.

Abstract P1530

Decline in Influenza-Associated Mortality Among Dutch Elderly Following the Introduction of a Nationwide Vaccination Program

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Background: Recently the effectiveness of the influenza vaccine on mortality in elderly has been questioned. Objective: To assess influenza-associated mortality among Dutch elderly before and after the introduction of a nationwide vaccination program. Design, Setting, and Participants: Retrospective nationwide study among persons aged 65 years and older over 1992 to 2003. National mortality data and viral surveillance data were used to estimate influenza-associated mortality for each year using rate difference methods (taking peri-seasonal rates as reference). Before the introduction of the nationwide primary care vaccination program in 1996, vaccination coverage was below 50%. From 1996 onwards, influenza vaccination was recommended for all elderly and uptake approximated 80%. Relative risks of influenza-associated mortality with corresponding 95% confidence interval (95%CI) were estimated over the influenza seasons before and after the introduction of the nationwide vaccination program. As a "control outcome", the same analyses were performed to estimate respiratory syncytial virus (RSV)-associated mortality over the years. Furthermore, we applied a Serfling-type regression analysis as a validation of the rate difference method. Results: Before the start of the program,

the average annual influenza-associated mortality was 131 (95%CI: 123-139) per 100,000 persons. Over the respiratory seasons from 1996 onwards, this was 105 (95%CI: 99-111) per 100,000 persons (relative risk 0.80, 95%CI: 0.78-0.82). The decline was largest in the age group 65-69 years (relative risk 0.54, 95%CI: 0.50-0.59) and less in those aged 75 years and older. RSV-associated mortality was similar in the 1992-1996 and 1996-2003 respiratory seasons (relative risk 1.03, 95%CI: 0.99-1.06). Serfling-type regression analysis demonstrated similar declines in influenza-associated mortality. Conclusion: Introduction of routine influenza vaccination among Dutch elderly was associated with a significant decrease in influenza-associated mortality, notably in those aged 65-69 years.

Abstract P1531

Survival of Influenza Viruses on Banknotes

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Background: To identify the potential of transmission in case of a pandemic, it is critical to assess the survival of influenza in different environments. Since billions of them are circulating every day, we considered banknotes as a possible way of transmission and design a study to assess the survival of human influenza viruses on banknotes. Methods: 50 µL of a stock solution of influenza A (H3N2), (H1N1) and B were deposed on banknotes conserved without any special precautions at room temperature. Banknotes were used to inoculate MDCK cells at different time points to assess the survival of fully competent virus. Stability of the viral RNA was also checked using real-time RT-PCR. Results: When inoculated at high concentrations (up to $5x10^{6.25}$ $TCID_{50}$/ml) A/Moscow/10/99 was recovered by culture up to 72 hours after deposition on the banknotes. Survival of A/Wisconsin/67/2005, A/New Caledonia/20/97 and B/Hong Kong/330/05 was significantly shorter and limited only a few hours. When virus was mixed with respiratory secretions the survival rate raised significantly up to 9 days for A/Moscow/10/99 and up to 1 day for B/Hong Kong/330/05. We observed also that higher inocula lead to longer survival time. RNA from influenza (detected by real-time PCR) stayed stable for at least 10 days. With respiratory secretions of individuals with a proven influenza illness, we could reproduce similar results. Clinical specimens of naturally infected individuals tested positive during the 2007 season were used. These specimens were directly deposed on banknotes. Influenza A (H3N2) was still recovered by cell culture in 50% after 24 hours in 36% after 48 hours. Conclusions: Our study provides some unexpected results. We showed that human influenza A virus can survive on banknotes up to 9 days when in respiratory secretions. In view of these results and with the knowledge that avian influenza in humans can be excreted in large quantities from different sites, this unusual way of transmission needs to be considered.

Abstract P1532

Clinical Features of Avian Influenza A (H5N1) Infection in Indonesia July 2005-April 2007

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Background: Since July 2005, clusters and sporadic cases of human infection with clade 2 sub-clade 1 H5N1 viruses continue to appear in Indonesia with an average of five cases per month. Methods: Epidemiologic, clinical and radiologic features of all confirmed H5N1 patients from July 2005 through mid-April 2007 were documented and analyzed. Results: Specimens from 1,265 patients with suspected AI were sent to NIHRD-Indonesia. H5N1 virus infection was confirmed in 7.4% (94/1,265) patients by real-time and gel-based RT-PCR. The case fatality proportion was 79% (74/94). The median age of the patients were 19 years, 46% female, 62% aged >15 years, and 76% had a history of close contacts with dead or sick poultry. Sixty-six patients had visited health services before being admitted to a referral hospital, but were not diagnosed as suspected AI and were sent home. The median time between onset of illness and the day of admission was 5 days for fatal cases, and 6 days for survivals. The median duration of hospitalization was 3 days and 17 days for fatal and non-fatal cases, respectively. The case fatality proportion for female and males was 88% and 71% ($p = 0.04$), for ≤15 years was 78% and for >15 years was 79% ($p = 0.5$). The prominent clinical features were fever and shortness of breath. Eighty-eight percent had radiologic evidence of pneumonia. Sixty five patients were given oseltamivir 75 mg bid, but of these, 71% (46/65) died. There was significant difference of survival outcome between those who were given oseltamivir and those who were not (29% vs. 4%; $p = 0.005$). No significant difference of clinical laboratory results found between the fatal cases and the survivals, although those without thrombocytopenia were more likely to survive ($p = 0.08$). Conclusion: The case fatality proportion of the AI human cases in Indonesia is currently the highest. Most of the patients sought medical care late in their illness, indicated by the shortness of length of hospitalization before deceased. However, the speed of patients' referral to hospital did not seem to have any influence to the outcomes.

Abstract P1533

Generation of a 7-Segmented Influenza A Virus Containing the C Glycoprotein HEF Instead of Hemagglutinin and Neuraminidase

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Influenza viruses are classified into three types: A, B and C. The genomes of A and B type influenza viruses consist of eight RNA segments, whereas influenza C viruses only have seven RNAs. Both A and B influenza viruses contain two major surface glycoproteins: the hemagglutinin (HA), possessing the receptor-binding and fusion activities, and the neuraminidase (NA), which destroys the receptor by removing the sialic acid and thereby releasing the virus particles from the host cell membranes. Influenza C viruses contain only one major surface glycoprotein HEF (hemagglutinin-esterase-fusion), which has receptor-binding, fusion as well as receptor-destroying/esterase activities. By using reverse genetics, we have generated a 7-segmented chimeric influenza virus possessing six influenza A/PR/8 RNA segments (PB1, PB2, PA, NP, M, and NS), and one RNA segment encoding the influenza C/Johannesburg/1/66 (C/JHG/66) HEF instead of the HA and NA. RT-PCR, Western blot, and acrylamide gel RNA electrophoresis analysis confirmed that the HEF viral RNA (vRNA) and proteins are incorporated into the rescued chimeric virus particles, whereas the HA and NA vRNAs and proteins are not. Although the HEF coding region is flanked by previously identified influenza A HA packaging sequences at both the 3' and 5' ends, the HEF vRNA is still suboptimally packaged. Furthermore, we were able to quantify the infectious titer of the 7-segmented chimeric virus in MDCK cells by using an immunostaining method. We are now exploring the possibility that this virus could serve as a live vector to carry foreign antigens.

Abstract P1534

A Novel Consensus HA-Based DNA Vaccine Against H5N1 Influenza Viruses

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The epidemic of highly pathogenic avian influenza H5N1 viruses in poultry and its transmission to human have posed a pandemic threat. Hundreds of humans have been infected and most infections have resulted in severe morbidity and mortality. The development of effective influenza vaccines against H5N1 human infection is paramount for pandemic preparedness. Although conventional vaccines based on inactivated viral particles can induce strain-specific antibody responses, real challenges remain concerning the large-scale production capacity of such vaccines and their ability to generate cross protective immunity against heterologous viruses. We hypothesize that a consensus hemagglutinin (HAc) based DNA vaccine would have distinct advantages. Such a strategy would be readily amenable to rapid modification/production, and may potentially induce cross protective immunity. The HAc was derived from ~500 available HA sequences (GenBank, early 2006). This HAc phylogenetically resides between the WHO suggested vaccine strains in clade 1 and clade 2 of H5N1 viruses. The synthetic codon-optimized HAc DNA vaccine (pHAc) was constructed and showed high levels of HA expression in vitro. Cells expressing this HAc were capable of inducing hemadsorption. BALB/c mice receiving two vaccinations of pHAc via electroporation developed high anti-HA Ab titers (>1:10,000). By IFNγ ELISpot, HA-specific T-cell responses to a CD8 epitope were also detected (~800 SFC/10^6 splenocytes), indicating that pHAc can induce both humoral and cell-mediated immune responses in vivo. Most importantly, pHAc vaccinated mice were fully protected from a mucosal challenge with a lethal dose (100 LD_{50}) of NIBRG-14 (a reassortant influenza A virus with H5N1 clade 1 Vietnam 1194 HA). Furthermore, the immune sera from pHAc vaccinated mice were capable of neutralizing the in vitro infectivity of multiple pseudoviruses composed of HA's from H5N1 viruses (both clade 1 and 2) and the core from replication-defective HIV-1. This observation indicates that the pHAc may confer broad, cross protection against different clades of H5N1 viruses. The challenge studies using additional viral strains are currently ongoing.

Abstract P1535

A Novel DNA-Based Vaccine Protects Mice Against Lethal Infection With H5N1 Influenza Virus Isolate A/Vietnam/1203/04

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Influenza A viruses are negative-sense, segmented RNA viruses that belong to the virus family Orthomyxoviridae. This

segmented genome structure allows these viruses to evolve by reassortment of gene segments exchanged between different strains. To date, over 200 human cases of highly pathogenic avian influenza in Southeast Asia in the periods 1996-1997 and 2003-2005 and later, 2005-6, extending to Turkey and the Middle East, have been confirmed by the World Health Organization. The avian H5N1 influenza viruses that are currently circulating in Asia represent the most immediate threat of the long-anticipated next influenza pandemic. Preventive and therapeutic options for highly pathogenic avian influenza A/H5N1 infections are limited. Several vaccine candidates against highly pathogenic avian influenza have been developed and are currently undergoing clinical trials, however, the testing of antiviral drugs is a high priority for preparedness against pandemic avian influenza. Traditionally, DNA vaccines are manufactured through fermentation process. CytoGenix has developed a proprietary cell-free technology for producing large quantity of DNA such as DNA vaccines. Using Syn™ DNA technology, we have generated several vaccine candidates including SynDNA™-H5N1. Intramuscular immunization with SynDNA™-H5N1 vaccine candidate protected 13 out of 15 mice that were challenged with H5N1 virus and all vehicle vaccinated animals succumbed to the disease within 8 days. Protected animals had less severe clinical disease and gained body weight and had no encephalitic disease. Our results from these studies indicate that SynDNA™-H5N1 vaccine candidate is safe and immunogenic in mice while inducing protective immune response against avian influenza A H5N1 virus.

Abstract P1536

Evaluation of the QuickVue Rapid Diagnostic Test for Influenza in a Community Setting

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Background: Rapid influenza diagnostic tests can be useful to facilitate optimal treatment of seasonal influenza, and in a pandemic scenario could potentially be important tools for control and mitigation as well as clinical management. We evaluated the performance of the QuickVue Influenza A+B test among a large community sample of patients presenting with influenza-like-illness in a sub-tropical setting. Methods: Patients older than 2 years whose chief complaint consisted of any combination of at least 2 influenza-like symptoms were recruited from 26 outpatient clinics in Hong Kong during the 2007 influenza season. Two sets of nasal and throat swabs were obtained using a standardized protocol from each patient. One set of swabs was combined and tested by QuickVue while the other was sent for viral culture (gold standard). Results: A total of 362 subjects have been enrolled as of 21 April 2007, of a total recruitment target of at least 500 subjects by 1 June 2007. Based on currently available laboratory results for 93 enrolled patients, the QuickVue test had sensitivity 52% and specificity 97%, with positive predictive value 93% and negative predictive value 80%. Stratifying the results by age showed that the rapid diagnostic test had much lower sensitivity in adults (45%) than in children (71%). Stratifying the results by symptom onset date showed that the rapid diagnostic test had much higher sensitivity in subjects who were tested within 24 hours of symptom onset (64%) versus 24-48 hours after onset (38%). Laboratory results for all subjects will be available by mid June. Discussion: The results of our study suggest that the QuickVue rapid diagnostic test has lower sensitivity in the community setting compared to most previous evaluations typically under more controlled conditions such as laboratories and hospitals. Our finding that sensitivity varies by age and time of symptom onset is likely explained by the greater degree of viral shedding in children and earlier in the course of illness, and this has important practical implications for the use of rapid diagnostic tests in routine care as well as potential relevance for pandemic planning.

Abstract P1537

Rescue of H5N1 Subtype Avian Influenza A Virus Lethal to Mice by Reverse Genetics

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Avian influenza virus is acquiring the ability to cross the species barrier between birds and mammals. However, the genetic mechanism of this ability is not well known. We successfully rescued an influenza A virus A/Chicken/Guangdong/03 (H5N1), which is highly pathogenic to both SPF chickens and BALB/c mice, by plasmid-based reverse genetics. The rescued virus, R-A/Chicken/Guangdong/03 (R-CG), and the wild-type A/Chicken/Guangdong/03 (W-CG) were found to share similar biological properties, such as in titres of 50% egg infectious dose (EID_{50}), 50% tissue culture infectious dose ($TCID_{50}$) and

intravenous pathogenicity index (IPVI). R-CG, like W-CG, is highly pathogenic in mice following natural route infection. Both R-CG and W-CG viruses can be isolated from many organs of mice such as brain, lung, kidney and spleen. As a result, the constructed R-CG reverse genetic system can be used as a tool in the investigation of the molecular mechanism of infection of the mammalian host by avian influenza virus.

Abstract P1538

2006-2007 Influenza Vaccine Coverage Among Participants in the U.S. Department of Defense (DoD) Global Influenza Surveillance Program

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Background: The seasonal influenza vaccine is a mandatory immunization for all DoD military personnel. Vaccine coverage approaches 98% in some services and is strongly encouraged for use in the beneficiary population. Vaccine failure may occur due to a mismatch in circulating virus strains and the current influenza vaccine. Identifying these mismatches in putative HA antigenic sites may be critical in determining future vaccine composition and in providing situational awareness to commanders in the field. This information is further communicated to the Centers for Disease Control and Prevention. Methods: Individuals with positive influenza results confirmed by viral culture were collected from the DoD Global Influenza Surveillance Program's 65 sentinel sites and compared to questionnaire results and influenza immunization status obtained from the Defense Enrollment Eligibility Reporting System (DEERS). Additionally, a majority of the isolates were further genetically sequenced. Vaccine protection was assumed to begin 14 days post-vaccination. A patient is considered as having a vaccine breakthrough if an influenza isolate was collected 14 days post-vaccination. For this review, patients with vaccination dates prior to 01 August 2006 were considered as not vaccinated. Results: Culture and/or PCR-confirmed influenza was observed in 19.4% (540 of 2,784) of the total respiratory samples submitted. Of these, 21.7% (117 of 540) had history of influenza vaccination during the 2006-2007 season. Sixty-four percent (75 of 117) patients received the vaccine >14 days prior to the collection of an influenza isolate. Of these, 86.7% (65 of 75) were sub-typed (Table 1) and 64.0% (48 of 75) were sequenced. Key influenza A/H3 isolates exhibited a R142G mutation, which has been characterized as a potential key contributor to the antigenicity of these strains (Graph 2). A small percentage of the isolates conferred mutations at several other of the reported antigenic sites. Based on sequence analysis, a vast majority of the A/H1 isolates were homologous to the vaccine's New Caledonia component. However, there were a few divergent strains (Clade 2-like) (Graph 3). Isolates from Clade 2 were obtained from CONUS. Conclusion: Of patients participating in the DoD Influenza Surveillance Program, 64% who had a history of influenza vaccination contracted an influenza infection >14 days post-vaccination. Numerous influenza A isolates appeared to show certain genetic changes. These changes have been of interest to the Centers for Disease Control and Prevention, where further characterization showed reduced hemagglutinin inhibition titers in some isolates.

Abstract P1539

Modulating the Expression and Function of Retinoic Acid Inducible Gene-I (RIG-I), an Intracellular Pathogen Sensor as a Preventive and Therapeutic Strategy Against Influenza Viral Infections

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The innate immune system is the host's first line of defense against a variety of pathogens. Pattern recognition receptors (PRR) of innate immune system recognize pathogen-associated molecule patterns (PAMPs) that are unique to microorganisms and initiate the induction of inflammatory and anti-microbial responses. Retinoic acid inducible gene I (RIG-I) has recently been identified as one of the key intracellular sensors of viral infections. RIG-I binds to cytosolic double-stranded RNA or 5′ triphosphate containing single-stranded RNA and initiates a signaling cascade that leads to the activation of transcription factors required for expression of type I interferon (IFN-I). We have shown recently that ectopic expression of N-terminal fragment of RIG-I in human lung epithelial cell line (A549), inhibited the replication of influenza viruses, including highly pathogenic avian influenza virus by two logs. We will present data from our recent studies that further substantiate the potential clinical utility of RIG-I– based strategies to prevent and treat infections caused by seasonal and pandemic influenza viruses. Reference Guo, G., Chen, L-M., Gomez, J.A., Fujita,T., Katz, J.M., Donis, R.O., and Sambhara, S. (2007). NS1 protein of Influenza A Virus inhibits the function of intracytoplasmic pathogen sensor, RIG-I. Am. J. Resp. Cell Mol. Biol. 36:236.

Abstract P1540

The Economic Impact of Providing Rapid Influenza Test Results to Physicians Caring for Ambulatory Adults With Influenza-Like-Illness (ILI)

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Background: Use of rapid influenza testing is becoming more widespread even though the economic impact of providing these results to physicians has not been studied. The objective of this study is to determine the economic impact of adding the cost of rapid influenza testing to the total cost of care for adult ambulatory patients with ILI. Methods: After providing informed consent, adults at an academic primary care practice were prospectively enrolled to undergo rapid influenza testing when presenting with fever, respiratory symptoms and illness of ≤7 days. Results of the Quidel QuickVue® Influenza A+B test were either immediately made available to (MD aware) or withheld from (MD unaware) treating physicians based on alternate day assignment. Demographics, symptoms, days of illness, tests, treatments, prescriptions, physician billing and return visits within 2 weeks were recorded. Total costs were comprised of the following: diagnostic, treatment/ medications, physician billing and productivity. Costs for diagnostic tests, treatments and physician billing were assigned using the Medicare fee schedule and medication costs were assigned using prescription drug average wholesale prices. Productivity estimates were made using the average U.S.employee compensation and based on the following: 1) age <65 years, with 2) culture proven influenza, 3) antiviral therapy initiated within 48 hours of symptom onset, which 4) allows an estimated ½ day earlier return to work. Results: From January-February 2006, 203 patients were tested for influenza. MD aware and unaware groups had similar total costs ($233 vs. $231, p=0.87), medication costs ($161 vs. $166, p=0.65) and physician costs ($67 vs. $63, p=0.30). The MD aware group had higher diagnostic ($21 vs. $5, p<0.01) and antiviral costs ($18 vs. $8, p=0.02), lower antibiotic costs ($23 vs. $36, p= 0.04) and increased productivity (mean difference $14, p<0.01). For patients treated within 48 hours of symptom onset, total costs were lower for the MD aware group ($216 vs. $256, p=0.05). Conclusion: Providing physicians with rapid influenza test results does not increase total costs for adults with ILI within the first week of symptoms, while provision of the test results within the first 48 hours has the potential to lower total patient care costs.

Abstract P1541

Benefits of Physician Knowledge of Rapid Influenza Test Results in Ambulatory Adults With Influenza-Like-Illness (ILI)

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Background: Rapid influenza testing has been shown in several studies to increase antiviral use and reduce diagnostic testing and antibiotic use in pediatric patients with influenza. To our knowledge there are no prospective studies that address these issues in adults. Objective: To determine the effect of providing rapid influenza test results on physician treatment of ambulatory adults with ILI. Methods: Adult patients were prospectively enrolled at an academic primary care practice during acute care visits for ILI. After informed consent was obtained, patients had nasal swab specimens obtained for rapid influenza testing and viral culture. Results of the Quidel QuickVue® Influenza A+B test were either immediately made available to (MD aware) or withheld from (MD unaware) treating physicians based on alternate day assignment. Data collected included demographics, symptoms/symptom days, tests and treatments during the visit, prescriptions, rapid test results and viral culture results. Continuous variables were compared using the two-sample t-test. Categorical variables were compared using the Chi-square test or the Fisher's Exact Test. The designated level of significance was 0.05. Results: From January-February 2006, a total of 203 patients were enrolled and tested for influenza. During the course of the study, several physicians raised ethical concerns over the effect of withholding test results on the safety of their patients. In these cases, physicians were granted access to the rapid influenza test results. Patients were grouped and analyzed based on whether or not the MD actually received the results of the rapid influenza test. There were no significant demographic or patient characteristic differences between the MD aware and unaware groups. Patients in the MD aware group were more likely to have antivirals prescribed than those in the MD unaware group 26.7% vs. 11.1% (P=0.009). Antibiotic prescribing in the MD aware group was 36.6% vs. 48.6% in the MD unaware group (P=0.097). Conclusion: Physicians aware of the rapid influenza test results made more appropriate decisions regarding treatment of adults with influenza and ILI. More research is warranted to validate these findings.

Abstract P1542

Isolation of Highly Pathogenic Avian Influenza H5N1 Viruses in Ukraine

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Background: Ukraine, as well as other countries in Europe, was at risk of being affected by highly pathogenic avian influenza (HPAI) H5N1 viruses in autumn 2005. Taking into account flyways of wild waterfowl and migratory birds, in particular birds flying from the territory of the Russian Federation, the south of Ukraine was at the highest risk. Virus monitoring in that area revealed a circulation of only low pathogenic H5N3 viruses in that area during the summer of 2005. At the end of November, 2005, information about high poultry mortality in the west part of the Crimean peninsula was received. Information about isolation of HPAI H5N1viruses in Romania at the same period of time increased our suspicion of a similar etiology for the outbreak in Crimea. The President of Ukraine declared a state of emergency for the region. Quarantine was introduced in villages where outbreaks were registered. Euthanasia and disposal of a large number of poultry was conducted in accordance with recommendations of FAO. Methods: Samples from dead and live poultry as well as from wild birds were regularly collected for lab investigation. Samples of RNA isolated directly from original clinical samples were run in real-time PCR with use of CDC influenza A universal and subtype specific (Asian H5 and Eurasian H7) primers and probes, as well as with primers and probes specific for Newcastle disease virus. Sera were collected from people with fever and respiratory symptoms. Results: All sera tested by an H5-specific microneutralization assay were negative for anti-H5 antibody. Avian samples from chickens, ducks, geese and 14 species of wild birds, positive in real-time PCR, were inoculated into embryonated eggs and 30 strains of HPAI H5N1 viruses were isolated and characterized genetically (sequencing) and antigenically (hemagglutination-inhibition test with use of reference post-infection ferret antisera). The analysis demonstrated the grouping of these isolates with other viruses from subclade 2-2 of HPAI H5N1 viruses (A/Qinghai Lake-like viruses). Mutations previously described for viruses resistant to adamantanes or neuraminidase inhibitors were not found. Conclusions: Circulation of subclade 2-2 HPAI H5N1 viruses in 2005 in Crimea, Ukraine, was confirmed. Although additional poultry outbreaks were registered later in some other regions of Ukraine, the wide spread of HPAI viruses was prevented due to effective control measures undertaken by the state government and local authorities.

Abstract P1543

An Influenza N1 Neuraminidase-specific Monoclonal Antibody With Broad Inactivating Activity Against H5N1 Viruses

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Influenza has a long history characterized by waves of pandemics and epidemics. Although flu epidemics occur nearly every year, pandemics fortunately do not occur very often. However, today we are faced with the potential of an influenza pandemic. An avian influenza virus of the type H5N1, currently causing an epidemic in poultry in Asia, is persistently spreading throughout the globe increasing the potential for outbreaks in human populations and perhaps leading to a pandemic. The virus is highly pathogenic in birds and humans and could potentially cause widespread illness and mortality. Several vaccine candidates based on potentially pandemic influenza strains and antiviral drugs have been tested in preclinical studies. The data obtained so far have shown some promise, but have also revealed some shortcomings of both approaches. We have identified and characterized an H5N1 neuraminidase-specific monoclonal antibody which specifically inhibited N1 neuraminidase activity of high pathogenic avian influenza strains from clades 1 and 2. This antibody could have potential for the treatment of oseltamivir- and zanamivir-resistant strains of H5N1, and could be developed into a diagnostic and therapeutic tool.

Abstract P1544

Development of a Multiplex RT-PCR Based System for Neuraminidase Subtyping and Detection of Genotypic Resistance to Neuraminidase Inhibitors in Influenzavirus A

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The influenza virus neuraminidase plays an essential role in viral pathogenesis. Furthermore, the development and

introduction into clinical practice of neuraminidase inhibitors (NAI) increases the emergence of resistant viruses to these antiviral agents. A multiplex nested-PCR based system was developed for subtyping and assessing susceptibility to NAI in the genus Influenzavirus A. The assay includes a generic RT-PCR with inosinated and degenerated primers set for detection of the nine neuraminidase subtypes. The inner amplification allows the recognition and differentiation of N1 and N2 subtypes in clinical samples. The subsequent sequencing is capable of detecting the main mutations implicated in the resistance to neuraminidase inhibitors at the residues E119V/A/D/G, R292K and N294S for N2 subtype and H274Y and N294S for N1 subtype. Different subtypes of animal and human influenza A virus strains and human clinical samples were assayed by this method to ensure specificity, sensitivity and reproducibility. The sequencing of the NA gene revealed an E119V substitution associated with resistance to NAI in a prolonged shedding influenza A virus immunocompromised patient. Genotypic resistance to adamantanes was detected in the same patient by sequencing of the M2 gene and detection of the V27A mutation. A multiplex nested-PCR based system was developed for subtyping N1 and N2 neuraminidase of Influenza A virus from animal or human origin, molecular surveillance support in the genus Influenzavirus A and assessment of susceptibility to NAI. The high sensitivity of this method allows detection and sequencing directly from clinical samples, avoiding the finding of mutations due to cell culture adaptation.

Abstract P1545

A Vaccine Based on the M2 Ectodomain: Heterosubtypic Protection, Intranasal Delivery and Mechanism of Action

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The 23 amino acid long ectodomain of matrix protein 2 (M2e) is highly conserved among all human influenza A strains. M2e-specific antibodies are barely induced following infection. Two M2e-consensus sequences can be discerned, a human-type (hM2e), and an avian-type (avM2e), differing at five positions. Of note, A/Brevig Mission/1/1918 virus and most isolates from H5N1-infected patients have an intermediate sequence. The strong conservation of these two types and the limited changes allowed in the avM2e to hM2e adaptation are mainly due to constraints imposed by the overlapping M1 (matrix) gene. M2e, when it is expressed in multiple copies on the surface of hepatitis B core-particles, is highly immunogenic and induces protective antibodies. This vaccine was engineered to improve particle stability and M2e immunogenicity, and it can be produced in high yields in E. coli. Parenteral immunization of mice protected against a lethal challenge with various virus subtype strains, prevented or limited morbidity, and reduced viral lung titer. We demonstrated that several adjuvants that are suitable for humans enhance the protective immune response. In addition, pre-existing anti-HBc antibodies did not significantly affect anti-M2e humoral responses to injected M2e-HBc particles. Vaccination also protected against a lethal challenge with a virus strain containing an avian type M2-protein, in accordance with the finding that hM2e-immune serum specifically binds to a large panel of avM2e-sequences. Intranasal vaccines have a number of advantages, including needle-free administration and the induction of mucosal immunity in the nasal and respiratory lymphoid tissues, an advantage for protection against a respiratory pathogen. We used CTA1-DD, a Cholera toxin-derived non-toxic recombinant mucosal adjuvant that is targeted to immune cells. Intranasal delivery of M2e-vaccine plus CTA1-DD induced high titers of circulating M2e-antibodies, and provided complete protection against a lethal challenge in a mouse model. Body temperature and weight loss were less pronounced than in the group vaccinated parenterally with M2-HBc. Finally, by using different knockout animals we demonstrated that anti-M2e IgGs provide protection, at least in part, by a cell-mediated immune mechanism.

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