isirv
Antiviral Group

Programme & Abstract Book

Severe Influenza: Burden, Pathogenesis and Management

29th – 31st October 2012
Melia Hotel, Hanoi, Viet Nam

The second isirv-Antiviral Group Conference
in conjunction with NIHE

NIHE

Front Cover Picture:

The National Institute of Hygiene and Epidemiology (NIHE) in Hanoi focuses on prevention and control of communicable diseases and operates under the authority of the Vietnamese Ministry of Health. In addition to scientific research in epidemiology, medical microbiology, immunology, and molecular biology, NIHE conducts various national programmes on infectious disease surveillance and control, develops vaccines, and conducts public health training and education at both undergraduate and post-graduate levels. Founded in 1926 as the Pasteur Institute of Hanoi, NIHE became a national public health institute in 1997. Today it employs some 300 people,

Many major infectious diseases, including tetanus, typhoid, mumps, meningitis, hepatitis, plague, rabies and malaria, have been significantly reduced or eradicated (polio) in Vietnam over the past 10 years. However, tuberculosis, dengue hemorrhagic fever, viral encephalitis, diarrhoea, measles, cholera and HIV/AIDS remain major public health problems, and the country is ever a geographical hot spot for emerging infectious diseases such as SARS and avian influenza.

Presently NIHE's main research focus is emerging, and re-emerging infectious disease including influenza, dengue, hand-foot and mouth disease, viral encephalitis, HIV/AIDS, rabies. Institute plans for the future are to build an International Center for Biomedical Research, which would serve as an epidemic intelligence center focused on early detection and rapid response for emerging diseases and pandemics that threaten Vietnam and other countries in the region.

NIHE is a member of the International Association of National Public Health Institutes (IANPHI), Pasteur Institute Network, Asian-African Research Network of Infectious Diseases, Emerging Infectious Diseases (EID) Program of ASEAN and currently has strong partnership with 9 international governments, neighbouring countries, and collaborates closely with 23 institutes and universities and international organizations, such as WHO, Unicef, PATH, JICA, FHI, US CDC, GAVI, NHI, IVI, NIID, Nagasaki and Oxford University.

Acknowledgements

We are most grateful for the support from the following companies and institutions – thank you.

BMG LABTECH
The Microplate Reader Company

Roche

DENKA SEIKEN CO., LTD.

SHIONOGI & CO., LTD.

FUJIREBIO INC.

TOYAMA CHEMICAL CO., LTD.
FUJIFILM
FUJIFILM Group

NIHE

Viroclinics biosciences

wellcome trust

Severe Influenza: Burden, Pathogenesis and Management

Monday 29th - Wednesday 31st October 2012, Hanoi

ORGANISING COMMITTEE

Chairman

Alan Hay
Chairman, isirv-AVG
National Institute for Medical Research
Mill Hill
London
UK

Peter Horby
Oxford University Clinical Research Unit
Hanoi
Viet Nam

Mai Le
National Institute of Hygiene and Epidemiology (NIHE)
Hanoi
Viet Nam

Arnold Monto
University of Michigan School of Public Health
Ann Arbor, MI
USA

John Nicholls
Hong Kong University
Hong Kong
SAR, China

Dung Tham
National Institute of Hygiene and Epidemiology (NIHE)
Hanoi
Viet Nam

John Watson
Heath Protection Agency
London
UK

Maria Zambon
Heath Protection Agency
London
UK

SCIENTIFIC PROGRAMME COMMITTEE

Chairman

Frederick Hayden
University of Virginia School of Medicine
Virginia
USA

Jeremy Farrar
Wellcome Unit Viet Nam/Oxford University Clinical Research Unit Viet Nam (OUCRU)
Ho Chi Minh City
Viet Nam

Nguyen Tran Hien
National Institute of Hygiene and Epidemiology (NIHE)
Hanoi
Viet Nam

Tran Tinh Hien
Oxford University Clinical Research Unit Vietnam (OUCRU)
In partnership with
Hospital for Tropical Diseases (HTD)
Ho Chi Minh City
Viet Nam

Peter Openshaw
National Heart and Lung Institute
Imperial College
London
UK

Nahoko Shindo
World Health Organisation
Geneva
Switzerland

Norio Sugaya
Keiyu Hospital
Yokohama
Japan

Welcome

On behalf of the Organising and Scientific Committees we take great pleasure in welcoming you to Hanoi for the second isirv Antiviral Group (isirv-AVG) conference on Severe Influenza: Burden, Pathogenesis and Management, organised in conjunction with the National Institute of Hygiene and Epidemiology (NIHE).

The 2009 A(H1N1) pandemic was a stark reminder of the unpredictability of influenza and the many gaps that remain in our understanding of the pathogenesis and management of severe influenza, whether due to pandemic, seasonal or H5N1 viruses. This recent experience, coupled with the persistent threat of highly pathogenic avian A(H5N1) and new information on its potential transmissibility, demands a renewed focus on the management of severe influenza.

This conference therefore brings together leading experts to present their research and discuss all aspects of severe influenza, including its epidemiology and clinical manifestations, virus and host factors in SARI pathogenesis, and clinical management, including intensive care strategies and the role of antivirals and other interventions in mitigating disease severity as well as the problem of antiviral resistance.

Its location in Hanoi reflects the continuing threat of H5N1, particularly in South-East Asia where some two-thirds of human cases have been reported, and the divergent evolution of the viruses in this part of the world, as well as the burden of seasonal influenza in countries with limited use of vaccine. Moreover, about a fifth of the 600 or so cases of H5N1, with its high fatality, have occurred in Viet Nam, and the medical services in this country were in the forefront in confronting the emerging threat during 2004-2005, treating over 60% of the H5N1- infected patients.

The programme incorporates a combination of symposium sessions to provide a comprehensive review of the various aspects of severe influenza and its management, workshops to provide a forum for more detailed discussion of particular themes, and more practical demonstrations of selected topics.

We thank all who have contributed to making this conference possible: the members of the Organising and Scientific Committees for their imaginative contributions; the generous financial support of our sponsors, including provision of travel grants for more than 20 participants; but particularly to the staff of NIHE and DeSouza Associates who have dealt so effectively with the logistics and organisational challenges to bring the conference to fruition.

We trust that their efforts will be rewarded by the success of the conference in enhancing your understanding of the various aspects of the clinical management of severe influenza and other respiratory disease. In addition, we hope that you have a stimulating and productive experience which will engender collaboration and encourage participation in isirv and the antiviral Group.

Alan Hay
Chair of isirv-AVG

Nguyen Tran Hien
Director of NIHE

John Watson
Chair of isirv

Severe Influenza: Burden, Pathogenesis and Management

Monday 29th - Wednesday 31st October 2012, Hanoi

Programme

Monday 29th October

07:00 onwards	Registration
09:00-09:20	Welcomes by Prof Nguyen Tran Hien (NIHE), Alan Hay (Chair AVG) & John Watson (Chair isirv)
09:20-09:40	**Special Remarks**: *Madam Nguyen Thi Kim Tien, Minister of Health, Viet Nam*
	Symposium Session 1: Burden of Severe Influenza and SARI Chairs: *Nguyen Tran Hien, Viet Nam & John Watson, UK*
09:40-10:00	• Incidence, Severity & Impact of Seasonal and Pandemic Influenza - summary of key conclusions from an isirv meeting, September 2012 *John Watson, UK*
10:00-10:30	• **Keynote Lecture** Epidemiology and Impact of Severe Influenza and SARI: Asian Perspectives *Takeshi Kasai, Viet Nam*
10:30-11:10	• Overview on Influenza Vaccines *Lance Jennings, New Zealand & Nguyen Tran Hien, Viet Nam*
11:10-11:30	Tea/Coffee
11:30-13:30	**Symposium Session 2: Influenza Pathogenesis and Acute Lung Injury** Chairs: *John Nicholls, HK, SAR China & Menno de Jong, The Netherlands* • Immunopathology of Acute Lung Injury and ARDS *Malik Peiris, HK, SAR China* • Observations from Avian H5N1 Patients *Tran Tinh Hien, Viet Nam* • Mechanisms of Severe Pandemic 2009 H1N1 Illness *Peter Openshaw, UK* • Host Genetic Factors in Susceptibility and Severity *Peter Horby, Viet Nam* • Viral-Bacterial Interactions: Therapeutic Implications *Jane Deng, USA*
13:30-14:30	Lunch
14:30-16:00	**Concurrent Workshops 1** **a.** Influenza Antivirals – effectiveness, safety, novel agents, usage, national policies, guidelines Chairs: *Nahoko Shindo, Switzerland & Bin Cao, China* *Nahoko Shindo* - Development of WHO Standard Guideline on Clinical Management of Influenza Virus Infection *Reiko Saito* – Clinical Effectiveness of Neuraminidase Inhibitors – Oseltamivir, Zanamivir, Laninamivir, and Peramivir – for Treatment of Influenza A(H3N2) and A(H1N1)pdm09 Infection *Jean-François Rossignol* - Nitazoxanide in the Treatment of Acute Uncomplicated Influenza *Jaap Goudsmit* – Human Monoclonal Antibodies to Prevent and Treat Influenza A and B, Including Infections by H5N1 Virus *Donna Ambrosino* – Design of a Broadly Neutralizing Antibody Targeting Influenza *Celine Deffrasnes* – Modulating the Expression of Host Molecules Decreases H5N1 Replication

b. Severe Influenza and SARI Epidemiology – impact, surveillance, healthcare utilization, vaccines

Chairs: *Abdullah Brooks, USA & Mai Le, Viet Nam*

Abdullah Brooks
Maciej Boni - Understanding Influenza Dynamics Through Serial Seroepidemiology
Ji Yun Noh – Laboratory Surveillance on Influenza-like Illness at Emergency Department in Seven Teaching Hospitals, South Korea: 2011-2012
Taro Kamigaki – Estimates of Severe Acute Respiratory Infections Incidence in a City of the Philippines, 2009-2011
Pham Quang Thai – Effect of Climate on ILI Dynamics and Seasonality in Vietnam from 1991 to 2010
Helen Green – Severe Influenza Critical Care Surveillance: Insights into the Impact and Severity of the 2010/11 Influenza Season Relative to the 2009/10 Pandemic Season in England

16:00-16:30 Tea/Coffee

16:30-18:30 **Demonstrations**

- WHO Training Materials on SARI Management
  Nahoko Shindo, Switzerland, Paula Lister, UK & Janet Diaz, USA
- National Surveillance Platforms in Viet Nam
  Nguyen Tran Hien, Viet Nam
- Neuraminidase Inhibitor Resistant and Sensitive Reference Viruses for Use in Susceptibility Testing
  Aeron Hurt, Australia
- Analysis of Antiviral Resistance Data – IC_{50} determination and statistical analysis
  Larisa Gubareva, USA & Adam Meijer, The Netherlands

19:30 Conference Dinner - Temple of Literature

Severe Influenza: Burden, Pathogenesis and Management

Monday 29th - Wednesday 31st October 2012, Hanoi

Tuesday 30th October

08:45-10:45 **Symposium Session 3: Advances in Clinical Management**
Chair: *Arnold Monto, USA*

- New Developments in Influenza and Respiratory Pathogen Diagnostics
  Christopher Wong, Singapore
- Influenza Antiviral Effectiveness in Hospitalised Patients (Including Intravenous NAIs)
  Norio Sugaya, Japan
- Detection and Management of Antiviral Resistance Emergence
  Guy Boivin, Canada
- Adjunctive Therapies and Immunomodulatory Agents in SARI Management
  David Hui, HK, SAR China
- Ventilatory Strategies, Fluid Management and Supportive Care in ALI/ARDS
  Andrew Luks, USA

10:45-11:15 Tea/Coffee

11:15-12:45 **Concurrent Workshops 2**

a. SARI Pathogenesis and Treatment – receptors, host responses, host-directed therapies, biologic response modifiers
Chairs: *Nelson Lee, HK, SAR China & Tim Uyeki, USA*

Nelson Lee – Pathogenesis of Severe Influenza: Virus, Host, and Virus-Host Interactions
Eric Bortz – Host RNA Binding Proteins Regulate Adaptation of H5N1 HPAI Polymerase to Human Cells
Hassan Zaraket – The Hemagglutinin Protein Acid Stability Regulates H5N1 Influenza Virus Pathogenicity and Host Adaptation
Amabel Tan – Immune Stimulants as Potential Agents Against Pandemic Influenza and Secondary Bacterial Infection
Annette Fox – Severe Pandemic H1N1 2009 Infection is Associated with Transient NK and T Deficiency and Abberant CD8 Responses
Sarah Fardy – Analysis of Inflammasome Related Molecules in the Response to H5N1 Avian Influenza

b. Antiviral Resistance – surveillance/monitoring, mechanisms, assay development
Chairs: *Jenny McKimm-Breschkin, Australia & Philippe Buchy, Cambodia*

Jenny McKimm-Breschkin – Screening Neuraminidase Inhibitor Susceptibility of Avian Influenza Isolates from SE Asia 2005-2008 Identifies H5N1 I222 Mutants with Reduced Oseltamivir Sensitivity
Philippe Buchy – Cross-Neutralization Activity of Anti-H5N1 Specific Polyclonal Immunoglobulins Against Heterologous Strains of H5N1 Virus
Angie Lackenby – Classification of Neuraminidase Inhibitor Susceptibility for Surveillance
Martin Schutten – Treatment-Emergent Oseltamivir Resistance in Influenza A Viruses in the Influenza Resistance Information Study (IRIS) is Uncommon and Associated with Patient Age and Year of Enrolment
Adam Meijer – Oseltamivir-Resistant A(H1N1)pdm09 Influenza in Travellers Returning from Spain
Mika Shigematsu – What will Influenza Surveillance tell you about Antiviral Use and Resistance?

12:45-14.00 Lunch

Severe Influenza: Burden, Pathogenesis and Management

Monday 29th - Wednesday 31st October 2012, Hanoi

14:00-16:00	**Symposium Session 4: Meeting Clinical Challenges** Chairs: *Frederick Hayden, USA & Tim Uyeki, USA* • Antiviral Studies and Viral Fitness in Animal Models of Influenza *Yoshihiro Kawaoka, USA* • Influenza Encephalopathy and Related Neuropsychiatic Syndromes *Masashi Mizuguchi, Japan* • Severe Influenza in Pregnancy: Mechanisms and Management *May Li Lim, Singapore* • Influenza Prevention and Treatment in Transplant Recipients and Immunocompromised Hosts *Michael Ison, USA* • Advances in Antivirals for Non-Influenza Respiratory Viruses *Frederick Hayden, USA*
16:00-17:00	isirv-AVG Annual General Meeting
17:00-19:00	Poster Reception

Severe Influenza: Burden, Pathogenesis and Management

Monday 29th - Wednesday 31st October 2012, Hanoi

Wednesday 31st October

08:30-10:15	**Concurrent Workshops 3** **a.** Clinical Issues – unusual syndromes, diagnostics, ventilatory strategies, clinical management, healthcare worker protection Chairs: *David Hui, HK, SAR China, Andrew Luks, USA & Shigeru Saito, Japan* *Shigeru Saito* – No Maternal Death Caused by Pandemic (H1N1) 2009 in Japan *Toshihisa Ishikawa* – Rapid and Sensitive Detection of Highly Pathogenic H5N1 Influenza Virus by The SmartAmp Method *Jiu-xin Qu* – Etiological Study of Community-Acquired Pneumonia Among Adolescents and Adults Patients With Low or Moderate Severity *Shu Qiao Yang* – Influenza Pneumonia: A Concurrent Comparison Between pH1N1 and H3N2 in the Post-Pandemic Period *Steven Rockman* – Efficacy of a Single Dose of Intravenous Immunoglobulin to Prevent Pandemic Influenza *Cam Binh* – The Ethics of Research in Rapidly Evolving Infectious Disease Epidemics **b.** Consequences of Antiviral Resistance Mutations, Fitness, Animal Models Chairs: *Jilong Chen, China & Elena Govorkova, USA* *Jilong Chen* – Identification of Novel Host Factors Involved in Regulating Influenza Virus Infection and Replication *Sylvie van der Werf* – Impact of Genetic Variations of the Neuraminidase of the H5N1 and Pandemic H1N1pdm09 Viruses on Their Enzymatic Activity and Sensitivity to Neuraminidase Inhibitors *Aeron Hurt* – Using Ferrets to Assess the Fitness of the Oseltamivir-Resistant A(H1N1)pdm09 Viruses Responsible for a Cluster of Community Cases in Australia in 2011 *Erhard van der Vries* – H1N1 2009 Pandemic Influenza Virus: Resistance of the I223R Neuraminidase Mutant Explained by Kinetic and Structural Analysis *Andrés Pizzorno* – Evaluation of Pandemic Influenza A/H1N1 Virus Mutations Conferring Resistance to Zanamivir *Tatiana Baranovich* – T-705 Suppresses Influenza A Virus Infectivity Via Lethal Mutagenesis
10:15-10:45	Tea/Coffee
10:45-13:00	**Symposium Session 5: Future Directions** Chairs: *Alan Hay, UK & Tran Tinh Hien, Viet Nam* • Influenza Evolution: Can We Predict What's Next? *Jesse Bloom, USA* • Antivirals in 2009 Pandemic – lessons and implications for future strategies *Maria Zambon, UK* • Antiviral Combinations and Biotherapeutics for Influenza *Menno de Jong, The Netherlands* • New Research Paradigms in Response to Emerging Infectious Threats *Jeremy Farrar, Viet Nam* • Panel Discussion: Future Research Needs *Frederick Hayden (USA), Jesse Bloom (USA), Menno de Jong (The Netherlands), Jeremy Farrar (Viet Nam), Malik Peiris (HK, SAR China), Nahoko Shindo (Switzerland), Maria Zambon (UK)*
13:00-13:15	Conclusions and Close of Meeting
13:15-14:00	Lunch
14:00-15:30	Tour of NIHE

Notes

Monday 29th October

Symposium Session 1: Burden of Severe Influenza and SARI
Chairs: Nguyen Tran Hien & John Watson

Incidence, Severity and Impact of Seasonal and Pandemic influenza - summary of key conclusions from an ISIRV meeting, September 2012
John Watson – Health Protection Agency, London, UK

Despite preparation during the preceding years, the 2009 influenza pandemic posed considerable challenges to countries around the world to determine the appropriate responses. Many lessons were learnt both during the pandemic itself and in the subsequent periods of 'seasonal' influenza activity. Particularly crucial in making decisions about the response was an understanding of how great a threat the new influenza virus posed: how likely was it that individuals would develop an illness as part of the pandemic, how likely was it that they would become seriously ill and how likely was it that they would die? What ultimately would be the burden of illness in different populations and how effective would different approaches be to controlling the pandemic? Central to answering these questions is an understanding of the true incidence of influenza in the population and the severity of the illness in different subgroups of the population. The ISIRV conference in September 2012 considered these issues by bringing together many of those engaged in work in this area since the pandemic. In addition to consideration of the technical aspects of measuring incidence, assessing likely severity and predicting impact, the conference considered the role of communication and how the information collected could be turned into effective public health action. The conference also highlighted approaches to strengthening surveillance for influenza in the future and avenues for further research.

Overview on Influenza Vaccines
Lance Jennings – Canterbury District Health Board, Christchurch, New Zealand

Influenza vaccination remains the most effective way to prevent influenza virus infection and the associated severe outcomes. Recent global public health treats from avian influenza and pandemic influenza have highlighted major gaps in our knowledge base and ability to produce safe and effective vaccines in a relevant time frame and our ability to communicate to policy makers, health care professionals and the public, the benefits and risks associated with their use. The seasonal influenza vaccines currently in use are predominantly egg-derived inactivated vaccines, which have consistently been shown to provide good protection against influenza infection and reduce the risk of hospitalisation and death. However their protection is antibody-mediated, strain-specific and is dependent on the closeness of the match between the vaccine strain and the circulating virus, so that developing vaccines with broader and longer lasting protection is a high priority, and the "Universal Vaccine" the ultimate goal.

The increased focus on pandemic preparedness planning following the 2004 avian influenza epizootic and the development of monovalent H5N1 vaccines, has lead to new knowledge surrounding pre-pandemic vaccines; the need for vaccines which are cross-protective across the H5N1 clades, the role of adjuvanted vaccines and cell culture derived vaccines. However, our response to the A(H1N1)pdm09 pandemic has highlighted the practical issues relating to novel vaccine development, production and global supply. Increased capacity and new technologies are needed to produce vaccines against a novel influenza virus in sufficient quantities to protect populations during an emerging pandemic, not just the second wave. Seasonal influenza provides an ongoing threat with the cumulative annual burden, eclipsing that of each pandemic. The WHO has recently reviewed the recommendations for influenza vaccination which now identify pregnant women as the highest priority group ahead of health care workers, children aged 6 months to 5 years, people aged 65 or more, and people with pre-existing chronic illnesses. However recommendations need to be translated into a countries policy, and then control strategies implemented. In the Asia-Pacific region, control strategies vary widely - from countries with an appreciation of influenza impact, good surveillance, extensive vaccination programs, and fairly good vaccine uptake; to those with no recommendations, no

programs, little information about impact, and very little vaccine use. A countries affluence is related to vaccine use, however policies for seasonal influenza control with effective communication strategies need to be in place.

Monday 29th October

Symposium Session 2: Influenza Pathogenesis and Acute Lung Injury

Observations from Avian H5N1 Patients

Tran Tinh Hien – Oxford University Clinical Research Unit, Ho Chi Minh City, Viet Nam

Avian influenza A (H5N1) occurred in poultry throughout Asia has had major economic and health repercussions. Infections with this virus have been identified since January 2004 and despite intensive clinical management the case fatality is still unacceptably high. We report the clinical findings among patients with confirmed cases of avian influenza A (H5N1) who presented to hospitals in Vietnam. In all cases, the diagnosis of influenza A (H5N1) was confirmed by means of viral culture or reverse transcriptase–polymerase chain reaction (RT-PCR). Patients presented to hospitals after a median duration of illness of 6 days with fever (75%) cough (89%) and dyspnea (98%). Diarrhea and mucosal bleeding at hospital admission were more common in fatal than in non-fatal cases. Common findings were bilateral pulmonary infiltrates on chest X-Ray (72%), lymphopenia (73%), and increased serum transaminase levels (ALT69%, ALT 61%). The most reliable predictor of a fatal outcome was the presence of both neutropenia and raised ALT level on admission (correctly predicted 91% of death and 82% of survival). Treatment of oseltamivir showed benefit but use of corticosteroid is associated with increased risk of death. We observed low peripheral blood T-lymphocyte counts and high chemokine and cytokine levels in H5N1-infected individuals, particularly in those who died, and these correlated with pharyngeal viral loads. Our observations indicate that high viral load, and the resulting intense inflammatory responses, is central to influenza H5N1 pathogenesis. The focus of clinical management should be established with early diagnosis and effective antiviral treatment. There is an urgent need for more coordination in clinical and epidemiologic research among institutions in countries with cases of influenza A (H5N1) and internationally.

Mechanisms of Severe Pandemic 2009 H1N1 Illness

Peter Openshaw – Imperial College, London, UK

Pandemics remain a major threat to security, health and wellbeing. To better understand the pathogenesis of influenza, we mounted a multicentre collaborative study of patients admitted with influenza like illness during and after the 2009 pandemic. Although illness was generally mild, there were over 8,000 flu-related hospitalisations in the UK. The disease was especially severe in some patients with asthma, in pregnant women and in children under 5 years of age, but many of those admitted to hospital had no known risk factor and were otherwise young and healthy.

To learn more about the causes of severe influenza we established a consortium of 45 principal investigators to conduct an in-depth study of the factors that affect disease severity. the 'Mechanisms of Severe Acute Influenza Consortium' (MOSAIC, funded by the Wellcome Trust and MRC). We recruited 257 patients with influenza-like illness from 11 hospitals (total 4800 beds), obtaining sequential samples from the respiratory tract and blood throughout illness and recovery.

Research teams were set up to focus on host (cellular immunology, soluble mediator responses, transcriptomics and genomics), pathogen (influenza virus genetic and antigenic characteristics, viral shedding and viral load) and co-pathogen (co-infecting or secondarily-infecting bacteria and viruses. MOSAIC allows a series of related research questions to be tested, linking the diverse information on a case-by-case basis from patients and controls.

Analysis will take time to complete, but we have so far identified a gene variant in IFITM3 that causes defective host responses and increased disease severity. There are currently more than 15 separate investigations underway using the core clinical data and archived biobank. Ultimately we hope to improve our understanding of influenza and its pathogenesis, allowing novel approaches to both diagnosis and treatment of SARI.

Host Genetic Factors in Susceptibility and Severity
Peter Horby – Oxford University Clinical Research Unit, Hanoi, Viet Nam

Data is accumulating to suggest an important role for host genetics in susceptibility to influenza in mice and humans. So far only one gene (Ifitm3) has been convincingly shown to play a role in susceptibility to severe influenza A in humans and a large number of candidate genes remain to be explored. Susceptibility to H5N1 may be less complex than 'human influenza', since the phenotype appears to be more dichotomous than continuous, immunity probably plays a lesser role, co-morbidity seems less important, and familial aggregation is more marked. Despite the challenges, the importance of understanding the pathogenesis of highly pathogenic influenza and the possibility that a rare genetic variant with a moderate to large effect underlies H5N1 susceptibility, makes efforts to assemble DNA from H5N1 cases worthwhile.

To identify single nucleotide polymorphisms associated with susceptibility to H5N1 we undertook a genome-wide, case-control discovery study in 51 H5N1 cases from Vietnam and Thailand. Five SNPs in two distinct genetic loci on Chromosome 2 were identified with odds ratios of 3-4 and P □ 5 x 10-5 (three SNPs at Interleukin-1 gene cluster and two SNPs at TRPM8). One SNP at TRPM8 (rs7560562) reached genome-wide significance at P □ 5 x 10-8. When genetic variation at both loci was considered simultaneously we found strong evidence of an association with susceptibility to H5N1 (P-trend = 7.80 x 10-12), with an odds ratio of 31 (95% ci = 9.84 – 98.18) for individuals carrying three copies of the risk alleles compared to wild-type individuals.

Viral-Bacterial Interactions: Therapeutic Implications
Jane Deng – University of California at Los Angeles, USA

Bacterial pneumonias have been a major lethal complication of all influenza pandemics over the last century. Even during non-pandemic years, substantial epidemiologic evidence links respiratory viral infections with the development of bacterial pneumonia, particularly among patients who require hospitalization. Immunologically, acute viral infections change the immune environment in the respiratory tract, thereby placing the host at risk for secondary bacterial infection. The pathogenesis of viral-bacterial co-infections appears to be complex - on the one hand, involving viral-mediated impairment of antibacterial innate immune responses, while on the other, creating a condition of dysregulated inflammation that leads to lung injury. At the present time, clinicians face several challenges to effective management of secondary bacterial infections following acute viral infections. During this talk, our current understanding of the pathogenetic mechanisms involved in viral-bacterial co-infections will be presented, as well as strategies that researchers and clinicians can use to combat this significant public health problem.

Funding: NIH R01 HL108949, CalTech-UCLA JCTM TAG award, Pfizer ASPIRE award to J.C.D.

Monday 29th October - Concurrent Workshop 1a

Influenza Antivirals – effectiveness, safety, novel agents, usage, national policies, guidelines

Chairs: *Nahoko Shindo, World Health Organization, Geneva, Switzerland & Bin Cao, Capital Medical University, Beijing, China*

O1. Development of WHO Standard Guideline on Clinical Management of Influenza Virus Infection
Nahoko Shindo

O2. Clinical Effectiveness of Neuraminidase Inhibitors – Oseltamivir, Zanamivir, Laninamivir, and Peramivir – for Treatment of Influenza A(H3N2) and A(H1N1)pdm09 Infection.
Reiko Saito

O3. Nitazoxanide in the Treatment of Acute Uncomplicated Influenza
Jean-François Rossignol

O4. Human Monoclonal Antibodies to Prevent and Treat Influenza A and B, including Infections by H5N1 Virus
Jaap Goudsmit

O5. Design of a Broadly Neutralizing Antibody Targeting Influenza
Donna Ambrosino

O6. Modulating the Expression of Host Molecules Decreases H5N1 Replication
Celine Deffrasnes

Abstracts

O1

Development of WHO Standard Guideline on Clinical Management of Influenza Virus Infection

Nahoko Shindo

World Health Organization, Geneva, Switzerland

Background: setting norms and standards, and promoting and monitoring their implementation is one of the core functions of WHO. In response to the threat of avian influenza A(H5N1) virus, and the emergence of pandemic influenza A(H1N1) 2009 virus, a series of "rapid advice" (emergency) and interim guidelines have been published. Many of these are time-limited, scope-limited, or have been developed without full review of evidence or documented peer review.

Aim & Scope: the aim is to consolidate interim and emergency guidelines and other guidance notes into a comprehensive standard guideline. In doing so, the evidence behind such guidelines will be more extensively reviewed, and cross referencing to other WHO guidelines better documented. The resulting guidelines will thus be valid over a longer time period, and will provide a strong reference base for any future emergency guidance in the event of new influenza outbreaks, epidemics or pandemic.

The overall scope of the WHO guideline will be the clinical management of severe influenza disease. The following aspects will be included within this scope:

- Treatment of severe influenza e.g. viral pneumonia, ARDS, multiple organ failure, septic shock;
- Pharmacological interventions for treatment, including influenza antiviral drugs, anti-inflammatory drugs and adjunctive therapies;
- Non-pharmacological clinical interventions, such as mechanical ventilation, oxygen and fluid management.
- Preventing development of severe influenza; including treatment of patients at higher risk of progression to severe disease and prevention of infection in highest risk patients

Formulating questions and choosing outcomes based on the PICOT framework:

Population	All patients presenting with severe or deteriorating influenza illness All patients in groups defined as at higher risk of severe or complicated disease
Intervention	Influenza antivirals (including investigational products) Adjunctive therapies, such as immunomodulators, serum or plasma products Other pharmacological and non-pharmacological clinical interventions
Comparator	There are currently few established standards; comparator is generally no intervention or placebo
Outcome	Prevention of infection (in higher risk individuals) Prevention of disease progression Time to resolution of severe illness Reduction in hospital or ICU admission or length of hospital stay Reduction in mortality
Time	Short term (to resolution of illness)

Process: the Standard Guideline has been developed in accord with WHO standard for guideline development. The development of a standard guideline requires substantial evidence review and assessment. Both the commissioning of systematic reviews, as well as GRADE assessments, are required. Areas to be addressed include:

- Evidence that antivirals reduce morbidity and mortality associated with severe or complicated influenza

- Evidence supporting definition of higher risk groups for severe or complicated influenza
- Data on investigational and adjunctive therapies
- Evidence that standard of care in viral pneumonia and ARDS are relevant for influenza infections
- Efficacy of antivirals and vaccines in preventing infection in higher risk groups
- Confirmation that secondary bacterial infections (community acquired and nosocomial) are similar in influenza cases to other presentations in comparable settings

Outcome: the standard guideline is due publication later in 2012.

O2

Clinical Effectiveness Of Neuraminidase Inhibitors Oseltamivir, Zanamivir, Laninamivir, and Peramivir — for Treatment Of Influenza A(H3N2) And A(H1N1)pdm09 Infection

R Saito[1], Y Shobugawa[1], I Sato[2], T Kawashima[3], C Dapat[1], IC Dapat[1], K Hiroki[1], Y Suzuki[1,4], K Saito[1] and H Suzuki[1,5]

[1]Department of International Health, Graduate School of Medical and Dental Sciences, Niigata University, 1-757 Asahimachi-dori, Chuo-ku, Niigata 951-8510, Japan; [2]Yoiko Pediatric Clinic, Niigata, Japan; [3]Kawashima Naika Clinic, Gunma, Japan; [4]Viral Disease and Epidemiology Research Division, National Institute of Animal Health, National Agriculture and Food Research Organization, Tsukuba, Japan; [5]Faculty of Nursing, Social Welfare, and Psychology, Niigata Seiryo University, Niigata, Japan

The clinical effectiveness of the newly released neuraminidase inhibitors (NAIs) laninamivir and peramivir has not been sufficiently evaluated in influenza-infected patients in clinical and practical settings. In this study, we analyzed the clinical data of 211 patients infected with influenza A virus subtype H3N2 (A(H3N2)) and 45 patients infected with influenza A virus subtype H1N1pdm (A(H1N1)pdm09) who received the NAIs oseltamivir, zanamivir, laninamivir, or peramivir during the 2010-2011 influenza season. The duration of fever from the first dose of the NAI to fever alleviation to less than 37.5oC was evaluated as an indicator of the clinical effectiveness of the NAIs in the influenza infected patients. For the A(H3N2)-infected patients, Kaplan–Meier analysis showed the peramivir treatment group had the fastest time of fever alleviation to less than 37.5 oC (median 17.0 h, 95 % confidence interval [CI] 7.2–26.8 h) of the four treatment groups. No significant difference was found in the time to fever alleviation among the other antivirals, oseltamivir, zanamivir, and laninamivir. Results of multivariate analysis, using a Cox proportional- hazards model (hazard ratio 3.321) adjusted for the factors age, sex, body weight, vaccination status, time from onset to the clinic visit, and body temperature showed significantly faster fever alleviation in the peramivir treatment group compared with the oseltamivir treatment group. For the A(H1N1)pdm09-infected patients, only the oseltamivir and zanamivir treatment groups were compared, and no significant difference in time to alleviation of fever was observed between the two groups. Further evaluation of the clinical effectiveness of the newly released NAIs for influenza-infected patients, including those infected with A(H1N1)pdm09, is needed.

Updated clinical results in the 2011-2012 season will also be presented.

O3

Nitazoxanide in the Treatment of Acute Uncomplicated Influenza

Jean-François Rossignol

Romark Laboratories, L.C., Tampa, Florida and University of Oxford Department of Biochemistry, Institute of Glycobiology, Oxford, United Kingdom

Nitazoxanide (NTZ) and its active circulating metabolite, tizoxanide (TIZ), inhibit in vitro replication of a broad range of orthomyxoviridae and paramyxoviridae including currently circulating influenza strains (H1N1, H3N2 and B), avian A/Vietnam/1203/04(H5N1), parainfluenza viruses and RSV. This activity is attributed to highly selective stimulation of innate immunity via interferon-stimulated-genes

pathways rather than a direct effect on the virus. Combinations of NTZ with oseltamivir are synergistic in inhibiting in vitro replication of influenza viruses (Combination Index = 0.47, CalcuSyn® software). Attempts to select for influenza virus resistant to NTZ in vitro have been unsuccessful suggesting a high barrier to resistance. A Phase 2b-3 randomized, double-blind, placebo-controlled, dose range-finding clinical trial was conducted to evaluate efficacy and safety of NTZ in the treatment of acute uncomplicated influenza. The trial was conducted in 74 outpatient centers throughout the United States during the 2010-2011 flu season. 624 subjects aged 12-65 years with fever, at least one respiratory symptom and one constitutional symptom were enrolled within 48 hours of symptom onset. Subjects were randomized to receive placebo, NTZ 300 mg or NTZ 600 mg orally twice daily for 5 consecutive days. The primary endpoint was time from first dose to alleviation of symptoms (all symptoms graded as mild or absent) in subjects with laboratory-confirmed influenza infection. Influenza strains were representative of the season with approximately 47% being influenza A 2009 H1N1, 23% influenza A H3N2 and 30% influenza B. Subjects receiving 600 mg NTZ orally twice daily for 5 days experienced a statistically significant reduction in duration of symptoms compared to the placebo group (median time to alleviation 90 hrs vs. 117 hrs, $P<0.001$). Subjects in the low dose group experienced a lesser reduction in duration of symptoms compared to the placebo group, demonstrating dose response. Dose-dependent reductions of viral titer were also observed during treatment with a statistically significant difference ($P<0.001$) observed for subjects in the 600 mg NTZ group compared to the placebo group. NTZ was well tolerated with only minor clinical side effects reported among the three groups. Because of these encouraging results, further studies of NTZ for influenza treatment are underway in the United States during the 2012-2013 flu season.

O4

Human Monoclonal Antibodies to Prevent and Treat Influenza A and B, including Infections by H5N1 Virus

J Goudsmit

Crucell Vaccine Institute, Janssen Center of Excellence for Immunoprophylaxis, Leiden, The Netherlands

Recently we described a series of human monoclonal antibodies that all bind to Hemagglutinin (HA). We recovered these antibodies from the IgM pool in the blood of adults that were vaccinated with inflexal, the virosomal seasonal influenza vaccine. Antibody CR6261 (Science 324, 246, 2009) bound to the stalk of HA of all influenza viruses of Group 1 and was able to prevent infection as well as cure mice up to 5 days after challenge with 25LD50 of H1N1 A/WSN/33.

Antibody CR8020 (Science, 333, 843 ,2011) bound to the stalk of HA of all influenza viruses of Group 2 and was able to prevent infection as well as cure mice up to 3 days after infection with 25LD50 of H3N2 A/HK/68. Finally we discovered another three human monoclonal antibodies, two that bound exclusively to the head of HA of all influenza B viruses, CR8033 and CR8071, and were able to protect mice from influenza B infection and one antibody, CR9114, that protected mice not only from influenza B, but also from influenza A (Science, in press). Clinical trials with the antibodies, CR6261 and CR8020 will commence in 2013. Proof of Concept Studies for therapy of severe influenza guided by point-of-care diagnostics will follow shortly thereafter. Simultaneously we are developing a mixture of two of these antibodies for prophylaxis to protect people at risk for severe influenza and prevent spread of infection in case of an epidemic.

We will present the newest results on the breadth of neutralization of these antibodies using field isolates of seasonal influenza viruses and the ability of these antibodies to prevent and treat H5N1 infection, including the recently described ferret-to-ferret transmissible viruses.

O5

Design of a Broadly Neutralizing Antibody Targeting Influenza

Donna Ambrosino, AN Zachary Shriver, AN Karthik Viswanatha

Visterra Inc., Cambridge, MA, USA

Background: discovery of broadly neutralizing antibodies against viral pathogens, including influenza A, HCV, RSV, Rabies and HIV, is a major goal for therapy, prevention, and vaccine design. Traditional approaches, using B-cell panning, transgenic mice, and phage libraries have been only partially successful, requiring development of novel approaches.

Methods: through structural analysis, we have identified a site on influenza hemagglutinin (HA) that is not only conserved across all influenza subtypes, but also appears to be resistant to mutation. Harnessing this analysis and using a protein engineering approach, we have designed >50 human antibodies that bind to and neutralize Group 1 and 2 influenza A virus strains, including an optimized candidate- VIS410.

Results: VIS410 targets the identified conserved region and demonstrates good physiochemical attributes, including solubility, stability, affinity and specificity. In vitro, VIS410 demonstrates dose-dependent viral inhibition with an IC50 in the low □g/ml range against both Group 1 and Group 2 virus strains. Mechanistic studies indicate that VIS410 inhibits HA fusion with the cell membrane, consistent with the design criteria. Furthermore, VIS410 potently and specifically protects mice infected with a lethal dose of influenza virus, both prophylactically and therapeutically. As a prophylactic, single doses at > 1-2.5 mg/kg are completely protective. Therapeutically, 100% of infected mice treated with a single dose of antibody (< 10 mg/kg) at 48 hours post-infection survived a lethal challenge with representative Group 1 and Group 2 viruses. Antibodies designed by this approach also demonstrate significant protection in a mouse viral/bacterial co-infection model; a single dose of antibody is sufficient to mitigate viral challenge and prevent opportunistic bacterial infection.

Conclusion: VIS410 provides the potential for a novel therapeutic and passive immunoprophylactic agent against influenza and a framework for the design of a universal influenza vaccine. These results also demonstrate the potential of targeting HA to prevent infection or in treating established infection In addition, the breadth of VIS410 neutralization opens the potential for a single monoclonal antibody to provide protection from all influenza A strains, including potential pandemic strains. Finally, this engineering approach to creating human antibodies defines a novel pathway for rapid identification of therapeutics against other infectious agents.

O6

Modulating the Expression of Host Molecules Decreases H5N1 Replication

C Deffrasnes[1], CR Stewart[1], A Oberendorf[1], C Rootes[1], K Birrer[1], P Monaghan[1], D Green[1], AG Bean[1]

[1]CSIRO Australian Animal Health Laboratory, Geelong, Victoria, Australia

Objectives of the study: Avian influenza is a pandemic threat and new anti-virals need to be developed. Studying the pathogenesis of H5N1 infection and focusing on the vital role that host proteins play in influenza replication will offer new therapeutic targets. Host proteins from the coat protein complex (COP) are involved in many cellular pathways such as endocytosis, protein transport between the endoplasmic reticulum and the Golgi apparatus, and protein glycosylation. COP proteins have been shown to play a role in the viral replication cycle and their role in H5N1 pathogenesis is of great interest. In this study, we aimed at modulating the expression of a COP protein and measure its impact on H5N1 replication in human cells.

Methods used: HeLa and Vero cell lines were used in this study and H5N1 infections were done in a BSL3 facility. Viral replication was measured by TCID50. Short interfering RNAs (siRNAs) were designed against COP subunit alpha (COPA). siRNA transfection efficiency was evaluated by flow

cytometry and siRNA-mediated gene expression knockdown was measured by quantitative real-time PCR (qPCR). Cytotoxicity was assessed using the high throughput imager CellInsight. Golgi disruption was quantified using the CellInsight (immunofluorescence) and confocal microscopy. Changes in cytokine production were measured by qPCR. Statistical analyses were performed using Prism software.

Results obtained: we successfully identified a concentration of COPA siRNA showing efficient downregulation of COPA expression without any associated cytotoxicity. We found that blocking COPA and Golgi using siRNA or Brefeldin A results in significant Golgi disruption. Furthermore, we showed that COPA knockdown by siRNA or Brefeldin A treatment inhibits H5N1 replication.

Conclusions: our study demonstrates that transient downregulation of COPA protein is not cytotoxic. Moreover, our results show that inhibiting host proteins such as COPA reduces avian influenza (H5N1) replication in human cells. We are currently investigating the impact of host protein knockdown on cytokine expression following H5N1 infection.

Monday 29th October - Concurrent Workshop 1b

Severe Influenza and SARI Epidemiology - impact, surveillance, healthcare utilization, vaccines

Chairs: *Abdullah Brooks, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA & Mai Le, National Institute of Hygiene and Epidemiology, Hanoi, Viet Nam*

O7. *Abdullah Brooks*

O8. Understanding Influenza Dynamics through Serial Seroepidemiology
Maciej Boni

O9. Laboratory Surveillance on Influenza-like Illness at Emergency Department in Seven Teaching Hospitals, South Korea: 2011-2012
Ji Yun Noh

O10. Estimates of Severe Acute Respiratory Infections Incidence in a City of the Philippines, 2009-2011
Taro Kamigaki

O11. Effect of Climate on ILI Dynamics and Seasonality in Vietnam from 1991 to 2012
Pham Quang Thai

O12. Severe Influenza Critical Care Surveillance: Insights into the Impact and Severity of the 2010/11 Influenza Season Relative to the 2009/10 Pandemic Season in England
Helen Green

Abstracts

O7

O8

Understanding Influenza Dynamics Through Serial Seroepidemiology

Maciej F Boni

Sir Henry Dale Fellow, Centre for Tropical Medicine, Oxford University Clinical Research Unit

The epidemic dynamics and evolution of influenza A virus occur on a global scale. Influenza epidemics in temperate zones are seasonal and more predictable than transmission dynamics in tropical and sub-tropical areas, but the dynamics of these two climatic zones are closely linked, with East and Southeast (E/SE) Asia likely playing a major role in driving global influenza circulation. Influenza evolution affects the dynamics of influenza epidemics globally, but we do not know which human populations or which epidemiological conditions drive antigenic evolution in influenza. To answer this question would need genetic and epidemiological data from regions of the world that are suspected to play a large role in global influenza dynamics. I will describe several such studies initiated in Vietnam -- with particular focus on a long-term serial seroepidemiology study -- that are aimed at understanding the circulation of human influenza viruses in Southeast Asia, as well as some of the new analytical methods we are developing. Understanding Vietnam's role in global influenza circulation will help us determine how important of a role E/SE Asia play in global flu dynamics, and it will help us identify which components of the Vietnam data would allow for similar analyses to be done in other Asian countries.

O9

Laboratory Surveillance on Influenza-like Illness at Emergency Department in Seven Teaching Hospitals, South Korea: 2011-2012

Ji Yun Noh[1], Hee Jin Cheong[1], Won Suk Choi[1], Jacob Lee[2], Jin-Soo Lee[3], Seong-Heon Wie[4], Young Keun Kim[5], Hye Won Jeong[6] and Woo Joo Kim[1,7]

[1]Korea University, Seoul, Korea; [2]Hallym University, Seoul, Korea; [3]Inha University, Incheon, Korea; [4]The Catholic University of Korea, Suwon, Korea; [5]Yonsei University, Wonju, Korea; [6]Chungbuk University, Cheongju, Korea; [7]Transgovernmental Enterprise for Pandemic Influenza in Korea, Seoul, Korea

Objectives: to evaluate the distribution and seasonality of respiratory viruses through the Hospital-based Influenza Morbidity & Mortality Surveillance (HIMM), a surveillance scheme for influenza-like illness (ILI) based on the teaching hospitals in Korea.

Methods: respiratory specimens were obtained from adult patients (≥18 years) who visited to emergency department in seven teaching hospitals with ILI during week 40 starting Sep 25, 2011 and week 22 ending Jun 2, 2012. Multiplex PCR was performed to detect respiratory viruses: influenza virus, adenovirus, coronavirus, respiratory syncytial virus, rhinovirus, metapneumovirus, parainfluenza virus, bocavirus, and enterovirus.

Results: during week 40 to week 22, 2011-2012, 1,983 patients who visited to emergency department with ILI were enrolled in this study. Male was 810 (40.9%) and mean age was 47.1±19.3. The distribution of age groups were as follows: 461 (23.3%), 18-30 years; 672 (33.9%), 31-49 years; 390 (19.7%), 50-64 years; 457 (23.1%), ≥65 years. Four hundred thirty patients (22.0%) received influenza vaccination during 2011-2012 season. At least one of comorbidity was found in 495 patients (25.0%). Positive rate of respiratory viruses was 52.1% and total number of detected viruses was 1,100. Influenza A virus was the dominant agent (677, 61.5%) followed by influenza B virus (169, 15.4%), rhinovirus (86, 7.8%), metapneumovirus (61, 5.5%), coronavirus (36, 3.3%), parainfluenza virus (34, 3.1%), RSV (30, 2.7%) and adenovirus (7, 0.6%). Hospitalization rate was 3.5% (70/1,983)

and mortality was 0.2% (4/1,983). The epidemic curve was correlated strongly with the national surveillance scheme based on primary care clinic.

Conclusion: this study is the first extensive laboratory surveillance for epidemiology of respiratory viruses in patients with ILI based on teaching hospitals in Korea. This surveillance scheme would be valuable as the first well-constructed surveillance system based on several teaching hospitals in Korea.

O10

Estimates of Severe Acute Respiratory Infections Incidence in a City of the Philippines, 2009-2011

Taro Kamigaki[1], Veronica L Tallo[2], Portia P Alday[2], Alvin G Tan[2], Edelwisa S Mercado[2], Jenaline B Javier[2], Hitoshi Oshitani[1], Remigio M Olveda[2]

[1]Tohoku University Graduate School of Medicine, Sendai, Japan; [2]Research Institute of Tropical Medicine, Department of Health, Manila, Philippines

Objectives: although public health significance of influenza has been widely recognized, there remains limited information on the burden of disease of influenza in tropical countries including the Philippines.

Methods: we conducted the influenza surveillance study from January, 2009 to December, 2011 in an urbanized highland city of the Philippines. Severe acute respiratory infections (sARI) surveillance was one of the study components and established to collect data and specimens from one government and four private hospitals since April, 2009. Demographic data as well as epidemiological data was obtained from patients by using the standardized forms. Nasal and/or oropharyngeal swabs were collected and tested for influenza A, influenza B and respiratory syncytial virus (RSV) by performing RT-PCR test. The incidence of sARI associated with targeted viruses was estimated as the proportion of each virus/subtype positive cases per 10,000 populations.

Results: we obtained 2,458 specimens from 10,726 sARI cases from throughout our study period and calculated a mean crude sARI incidence of 12.1 per 100,000 populations (95% confidence interval 0.5-59.4). Sixty three percent of all sARI cases were children under 5 years old. Overall influenza detection rate was 8.5% and there was an observed year round influenza activity with two possible peaks each year. During study period, influenza A (H1) pdm09 brought higher sARI incidence followed by influenza B. Surprisingly, there were only 14 deaths reported among sARI cases during our study period.

Conclusions: Influenza posed a certain disease burden on hospitalization especially with children less than 5 years old in an urbanized tropical city of the Philippines. It is important to continue to monitor the disease burden of influenza with ILI and sARI surveillance.

O11

Effect of Climate on ILI Dynamics and Seasonality in Vietnam from 1991 to 2010

Pham Quang Thai[1], Maciej M Boni[2], Marc Choisy[3], Peter Horby[2]

[1] NIHE, Hanoi,Viet Nam; [2]Oxford University Clinical Research Unit (OUCRU),Vietnam; [3]IRD Viet Nam

Background: the respective influences of climatic and immunological factors on the epidemiological dynamics of influenza is an on-going debate. In this study we propose to test the effect of climatic factors on time series of influenza like illness (ILI) notifications that have been recorded monthly in each of the 64 provinces of Vietnam since 1991. With a latitude ranging from 8 to 24 degrees north and an altitude ranging from sea level to more than 3,000 m, Vietnam exhibits a high variety of climates on an medium-size surface (300,000 km2) and an important population size (~90 millions). All these make Vietnam a perfect country to empirically test the effect of climatic factors on the epidemiology of influenza.

Data: monthly ILI notifications have been passively recorded and aggregated by provinces (64) since 1991 by the National Institute of Hygiene and Epidemiology and 3 other regional institute from each region of Viet Nam under the supervision of General Department of Preventive Medicine MOH. Yearly estimates of province population size and census of communes populations sizes in 1989 and 1999 were available from GSO Viet Nam. Monthly average, minimal, and maximal temperatures, number of hours of sunshine, rainfall and absolute and relative humidity for 64 meteorological stations across the country were available from Viet Nam Institute of Meteorology Hydrology and Environment.

Methods: we used empirical mode decomposition on ILI time series for each province. Pairwise correlations between the dominant components of each pair of provinces were related to distances and population products in order to detect gravity coupling signatures such as traveling waves. The effect of climatic variables on the components of the decomposed ILI time series were tested in a Poisson regression, accounting for spatial and temporal auto-correlation, different periodicity by latitude; the presence of an influenza travelling wave; correlation between hours of sunshine and ILI also be tested.

Results: during the study period, 26,023,574 cases of ILI were reported. ILI reporting incidence for the whole country show a larger peak from September to November, and a smaller one from February to April. Other results under calculation and will be available at the time of the conference.

O12

Severe Influenza and Critical Care Surveillance: Insights into the Impact of the 2010/11 Influenza Season Relative to the 2009/10 Pandemic Season in England

HK Green[1], RG Pebody[1], J Ellis[1], M Galiano[1], JM Watson[1]

[1]Health Protection Agency, London, UK

Background: in December 2010/11, despite apparent widespread A(H1N1)pdm09 infection in the 2009/10 pandemic, the first post-pandemic influenza season in England was marked by reports of a rapid increase in ICU admissions due to A(H1N1)pdm09 influenza. The impact on these services over the winter was greater than seen during the 2009 pandemic across all regions in England. To interpret this unexpected observation, surveillance data on suspected influenza critical care admissions, winter circulating respiratory viruses and influenza vaccine uptake in 2009/10 and 2010/11 were examined.

Methods: data on the total daily number of patients in critical care with suspected influenza from week 51 2010 to 7 2011 and from week 29 2009 to 8 2010 across England were collected by age group (<5yrs, 5-15yrs, 16-64yrs and 65+yrs). The cumulative number of critical care influenza bed days were calculated for 2010/11 and 2009/10. To validate the likely cause, a regression analysis including weekly positivity of influenza and RSV investigated samples was undertaken. Additional data on respiratory bacterial laboratory reports, temperature and vaccine uptake of monovalent pandemic influenza vaccine in 2009/10 and trivalent seasonal influenza vaccine in 2010/11 was examined.

Results: there was a notable age shift in critical care admissions of suspected influenza from children (<15yrs) to young adults (16-64yrs) in 2010/11 compared to 2009/10. The peaks in critical care cases in both seasons coincided with peaks in A(H1N1)pdm09 activity, and additionally in 2010/11 with influenza B, notably cold weather and increased reports of bacterial coinfection. Regression analysis attributed the majority of suspected admissions to A(H1N1)pdm09 in both seasons with little contribution from infections due to RSV. Overall vaccine uptake in target groups with an A(H1N1)pdm09-containing vaccine was much higher at the peak level of critical care activity in 2010/11 compared with 2009/10.

Conclusions: the majority of suspected critical care admissions in 2010/11 are likely to have resulted from A(H1N1)pdm09 infection. A difference in vaccine uptake between seasons is unlikely to explain the change in impact observed. The A(H1N1)pdm09 infection age shift coupled with low temperatures favouring transmission and reports of secondary bacterial infection are likely to explain the increased impact in critical care in 2010/11 compared to 2009.

Tuesday 30th October

Symposium Session 3: Advances in Clinical Management
Chair: Arnold Monto

New Developments in Influenza and Respiratory Pathogen Diagnostics
Christopher Wong – Genome Institute of Singapore, Singapore

Identifying the microbial and viral pathogens causing clinical disease is currently still a difficult combination of clinical expertise and technical challenges. In respiratory disease, typically 60% of cases have no agent identified and even after 2 weeks, 10% of all patients hospitalized for infectious causes do not have the agent identified. Despite the rapid growth of products offering panels of molecular diagnostics, this problem has remained as the current generation of molecular diagnostics suffers from technical problems that prevent them from detecting: pathogens that have mutated through evolution or are novel strain type variants. With the high possibility of missing a pathogen, doctors and researchers are reluctant to order diagnostic tests; while companies are reluctant to develop new drugs to pathogens of unknown importance.

I will describe an overview of some of the newer multi-plexed molecular diagnostics tests which are available either as lab-developed assays or recently approved by the FDA. I will also describe the PathGEN® PathChip system developed at the Genome Institute of Singapore, which is a nucleic acid chip-based molecular method that can amplify all viruses and bacteria (whatever their genomic diversity) from clinical samples; and automatically detect and annotate the presence of any of more than 70,000 virus and bacteria genomes in a single test, that can additionally detect any co-infecting pathogens and simultaneously strain type them all.

In a recent trial at a university hospital in Denver, US on >250 samples, the chip achieved average specificity of 98% ± 2% and average sensitivity of 83% ± 14% across 11 groups of viruses. Significantly, the chip had an average negative predictive value of 98% ± 2%. We describe the performance of this chip, comparison with other multiplex respiratory diagnosis platforms, and highlight applications for molecular epidemiology and novel virus discovery.

Disclosures: The work on the PathChip is funded by grants from Exploit Technologies Pte Ltd, A*STAR and SPRING Singapore. CW is founder of PathGEN Dx Pte Ltd which has licensed the technology from A*STAR.

Influenza Antiviral Effectiveness in Hospitalised Patients (including Intravenous NAIs)
Norio Sugaya – Keiyu Hospital, Yokahama City, Japan

A meta-analysis showed that neuraminidase inhibitors (NAIs) have modest effectiveness against influenza illness in otherwise healthy adults and children and that there is insufficient evidence in regard to reducing complications of influenza. However, after the pandemic caused by H1N1/09, early treatment with NAIs has been reported to be effective in reducing serious cases and deaths in many countries.

Since the approval of zanamivir and oseltamivir for use in influenza virus infection in Japan in 2000, rapid diagnostic tests have been routinely performed by clinicians in patients who exhibit an influenza-like illness, and patients with positive test results, including otherwise healthy adults and children without any underlying illness, have usually been treated with NAIs. Even though 20.7 million cases of pandemic H1N1/09 infection were reported in Japan during the 2009-2010 season, only 198 deaths were reported nationwide. Japan may have had the lowest case fatality rate for symptomatic illness (<0.001%; 198/20.7 million) among the countries where a widespread epidemic was reported. The very low mortality rate that resulted from the H1N1/09 epidemic in Japan was mainly attributable to the universal implementation of early treatment with NAIs since the year 2000.

In addition to oseltamivir and zanamivir, the newly approved inhaled drug laninamivir and newly approved intravenous drug peramivir were used in Japan during the 2010-2011 season, bringing to four, the total number of NAIs currently being used in hospitals and clinics nationwide. The Japanese Association for Infectious Diseases recommends that influenza patients who have been hospitalized, especially patients with pneumonia, be treated with oseltamivir or peramivir. We compared the clinical effectiveness of the four NAIs currently being used in Japan on the basis of the duration of fever after the start of treatment. The results showed that peramivir was the most effective of the four NAIs against influenza A/H1N1/09 and A/H3N2, but that all four NAIs were much less effective against influenza B than against influenza A.

Assessment on the basis of their clinical effectiveness and viral shedding pattern showed that the NAIs were probably highly efficacious against serious illness caused by pandemic H1N1/09. Because of their lower clinical effectiveness and the longer viral shedding period, NAIs may not be effective in protecting against severe illness caused by influenza B virus infection. Intravenous peramivir may be the best option for the treatment of severe influenza virus infection.

Detection and Management of Antiviral Resistance Emergence
Guy Boivin – Laval University, Quebec, Canada

Influenza viruses are major human pathogens with a global distribution, accounting for more than 500 000 annual deaths worldwide and with considerable impact on the quality of life and productivity of the society. Due to the limited efficacy of vaccination, antiviral drugs constitute a complementary approach in the control and prevention of influenza infections and thus play an important role in the management of influenza outbreaks and pandemics. Currently, adamantanes and neuraminidase inhibitors (NAIs) are the only two classes of anti-influenza agents approved for clinical use. However, the worldwide emergence and high prevalence of adamantane-resistant virus variants has discouraged the use of the former drugs. NAIs have proved to be very effective against influenza A and B viruses. Nevertheless, oseltamivir-resistant strains have also been reported quite frequently, as in the case of seasonal A/H1N1 viruses that circulated between 2007 and 2009. The recent identification of permissive mutations in the neuraminidase gene has helped our understanding of the fitness and transmissibility of some drug-resistant influenza A mutants. Furthermore, new sensitive methods such as deep sequencing have shown that drug-resistant mutants could be present at low levels in infected individuals even in the absence of antiviral therapy and then be successfully transmitted. The emerging problem of NAI resistance highlights the need for continuous monitoring of drug resistance markers, as well as the development of new anti-influenza drugs and combination therapies.

Adjunctive Therapies and Immunomodulatory Agents in SARI Management
David Hui – The Chinese University of Hong Kong, Hong Kong, SAR China

Cytokine dysregulation has been reported in severe viral infections such as SARS, H5N1 influenza, and pandemic 2009 H1H1 influenza.1-3 During the major outbreak of SARS in 2003, systemic steroid was widely used in Asia based on CT scan and histopathological evidence of bronchiolitis obliterans organizing pneumonia,4,5 but high dose of systemic steroid was associated with increased risks of nosocomial infections (including fatal disseminated fungal infection), and avascular osteonecrosis.6-8 The use of systemic steroids was also associated with increased risk of nosocomial infections and higher mortality in patients hospitalized with severe pandemic 2009 H1N1 influenza esp with late or no administration of neuraminidase inhibitors.9-12 Use of systemic steroid for SARS or seasonal influenza may delay viral clearance.13,14 Passive immunotherapy in the form of convalescent plasma appeared useful as rescue therapy in SARS,15 H5N1,16 and pandemic H1N1 2009 influenza17 although such treatment would be limited by the supply from suitable donors who have fully recovered from the infections. There are conflicting data whether chronic users of statins are protected from developing more severe influenza and there is a lack of data on the role of acute use of statins in severe viral infections.18-20 More data are needed to explore the potential role of intravenous gammaglobulin, and other drugs with immuno-modulating properties such as gemfibrozil, pioglitazone, N-acetyl-cysteine, clarithromycin, celecoxib and mesalazine.21 Other modalities such as therapeutic plasma exchange, and polymyxin B-immobilized fiber column hemoperfusion were

reported as useful for severe pandemic 2009 H1N1 infection and these would require further investigation.

References:

1. Wong CK, et al. Clin Exp Immunol 2004
2. Peiris JS, et al. Lancet 2004
3. Lee N, et al. Antiviral therapy 2010
4. Wong KT, et al. Radiology 2003
5. Tse GM, et al. J Clin Pathol 2003
6. Wang H, et al. NEJM 2003
7. Hong N, et al. Clin Radiol 2004
8. Griffith J, et al. Clin Radiol 2005
9. Martin-Loeches et al.. Intens Care Med 2011
10. Brun-Buisson C, et al. AJRCCM 2011
11. Kim SH, et al. AJRCCM 2011
12. Han K, et al. Clin Infect Dis 2011
13. Lee N, et al. J Clin Virol 2004
14. Lee N, et al. J Infect Dis 2009
15. Soo Y, et al. Clin Microbiol Infect 2004
16. Zhou , et alB. NEJM 200
17. Hung IF, et al. Clin Infect Dis 2010
18. Kwong JC, et al. Plos One 09
19. Brett SJ, et al. PlosOne 2011;6(4):e18120
20. Vandermeer et al.. J Infect Dis 2012
21. Hui DS, et al. Chest 2010;137:916-925

Ventilatory Strategies, Fluid Management, and Supportive Care in ALI/ARDS

Andrew Luks – Harborview Medical Center, Seattle, USA

Patients who develop the Acute Respiratory Distress Syndrome (ARDS) in the setting of influenza infection are at increased risk for morbidity and mortality. While antiviral therapy is an important component of care for these patients, it is by no means sufficient to ensure a good outcome and additional measures are necessary. Patients who develop ARDS require high levels of supplemental oxygen to counteract the hypoxemia that results from extensive shunt physiology. Many patients require invasive mechanical ventilation for support of oxygenation, but in resource-limited settings supplemental oxygen via face mask may be the only available intervention. The use of non-invasive positive pressure ventilation in cases of severe hypoxemia remains without support in the literature. Patients deemed to have ARDS who receive mechanical ventilation should be placed on lung protective ventilation, whereby the tidal volume is decreased to 6 mL/kg of their ideal body weight and distending pressures are maintained below 30 cm H2O. Patients will require increased inspired oxygen concentrations and increased levels of positive end-expiratory pressure (PEEP) to support oxygenation and counteract atelectasis, although there is no clear evidence to support particular PEEP strategies. While high inspired oxygen concentrations and increased PEEP are sufficient in most patients to maintain adequate oxygenation, a minority of patients develop refractory hypoxemia and require one of several additional therapies, including prone positioning, inhaled vasodilators, infusion of paralytic medications or extracorporeal membrane oxygenation (ECMO). Application of these “rescue strategies” is complicated, however, by the lack of clear guidance in the literature as to what level of oxygenation problems constitutes “critical hypoxemia” and, therefore, necessitates use of these strategies, and the proper approach to selecting between these options. While much attention was devoted to these strategies in the recent H1N1 epidemic, clinicians must not lose sight of simple measures that also make a difference in patient outcomes by decreasing complications that lead to increased morbidity and mortality, including elevating the head of the patient’s bed, ensuring adequate prophylaxis against venous thromboembolism and gastrointestinal bleeding and conservative fluid management in the post-resuscitative phase of the patient’s illness. Attention to details such as these, along with appropriate implementation of lung protective ventilation, go a long way toward improving patient outcomes in patients with severe lung injury.

Tuesday 30th October - Concurrent Workshop 2a

SARI Pathogenesis and Treatment – receptors, host responses, host-directed therapies, biologic response modifiers

Chairs: *Nelson Lee, The Chinese University of Hong Kong, SAR China & Tim Uyeki, National Center for Immunization and Respiratory Diseases, Atlanta, USA*

O13. Pathogenesis of Severe Influenza: Virus, Host, and Virus-Host Interactions
Nelson Lee

O14. Host RNA Binding Proteins Regulate Adaptation of H5N1 HPAI Polymerase to Human Cells
Eric Bortz

O15. The Hemagglutinin Protein Acid Stability Regulates H5N1 Influenza Virus Pathogenicity and Host Adaptation
Hassan Zaraket

O16. Immune Stimulants as Potential Agents against Pandemic Influenza and Secondary Bacterial Infection
Amabel Tan

O17. Severe Pandemic H1N1 2009 Infection is Associated with Transient NK and T Deficiency and Abberant CD8 Responses
Annette Fox

O18. Analysis of Inflammasome Related Molecules in the Response to H5N1 Avian Influenza
Sarah Fardy

Abstracts

O13

Pathogenesis of Severe Influenza: Virus, Host, and Virus-Host Interactions

Nelson Lee

The Chinese University of Hong Kong, SAR China

For ‘seasonal’ influenza severe and fatal infections occur predominantly in the elderly and compromised individuals. For ‘pandemic’ influenza (e.g. H1N1pdm09), an increased proportion of younger (35□65 years), previously healthy adults are observed to develop severe diseases. Besides persons with conventional risk factors, pregnant women and obese individuals are found to be at increased risks. For ‘avian’ influenza (e.g. H5N1), persons with all ages are affected. A fulminant course of illness occurs in most patients, except perhaps in some younger children, such as those less than 5 years of age. Certain symptoms like sore throat and runny nose seem to indicate the predominant site of infection. However what factors determine these different manifestations are not entirely clear. Emerging data suggest that a combination of virus factors (e.g. genetic constellation, virulence, receptor affinity or tropism), host factors (e.g. underlying immunosuppression; pre-existing or cross immunity) and virus-host interactions (e.g. innate□TLRs, RLRs, NLRs, inflammasomes, DAMPS, Type-I interferons, pro-inflammatory cytokines; adaptive□Th1, Th17, B-cell responses) are likely involved. Proposed disease models include lack of pre-existing/cross immunity to the invading virus, inefficient viral clearance by host, continuous excitation of pro-inflammatory cytokines (e.g. IL-6, CXCL8/IL-8, CCL2/MCP-1, TNF-α), and aberrant, dysregulated adaptive immune responses (e.g. depressed Th1/Th17 immunity, pathogenic immune-complexes formation). Further immunosuppression such as corticosteroid administration and bacterial superinfection consequent to enhanced viral-bacterial interactions in influenza may also affect the disease course. In this workshop, investigators will present their research on the pathogenicity of viral HA proteins, the roles of host TLRs, inflammasomes, and RNA-binding proteins in controlling influenza, and cellular response dysregulation in severe infections. Better understanding of pathogenesis of severe influenza may allow hypothesis generation on new therapeutic (e.g. anti-viral, anti-inflammatory) and preventive (e.g. pre/post-exposure prophylaxis, vaccination) approaches.

Key references:

1. Lee N et al. J Infect Dis. 2011;203:1739-47
2. Oner AF et al. Clin Infect Dis. 2012;55:26-32
3. de Jong MD et al. Nat Med 2006;12:1203-7
4. Peiris JS et al. Trends Immunol 2009;30:574–584
5. Takeuchi O et al. Immunol Rev. 2009;227:75-86
6. Lee N et al. Clin Infect Dis 2007;45:723-731
7. Everitt AR et al; GenISIS and MOSAIC Investigators. Nature 2012;484:519-23
8. Monsalvo AC et al. Nat Med. 2011;17:195-9
9. Lee N et al. PLoS One. 2011;6:e26050
10. Lee N, Hui DSC. Lancet 2011;378:979-80

O14

Host RNA Binding Proteins Regulate Adaptation of H5N1 HPAI Polymerase to Human Cells

Eric Bortz[1], John Steel[2], Balaji Manicassamy[3], Randy A. Albrecht[4], Martin Schwemmle[5], Adolfo García-Sastre

[1]Department of Biological Sciences, University of Alaska, Anchorage, AK; [2]Dept. of Microbiology and Immunology, Emory University, Atlanta, GA.; [3]Dept. of Microbiology, University of Chicago, Chicago, IL.; [4] Department of Microbiology, Mount Sinai School of Medicine, New York, NY.; [5] Department of Virology, University of Freiburg, Germany

H5N1 highly pathogenic avian influenza A (HPAI) viruses are capable of crossing the species barrier from avian hosts into humans, causing a severe pulmonary disease often characterized by pneumonia and uncontrolled inflammatory responses. The influenza viral polymerase complex (PB1, PB2, PA, NP, viral RNA) associates with a network of more than 300 human host proteins. Taking a functional genomics (RNAi) approach, we have sought uncover mechanisms by which these host factors regulate HPAI virus RNA synthesis, induction of innate immune responses, and adaptation to human cells. In a subnetwork enriched in RNA binding proteins, 18/31 (58%) were required for both H1N1 and H5N1 polymerase function. One protein, DEAD-box RNA helicase 17 (DDX17), regulated H5N1 polymerase's synthesis of viral mRNA and vRNA in human cells. Remarkably, RNA binding proteins including DDX17 also governed H5N1 HPAI polymerase's adaptability to human cells according to genotype at the mammalian-adaptive PB2 residue 627. Thus, this network of viral polymerase-host interactions in part controls adaptation, replication and pathogenicity of H5N1 HPAI viruses in humans. Functional genomics can provide a molecular profile for assessing the replicative and pathogenic potential of emerging H5N1 viruses in human cells.

O15

The Hemagglutinin Protein Acid Stability Regulates H5N1 Influenza Virus Pathogenicity and Host Adaptation

Hassan Zaraket, Susu Duan, Olga A. Bridges, Charles J. Russell

Department of Infectious Diseases, St Jude Children's Research Hospital, Memphis TN 38105, USA.

Highly pathogenic avian influenza H5N1 has become endemic in several countries and continues to cause sporadic infections in humans. The hemagglutinin (HA) protein mediates receptor binding and fusion to endosomes by changing confirmation under acidic environment of the endosomes. We have previously shown that the activation pH of the HA protein is a determinant for H5N1 influenza virus virulence in ducks and chickens. To determine the effect of HA protein acid stability on virulence in mammalian species, we generated mutant H5N1 viruses with pH altering mutations in the HA protein stalk domain and compared their replication to the wild-type (WT) virus in vivo. The WT virus was found to be activated for fusion at pH 5.9 and replicate efficiently in the lungs of mice, causing significant morbidity and mortality. An Y231H mutation destabilized the HA protein (pH of activation = 6.3) and attenuated virus replication in mice, resulting in less weight loss and delayed mortality compared to WT. Stabilizing the HA protein by a K582I mutation, which lowered the activation pH of the WT virus by 0.5 pH units, resulted in greater virus replication in the lungs of mice and increased weight loss and mortality. Moreover, the K582I virus displayed enhanced replication in the upper respiratory tract of infected mice compared to the WT virus. Stabilizing the HA protein also enhanced H5N1 virus replication in the ferret upper respiratory tract early during infection. However, the virus replicated poorly in the lungs of infected ferrets and caused less weight loss than the WT virus. We have previously shown that increasing the acid stability of the HA protein significantly diminishes H5N1 virus shedding and virulence in Mallard ducks. Overall, we conclude that mammalian species may support a broad range of HA protein activation pH and that stabilization of the HA protein enhances H5N1 influenza virus replication in the upper respiratory tract of mammalian species. This could be particularly useful in optimizing live attenuated vaccines for better replication and thus immunity in the nasal cavity. The data also suggest that molecules that alter the acid stability of the HA protein may be attractive as antiviral drugs by either inactivating or preventing activation of the HA protein.

O16

Immune Stimulants as Potential Agents against Pandemic Influenza and Secondary Bacterial Infection

AC Tan[1], EJ Mifsud[1], W Zeng[1], DJ Jackson[1]

[1]Department of Microbiology and Immunology, The University of Melbourne, Melbourne, Australia

The pandemic of 1918 and the recent 2009 pandemic has highlighted the need to continue our search for agents against Influenza A virus. It is important to consider that the acquisition of a secondary bacterial infections such as Streptococcus pneumonia has been causally associated with the mortality and disease severity that occurs during influenza pandemics. Therefore, targeting both IAV infection as well as secondary bacterial infection could be an effective treatment strategy to reduce the severity impact of pandemic influenza.

The innate immune system plays a key role in control of early influenza infection, and disruption of compartments of the innate immune response has a significant impact on the severity and outcome of disease in mouse models of infection. The objective of this study was to investigate whether activation of the innate immune response could provide a generic antiviral or antibacterial “environment” that would be effective against respiratory infection. To test this theory, we administered synthetic compounds containing the TLR-2 agonist, Pam2Cys via the intranasal route to anesthetized mice enabling their deposition into the lung. We demonstrate that Pam2Cys administration induces an immune-enhanced environment in the lung, characterized by the induction of neutrophils, macrophages, NK cells and lymphocytes and Th1 and inflammatory cytokines (but not IFN-alpha) and this occurs in a TLR-2-dependent manner. Furthermore, Pam2Cys prophylaxis protected mice against weight loss and death associated with virulent PR8 H1N1 influenza A virus and reduced viral burden following challenge with H3N2 and H3N1 strains of influenza A virus as well as respiratory syncytial virus. We have recently developed a mouse model of influenza and secondary S. pneumoniae bacterial infection and shown that prophylaxis with Pam2Cys can prevent the lethality associated with secondary bacterial infection.

This study has demonstrated the potential for such agents to offer a broad nature of anti-viral activity, and our findings in a model of influenza- bacterial co-infection further support the development of such agents against pandemic influenza.

O17

Severe Pandemic H1N1 2009 Infection is Associated with Transient NK and T Deficiency and Abberant CD8 Responses

A Fox [1,2], LNM Hoa [1], P Horby, R van Doorn [1,2], NV Trung [3], N Hong Ha [3], NT Cap [3], VD Phu[3], NM Ha[3], NNT Diep[1], NVT Bich [1], KTT Huong [1], WR Taylor [1,2], J Farrar [1,2], H Wertheim [1,2], NV Kinh [3]

[1]Oxford University Clinical Research Unit, Wellcome Trust Major Overseas Programme, Dong Da, Ha Noi, Viet Nam; [2]Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, OX3 7LJ, UK; [3]. National Hospital for Tropical Diseases, Dong Da, Ha Noi, Viet Nam; [4]South East Asia Infectious Diseases Clinical Research Network, Jl Diponegoro no 69, Jakarta, Indonesia, 10430

Background: it is unclear why the severity of influenza varies in healthy adults or why the burden of severe influenza shifts to young adults when pandemic strains emerge. One possibility is that cross-protective T cell responses wane in this age group in the absence of recent infection. We therefore compared the acute cellular immune response in previously healthy adults with severe versus mild pandemic H1N1 infection.

Methods and findings: 49 previously healthy adults admitted to the National Hospital of Tropical Diseases, Viet Nam with RT-PCR-confirmed 2009 H1N1 infection were prospectively enrolled. 39 recovered quickly whereas 10 developed severe symptoms requiring supplemental oxygen and prolonged hospitalization. Peripheral blood lymphocyte subset counts and activation (HLADR, CD38)

and differentiation (CD27, CD28) marker expression were determined on days 0, 2, 5, 10, 14 and 28 by flow cytometry. NK, CD4 and CD8 lymphopenia developed in 100%, 90% and 60% of severe cases versus 13% ($p < 0.001$), 28%, ($p = 0.001$) and 18% ($p = 0.014$) of mild cases. CD4 and NK counts normalized following recovery. B cells counts were not significantly associated with severity. CD8 activation peaked 6-8 days after mild influenza onset, when 13% (6-22%) were HLADR+CD38+, and was accompanied by a significant loss of resting/CD27+CD28+ cells without accumulation of CD27+CD28- or CD27-CD28- cells. In severe influenza CD8 activation peaked more than 9 days post-onset, and/or was excessive (30-90% HLADR+CD38+) in association with accumulation of CD27+CD28- cells and maintenance of CD8 counts.

Conclusion: severe influenza is associated with transient T and NK cell deficiency. CD8 phenotype changes during mild influenza are consistent with a rapidly resolving memory response whereas in severe influenza activation is either delayed or excessive, and partially differentiated cells accumulate within blood indicating that recruitment of effectors cells to the lung could be impaired.

O18

Analysis of Inflammasome Related Molecules in the Response to H5N1 Avian Influenza

Sarah J. Fardy[1,2], Simon Burggraaf[1,2], Wayne G. Kimpton[2], John W. Lowenthal[1] and Andrew G.D. Bean[1]

[1]Australian Animal Health Laboratories, CSIRO, Geelong, VIC, Australia; [2]Veterinary Science, The University of Melbourne, Parkville, VIC, Australia

Highly pathogenic avian influenza (HPAI) infection is extremely acute and associated with severe mortality in both humans and poultry. The rapid onset of disease suggests that virus-host interactions, such as the immune response to virus, might contribute to the severity. With this in mind, it is critical to understand the host-pathogen interactions in order to develop novel approaches to managing infection. Pro-inflammatory cytokines, such as interleukin (IL)-1β and IL18, are crucial to an anti-viral response, however, the control of the expression of these molecules is vital, as exacerbated levels can lead to deleterious outcomes. In mammals these cytokines are regulated by a complex cytoplasmic protein scaffold known as the Nalp3 inflammasome. Currently, the mechanisms for Nalp3 activation are largely unknown, however, it appears Nalp3 and the associated cleavage enzyme caspase 1 (ICE) are important regulators. To elucidate the role of the inflammasome in the chicken response to H5N1 HPAI, we identified and characterized chicken Nalp3, ICE, IL1β and IL18 and investigated their role during infection. Through qRT-PCR, siRNA gene knockdown and ELISA analysis, the chicken Nalp3 inflammasome has been identified and its role in the response to H5N1 HPAI analysed. All 4 genes are greatly upregulated, particularly Nalp3 and ICE. In tandem with this was increased IL1β and IL18 protein secretion, measured in chicken sera. Together, these data indicate a strong Nalp3 inflammasome response, following HPAI infection in the chicken. This work provides an insight into strategies that target the immune system for improving resistance to avian influenza.

Tuesday 30th October - Concurrent Workshop 2b

Antiviral Resistance – surveillance/monitoring, mechanisms, assay development

Chairs: *Jenny McKimm-Breschkin, CSIRO Material Science and Engineering, Melbourne, Australia & Philippe Buchy, Institut Pasteur in Cambodia, South East Asia*

O19. Screening Neuraminidase Inhibitor Susceptibility of Avian Influenza Isolates from SE Asia 2005-2008 Identifies H5N1 I222 Mutants with Reduced Oseltamivir Sensitivity
Jenny McKimm-Breschkin

O20. Cross-Neutralization Activity of Anti-H5N1 Specific Polyclonal Immunoglobulins against Heterologous Strains of H5N1 Virus
Philippe Buchy

O21. Classification of Neuraminidase Inhibitor Susceptibility for Surveillance
Angie Lackenby

O22. Treatment-Emergent Oseltamivir Resistance in Influenza A Viruses in the Influenza Resistance Information Study (IRIS) is Uncommon and Associated with Patient Age and Year of Enrolment
Martin Schutten

O23. Oseltamivir-Resistant A(H1N1)pdm09 Influenza in Travellers Returning from Spain
Adam Meijer

O24. Antiviral Use and the Impact on Drug Resistance
Mika Shigematsu

Abstracts

O19

Screening Neuraminidase Inhibitor Susceptibility of Avian Influenza Isolates from SE Asia 2005-2008 Identifies H5N1 I222 Mutants with Reduced Oseltamivir Sensitivity

Jennifer McKimm-Breschkin[1,2], Susan Barrett[1,2], Pak Pudjiatmoko[3], Muhammad Azhar[3], Peter Mohr[1], Frank Wong[1], Paul Selleck[1], Kerri Bruce[1], Julie Cooke[1], Mia Kim[4,5] and James McGrane[4]

[1]CSIRO Australian Animal Health Laboratory, Geelong, Australia; [2]CSIRO Materials Science and Engineering, Parkville, Australia; [3]Directorate of Animal Health, Directorate General of Livestock and Animal Health Services, Ministry of Agriculture, Jakarta, Indonesia; [4]Food and Agriculture Organization of the United Nations, Animal Health Service (FAO-AGAH); [5]OIE-FAO Global Network of Expertise on Animal Influenzas (OFFLU)

Objectives: H5N1 viruses continue to spread and evolve, and surveillance is of critical importance to know both the antigenic variation for vaccine preparation, as well as their antiviral susceptibility. We previously reported that clade 2 H5N1 isolates from Indonesia have reduced susceptibility to oseltamivir compared to clade 1 isolates. We also identified isolates from Cambodia which had reduced oseltamivir sensitivity. In collaboration with the Indonesian Ministry of Agriculture CSIRO has been involved with an FAO-implemented OFFLU project monitoring hemagglutinin genetic and antigenic changes in Indonesian H5N1 isolates. These isolates also provided a rare and valuable source for screening for susceptibility to the neuraminidase inhibitors, oseltamivir and zanamivir, which form part of many countries' pandemic stockpiles.

Methods: samples were screened in the MUNANA based enzyme inhibition assay for sensitivity to zanamivir and oseltamivir. Isolates showing reduced susceptibility were screened in the IC50 kinetics assay against zanamivir, oseltamivir and peramivir to determine if mutations led to changes in the rates of inhibitor binding. Sequences were analyzed to identify mutations associated with reduced susceptibility.

Results: we have screened more than 160 H5N1 isolates from Indonesia, Cambodia and Thailand and found no virus with an IC50 > 5 nM for zanamivir. In contrast the median IC50 for oseltamivir for the Indonesian isolates was~25 nM, around 30-fold higher than for the clade 1 viruses. We found that an S246G mutation correlated with reduced oseltamivir sensitivity in some Cambodian isolates. Of more concern is that we identified 8 clade 2 isolates from Indonesia which were mild or extreme outliers to oseltamivir with I222T/V/M mutations. IC50s for oseltamivir for I222T/V mutants ranged from 43-63 nM and for I222M mutants were >250 nM. Outliers were from different geographic locations in Indonesia. Mutations at I222 led to loss of slow binding of oseltamivir, but had minimal effect on peramivir or zanamivir binding. We also detected another 4 Indonesian isolates with IC50s around 30 nM also demonstrating loss of slow binding, including one with an I117V mutation.
Conclusions: As H5N1 still remains a pandemic threat the incidence of mutations conferring reduced oseltamivir sensitivity is a concern and emphasizes the need for greater surveillance.

O20

Cross-Neutralization Activity of Anti-H5N1 Specific Polyclonal Immunoglobulins against Heterologous Strains of H5N1 Virus

Philippe Buchy[1], Cécile H. Herbreteau[2], Vincent Deubel[1] and Jean-François Saluzzo[2]

[1]Institut Pasteur du Cambodge, 5 Monivong blvd, PO Box 983, Phnom Penh, Cambodia; [2]Fab'entech, 321 avenue Jean Jaurès, Bâtiment Domilyon, 69007 Lyon, France

Objectives: highly pathogenic avian influenza virus (H5N1) remains a major global health concern. Since 2003, the regular emergence of new outbreaks is observed in Southeast Asia and over 600 human cases (with almost 60% of deaths) were recorded. Polyclonal immunoglobulins are known for their ability to present cross-reactivity capacities. The objective of this study is to confirm the

neutralization activity of anti-H5N1 specific polyclonal immunoglobulins on heterologous H5N1 viral strains representative of virus evolution since 2004 in Cambodia.

Methods: we used classical sero-neutralization in vitro assay to investigate the neutralization activity of anti-H5N1 immunoglobulins derived from horse plasma immunized with inactivated H5N1 A/Vietnam/1194/04 strain. 100 TCID50 of 10 different clade 1 H5N1 strains belonging to 6 distinct lineages were incubated with a range of dilution of immunoglobulins and then transferred to MDCK cells for neutralization analysis. Results obtained were confirmed by using a hemagglutination inhibition assay (HIA).

Results: incubation of specific anti-H5N1 immunoglobulins developed on A/Vietnam/1194/04 inactivated strain with various H5N1 strains isolated in Cambodia between 2004 and 2011 provided in vitro neutralization with similar titer comprised between 1:2000 to 1:4000 for all tested strains. Results were confirmed by HIA.

Conclusion: these data underpin the excellent cross-reactivity of these specific polyclonal immunoglobulins on various H5N1 strains isolated in Cambodia and representative of different lineages of clade 1 H5N1 virus circulating strains in Southeast Asia.

O21

Classification of Neuraminidase Inhibitor Susceptibility for Surveillance

A Lackenby, R Daniels, L Gubareva, A Hurt, J Lo, A Meijer, H Rebelo de Andrade, M Siqueira, E Takashita, M Tashiro, D Wang

WHO Expert Working Group on Surveillance of Antiviral Susceptibility for the Global Influenza Surveillance and Response System

Influenza neuraminidase inhibitor (NI) resistance is caused by numerous neuraminidase (NA) amino acid substitutions but only H275Y in N1-NA is consistently associated with treatment failure. As such, except for H275Y detection, genotypic assays are of limited use. Increasingly, laboratories are performing NA inhibition assays for in vitro surveillance of NI phenotypic susceptibility. Lack of a clear definition of reduced susceptibility has resulted in varied interpretations lab-to-lab. We propose criteria and terminology to define reductions in susceptibility, to provide consistency in reporting.

These criteria must be flexible enough to apply across expected lab-to-lab variations and identify but not overstate minor variations. A definition based on fold change, calculated from either the IC50 value of a validated sensitive control virus or the mean/median IC50 value for type/subtype-matched sensitive viruses in circulation was considered most applicable.

Using data from several sources (CDC USA, NIID Japan, the European CNRL, and European EQA panel), fold-criteria were set for normal (<10-fold increase), reduced (10-100-fold increase) and highly-reduced (>100-fold increase) inhibition for influenza A. For influenza B the same criteria were set at <5-fold, 5-50-fold and >50-fold increases respectively. Interpretation of an IC50 indicating reduced or highly reduced inhibition was consistent whether the fold-change was calculated on sensitive control virus, seasonal/subtype mean without outliers or seasonal/subtype median including outliers. Peramivir sensitivity for a minority of viruses was an exception.

These NI susceptibility criteria are for reporting surveillance data. The NA inhibition assay provides an in vitro measure of drug inhibition. While several NA substitutions causing IC50 changes in vitro have been identified, the impact of host (underlying conditions/immunocompromise) and other viral (replication capacity) factors on clinical outcome is unclear, as is the clinical significance of mixed virus populations. As clinical data have demonstrated the reduced effectiveness of oseltamivir when treating H275Y-N1 viruses, this variant is classified as resistant (>100-fold increase) to oseltamivir. Currently there is insufficient clinical data to indicate the effectiveness of NI treatment of viruses with other mutations associated with IC50 changes. As natural susceptibility of influenza viruses varies, these criteria will be reviewed to ensure that they remain applicable.

O22

Treatment-Emergent Oseltamivir Resistance in Influenza A Viruses in the Influenza Resistance Information Study (IRIS) is Uncommon and Associated with Patient Age and Year of Enrolment

M Schutten[1], C Boucher[1], R Dutkowski[2], J Ives[3], K Klumpp[2], B Lina[4], AS Monto[5], J Nguyen-Van-Tam[6], A Osterhaus[1], X Tong[2] and RJ Whitley[7]

[1]Erasmus MC, Rotterdam, The Netherlands; [2]Hoffmann-La Roche, Inc., Nutley, NJ, USA; [3]Roche Products Ltd, Welwyn, UK; [4]University of Lyon, Lyon, France; [5]University of Michigan School of Public Health, Ann Arbor, MI, USA; [6]University of Nottingham, Nottingham, UK; [7]University of Alabama at Birmingham, Birmingham, AL, USA

Background: a global observational trial, the Influenza Resistance Information Study (IRIS; NCT00884117), was initiated in 2008 to study neuraminidase inhibitor (NAI) resistance and influenza disease course.

Methods: patients with influenza-like illness and/or positive rapid test result had throat/nose swabs collected on Days 1, 3, 6 and 10 for real-time RT-PCR analyses of influenza type, subtype and NAI resistance. Positive samples were cultured, sequenced (HA, NA and M2) and phenotypically tested for NAI resistance. Patients recorded influenza symptom scores (scale, 0 [absent] to 3 [severe]) on diary cards (Days 1–12).

Results: of 1855 RT-PCR-positive patients infected with single influenza strains in years 1 to 3 (925 aged 1–12 years), 1310 had influenza A, of whom 700 received oseltamivir monotherapy within 2 days of symptom onset (seasonal H1, 9/47 [19%]; H3N2, 222/335 [66%]; H1N1pdm09, 469/928 [51%]). Genotypic resistance (post-Day 1) was detected by mutation-specific RT-PCR in 19 oseltamivir-treated influenza A patients (H275Y in H1N1pdm09, 17; R292K in H3N2, 2). Fourteen were aged 1–5 years, one 6–12 and four ≥13. Seventeen were enrolled in Year 3 (2010–11). Genotypic resistance in H1N1pdm09 cases was associated with later enrolment year and lower age (logistic regression). In H1N1pdm09 patients treated with oseltamivir monotherapy within 48 hours of illness onset, H275Y occurrence was significantly associated with the proportion of patients who shed virus for >6 days: 16/17 (94.1%) of H275Y patients and 250/417 [60%]) of wt-strain patients (p=0.004). Symptoms resolved before Day 6 (score of <1 for each symptom) in 10/17 (58.8%) H275Y and 240/409 (58.7%) wt-strain patients (not significant).

Conclusions: in years 1–3 of IRIS, treatment-emergent resistance to oseltamivir in influenza A viruses was found mostly in 1–5-year-olds and in patients enrolled in Year 3. Slower viral clearance might have increased the risk of developing H275Y. The presence of H275Y did not affect symptom resolution.

O23

Oseltamivir-Resistant A(H1N1)pdm09 Influenza in Travellers Returning from Spain

Adam Meijer[1], Marcel Jonges[1], Paul van Beek[1], Albert Osterhaus[2], Marion Koopmans[1,2]

[1]National Institute for Public Health and the Environment, Bilthoven; [2]Erasmus Medical Centre, Rotterdam; The Netherlands

Background: in the Netherlands, 20 H275Y oseltamivir-resistant A(H1N1)pdm09 influenza cases were found during the 2009/2010 pandemic. Most cases were linked to prolonged oseltamivir treatment and were immunocompromised. Since then, year-around influenza surveillance in the Netherlands did not identify more oseltamivir-resistant A(H1N1)pdm09 viruses. Australia reported in 2011 transmission of oseltamivir-resistant A(H1N1)pdm09 virus, associated with H275Y-permissive substitutions V241I, N369K, and N386S in the neuraminidase.

Objectives: to monitor influenza virus incidence, diversity, and virulence as well as antiviral resistance for public health decision-making.

Methods: Influenza virus positive specimens from sentinel surveillance or hospitalized patients are routinely characterized by sequencing, SNP-detection RT-PCR, or IC50 determination. Background information on exposure, treatment, risk factors and symptoms is collected for patients with unusual strains.

Results: two Dutch patients, a woman aged 17 (case 1) and a men aged 20 (case 2), were diagnosed with oseltamivir-resistant A(H1N1)pdm09 influenza virus without prior oseltamivir exposure. Direct sequencing showed that both viruses carry genetic clade 6 HAs, while the NA genes carry mutations that encode the H275Y substitution, associated with oseltamivir resistance, along with the permissive substitutions V241I, N369K and N386S. The viruses have identical partial sequences for HA, NA, M and PB2 genes. Dates of onset of influenza-like illness with high fever, cough and malaise were 13 and 14 August 2012 respectively, with prior mild symptoms starting around one week before, during holiday in Spain (Catalonia). However, holiday destinations were about 200 km apart, with no known links. The younger sister, who showed mild symptoms, probably infected case 1. Concurrently with case 1, the father and a Dutch friend showed similar mild symptoms.

Conclusions: two patients were identified infected by identical A(H1N1)pdm09 influenza viruses that apparently had naturally acquired oseltamivir-resistance. A common source was not found. HA clade 6 A(H1N1)pdm09 viruses carrying H275Y-permissive substitutions pose a risk for the emergence of transmissible oseltamivir-resistant A(H1N1)pdm09 virus, leaving zanamivir as the only treatment option in most countries world-wide. Given the popularity of the holiday locations where these two infections most likely have been acquired, diagnostic evaluation of patients with influenza-like illness and characterization of A(H1N1)pdm09 viruses should be encouraged for -but not be limited to- patients travelling from this area, to monitor possible spread of this oseltamivir-resistant strain.

O24

What will Influenza Surveillance tell you About Antiviral use and Resistance?

Mika Shigematsu

National Institute of Infectious Diseases, Tokyo, Japan

Surveillance is the mode to capture burden and trend of diseases occurring. The design of the surveillance determines what may be able to learn from it. Influenza is a beautiful example of the case. Influenza affects all age group, all gender, all ethnics, all geographic area in different scale, and throughout the year with or without seasonality. Not only from global collaboration, but also from regional collaboration and data incorporation from the existing surveillance will enhance surveillance capability.

History of the influenza-like illness surveillance goes back hundred years in Japan. Sound bases of notification system exist for sentinel case reporting and viral strain surveillance. As over 30% of Japanese population is aged above 65, interest in the influenza related death such as sever pneumonia and encephalitis cases rose and additional surveillance was introduced. Moreover, multiple case-surveillances and syndromic surveillances by different definition were introduced during a decade to prepare for expected influenza pandemic after sever acute respiratory syndrome global epidemic.

On the other hand, pathogen surveillance to sample and observe changing trend of influenza virus has been carried out steadily alongside with the national notification system. With knowledge of Japan as the world's largest consumer of anti-influenza medication, concern of the resistance induction arose among public health personnel. Surveillance for antiviral resistance in isolated influenza strains was initiated during 2009 by the National Institute of Infectious Diseases and Japan Association of Prefectural and Municipal Health Institutes. Started from Oseltamivir and Zanamivir resistance and then included Peramivir and Laninamivir from late 2010.

In order to link surveillance information and answer to the title question, one more data from clinical site is required. The information on usage of the flu remedy to hospitalized and outpatient influenza cases. A hospital based surveillance is outside of national infectious diseases surveillance system in Japan, so we rely on study based survey and voluntary surveillance for this information.

Introduce three types of surveillance data from Japan and discuss what is able to understand through existing surveillance from the perspective of impact of antiviral use to drug resistance induction. Then try to identify how the gap could be filled without increasing burdens to already over whelmed public health sector based on existing efforts taken in Japan.

Tuesday 30th October

Symposium Session 4: Meeting Clinical Challenges
Chairs: Frederick Hayden and Tim Uyeki

Influenza Encephalopathy and Related Neuropsychiatic Syndromes
Masashi Mizuguchi – University of Tokyo, Tokyo, Japan

Patients with influenza, in particular children, occasionally show neuropsychiatric symptoms, especially impairment of consciousness with severity and duration highly variable among cases.
Acute encephalopathy (AE) refers to coma or stupor lasting for more than 24 hours, accompanied by seizures in the vast majority of cases. In Japan, the incidence of influenza-associated AE is 100-500 cases every year. Based on clinical, radiologic and pathologic findings, AE is classified into many syndromes, such as Reye syndrome (RS), acute necrotizing encephalopathy (ANE), acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) and mild encephalitis/encephalopathy with reversible splenial lesion (MERS). RS is caused by metabolic disorder, ANE by systemic cytokine storm, and AESD by excitotoxicity. ANE is characterized by high fatality, and AESD by high probability of neurologic handicaps. Japanese investigators published a guideline for influenza associated AE.

Mild and transient alterations of consciousness are more common than AE. They consist of transient delirium during febrile illnesses, MERS (mild cases) and several other conditions. In Japan, where oseltamivir has been prescribed to numerous patients, some teenagers jumped from a high-rise apartment and others rushed into a highway. Causality between oseltamivir and delirium in these cases remains controversial. Except for rare cases of fatal accidents, these patients showed full recovery.

Severe Influenza in Pregnancy: Mechanisms and Management
May Li Lim – KK Women's and Children's Hospital, Singapore

Data from influenza pandemics and epidemics indicate that pregnancy is a risk factor for complications. Pandemics of 1918 and 1957 saw significant mortality in the pregnant cohort. Influenza epidemics have resulted in greater incidence of morbidity such as acute cardiopulmonary event and hospitalisation. The increased susceptibility to complications has been attributed to the immunological suppression associated with pregnancy. The immune suppression theory lends support to the idea that exacerbated viral burden is the reason behind lethality of influenza infection. There is suggestion however that the severity of infection is related to immune-based tissue injury. The immunomodulatory role of estrogen is thought to be implicated. Prompt institution of medical therapy is an imperative in pregnant women with influenza infection as it reduces the risk of complications. Education of women will help to raise awareness and reinforce the importance of early presentation for medical attention. Vaccination must be recommended to all pregnant women and women of child-bearing age as this will minimise the risk of infection.

References:

Neuzil KM et al. Impact of influenza on acute cardiopulmonary hospitalizations in pregnant women. Am J Epidemiol 1998; 148: 1094-1102.

Marcelin G et al. Fatal outcome of pandemic H1N1 2009 influenza virus infection is associated with immunopathology and impaired lung repair, not enhanced viral burden, in pregnant mice. J of Virology 2011; 85(21): 11208-11219.

Lim ML et al. Influenza A/H1N1 (2009) infection in pregnancy - an Asian perspective. BJOG 2010; 117(5): 551-556.

Influenza Prevention and Treatment in Transplant recipients and Immunocompromised Hosts

Michael Ison – Northwestern University, Chicago, USA

The host immune response is critical for the control and clearance of influenza virus after initial infection. Unfortunately, key components of the adaptive response to influenza is compromised in solid organ and hematopoietic stem cell transplant recipients. As a result, influenza in these key patient population is associated with prolonged viral shedding, more frequent complications, including bacterial and fungal superinfections and rejection, and increased mortality. While vaccine is the critical prophylaxis strategy in other populations, response rates are diminished, particularly early post-transplant, among immunocompromised patients. Prospective data suggests that antiviral prophylaxis represents an effective and safe alternative to vaccine in patients who would be predicted to have poor responses to influenza vaccine. While there have not been prospective studies completed of antiviral therapy in solid organ or hematopoietic stem cell patient populations, retrospectively collected data clearly demonstrates that early therapy is associated with reduced rates of progression to lower airway involvement, morbidity and mortality. Therapy may also be associated with reduced rate of chronic rejection in lung transplant patient populations. Further studies are needed to define the optimal regimen (including combination therapies), dose, duration and endpoint to define successful treatment. Lastly, emergence of antiviral resistance appears to be more common among transplant recipients and studies are needed to better define risk factors, kinetics of emergence of resistant variants, and the optimal therapeutic intervention to prevent and manage resistant variants.

Advances in Antivirals for Non-Influenza Respiratory Viruses

Frederick Hayden – University of Virginia School of Medicine, Charlottesville, USA

A modest number of antivirals have been tested for treating non-influenza respiratory viral infections (RVIs), and currently agents of proven therapeutic value are largely limited to influenza. Seasonal prophylaxis with RSV-neutralizing monoclonal antibody against the viral F protein (palivizumab) provides partial protection against severe RSV disease in high-risk infants (prematurity, those with a congenital heart disease or bronchopulmonary dysplasia), but palivizumab is not effective for treatment in hospitalized children and is extremely costly. Aerosolized ribavirin (AE) is approved for treating RSV infections in infants but is difficult to administer, costly, and of unclear effectiveness. Most data on antivirals for non-influenza RVIs derives from observational reports in immunocompromised hosts, particularly hematopoietic stem cell transplant (HSCT) and lung transplant (LTx) recipients, and in other at-risk populations

Use of AR, especially when combined with anti-RSV immunoglobulins, reduces the likelihood of RSV progression from upper respiratory tract (URT) illness to pneumonia and mortality in HSCT patients, but AR is not effective in parainfluenza virus infection (PIV). An inhaled sialidase conjugate (DAS181) under study for influenza is active against PIV in pre-clinical studies and has been used in treating severe PIV infection in HSCT and LTx recipients. Early AR or systemic (IV or oral) ribavirin appear effective in reducing risk of bronchiolitis obliterans syndrome (BOS) progression or development in paramyxovirus (RSV, PIV, or HuMPV)-infected LTx patients. Recent phase 2 studies indicated that an inhalation treatment with a small inhibitory RNA directed to the N gene of RSV (ALN-RSV01) reduced the risk of BOS development in LTx recipients. Intravenous cidofovir is the primary treatment for severe adenovirus infections in HSCT recipients and other immunocompromised hosts but causes nephrotoxicity and is incompletely effective. An oral cidofovir derivative (CMX001) has much improved tolerability and appears to benefit HSCT patients failing cidofovir. Human rhinoviruses (HRVs) are the most common cause of RVIs across all age groups and major cause of asthma and CODP exacerbations. Two recent controlled studies reported that early HRV colds treatment with an oral capsid binding HRV inhibitor (BTA798) or with inhaled interferon-beta reduces the risk of exacerbations in asthmatic adults. Further studies on these antivirals are anticipated.

Improved understanding of the structure and function of viral proteins and of the pathogenesis of RVI infections in different clinical syndromes and patient groups will be fundamental to achieving rationally designed therapeutics and antiviral drugs for RVIs. Viral replication patterns and innate immune responses are thought to account for much of the symptomatology of acute RVIs, and, in some cases, contribute to tissue damage in key target organs. Some of the pathways involved in the host responses to RVIs are also those necessary for efficient viral replication. Consequently, a drug has the potential to inhibit both viral replication and potentially deleterious host responses.

Notes

Wednesday 31st October - Concurrent Workshops 3a

Clinical Issues – unusual syndromes, diagnostics, ventilatory strategies, clinical management, healthcare worker protection

Chairs: *David Hui, The Chinese University of Hong Kong, SAR China, Andrew Luks, Harborview Medical Centre, Seattle, USA & Shigeru Saito, University of Toyama, Toyama, Japan*

O25. No Maternal Death caused by Pandemic (H1N1) 2009 in Japan
Shigeru Saito

O26. Rapid and Sensitive Detection of Highly Pathogenic H5N1 Influenza Virus by The SmartAmp Method
Toshihisa Ishikawa

O27. Etiological Study of Community Acquired Pneumonia among Adolescents and Adults Patients with Low or Moderate Severity
Jiu-xin Qu

O28. Influenza Pneumonia: A Concurrent Comparison between pH1N1 and H3N2 in the Post-Pandemic Period
Shu Qiao Yang

O31. Efficacy of a Single Dose of Intravenous Immunoglobulin to Prevent Pandemic Influenza
Steven Rockman

O32. The Ethics of Research in Rapidly Evolving Infectious Disease Epidemics
Cam Binh

Abstracts

O25

No Maternal Death caused by Pandemic (H1N1) 2009 in Japan

Shigeru Saito[1], Akihito Nakai[2], Nobuya Unno[3], Takahiko Kubo[4], Hisanori Minakami[5]

[1]Dept. of OB/GYN, Univ. of Toyama, [2]Dept. of OB/GYN, Nippon Medical School, [3]Dept. of OB/GYN, Kitasato Univ., [4]Dept. of Maternal-Fetal and Neonatal Medicine, National Center for Child Health and Development, [5]Dept. of Obstetrics, Hokkaido Univ., Japan

Objectives: it is well known that novel pandemic (H1N1) 2009 virus infection increase the hospital admission rate and the number of maternal mortality. However, no maternal mortality was observed among pregnant Japanese women. We discuss what factors contributed to the lack of maternal mortality in Japan during this pandemic.

Patients and Method: total 53 maternal death cases in 1.10 million of total birth and 45 maternal death cases in 1.10 million of total birth maternal were reported in 2009 and 2010, respectively in Japan. National data showed no maternal mortality due to pandemic (H1N1) 2009 among pregnant Japanese women in 2009 and 2010. The Japan Society of Obstetrics and Gynecology (JSOG) recommended the following; (i) prompt use of antiviral drugs for treatment of pregnant women on May 8, 2009 via website, (ii) an early visit to the general practitioner when febrile on June 16, 2009, (iii) active use of antiviral drugs for prophylaxis after close contact with an infected person on Aug 4, 2009, and (iv) vaccination against the pandemic (H1N1) 2009 strain on Sept 7, 2009. The number of visit to the JSOG website increased to 193,705 in Oct 2009. Yamada's paper (JOGR 2012:38:130-136) showed that antiviral medicines for prophylaxis was performed in 40,000 – 50,000 pregnant women, and 60% of pregnant women were vaccinated within 1.5 months after availability of a vaccine against pandemic H1N1 influenza. These treatment reduced the infection rate in Japanese pregnant women from general infection rate in Japan; 12% to 3.5% in pregnant women. Nakai et al. (J. Infect. 2011:62:232-233) reported the clinical data of 181 hospitalized women. Seventeen (9.4%) developed pneumonia, two of these required admission to an ICU, and all 181 cases recovered completely. These rates were quite low compared to those in other countries.

Conclusions: the information by JSOG was very effective to inform the prevention and treatment of H1N1 infection. High rate of vaccination to pregnant women, antiviral medicines for prophylaxis and antiviral medicines within 48hr after symptom onset may have contributed to the maternal mortality zero.

O26

Rapid and Sensitive Detection of Highly Pathogenic H5N1 Influenza Virus by The SmartAmp Method

Kengo Usui[1], Yasumasa Kimura[1], Chiharu Kawakami[2], Yuko Sakai-Tagawa[3], Takeshi Hanami[1], Takahiro Soma[1], Hiroko Kinoshita[1], Tsukasa Kouno[1], Erik Arner[1], Yoshiyuki Nagai[4], Yoshihiro Kawaoka[3], Yoshihide Hayashizaki[1], and Toshihisa Ishikawa[1]

[1]Omics Science Center, RIKEN Yokohama Institute, Yokohama 230-0045, Japan; [2]Yokohama City Institute of Health, Yokohama 235-0012, Japan; [3]Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan; [4]Center of Research Network for Infectious Diseases (CRNID), RIKEN Yokohama Institute, Tokyo 101-0051, Japan

The possibility of human-to-human transmission of highly pathogenic avian influenza A(H5N1) viruses is becoming a fear for human health and society. Since the first human case emerged in 1997 in Hong Kong, A(H5N1) viruses have been circulating among avian species and have spread throughout Asia, Europe, and Africa, with sporadic transmission to humans and reports of nearly 60% mortality. In this context, simple, cost-effective, and highly sensitive methods should be developed to detect influenza A(H5N1) virus.

To address the clinical need for rapid diagnosis, we have developed the “RT-SmartAmp” assay method to rapidly detect the highly pathogenic A(H5N1) influenza virus from patient swab samples. The RT-SmartAmp assay comprises both reverse transcriptase (RT) and isothermal DNA amplification reactions in one step. Thus, the RT-SmartAmp assay method requires neither RNA extraction nor PCR reaction. We have tested its specificity and sensitivity to A(H5N1) virus. By the RT-SmartAmp method, we could specifically detect the HA segment of A(H5N1) virus within 40 minutes without cross-reacting with the seasonal A(H1N1), 2009 pdm A(H1N1), A(H3N2), or B-type viruses. The minimal level for detection of A(H5N1) virus was estimated to be 50 copies of viral RNA encoding the HA segment in the RT-SmartAmp reaction mixture.

In addition, we have developed an exciton-controlled hybridization-sensitive fluorescent primer, called “Exciton Primer”, to specifically detect the HA segment of A(H5N1) virus. The exciton primer functions as a sequence-specific dye. After hybridization to complementary sequences, the exciton primer provides a sequence-specific fluorescent signal. Owing to its high signal/noise ratio, the exciton primer enables visual end-point detection of the RT-SmartAmp reaction. We have designed a small-sized visual detection device for on-site detection of the A(H5N1) influenza virus. In conclusion, the RT-SmartAmp assay method would provide a practical tool for rapid and sensitive detection of highly pathogenic A(H5N1) influenza virus. This study was supported by the J-GRID Project of Japanese MEXT “Detection of highly pathogenic H5N1 influenza virus by the SmartAmp method”.

O27

Etiological Study of Community-Acquired Pneumonia Among Adolescents and Adults Patients with Low or Moderate Severity

Jiu-xin Qu[1], Shu-qiao Yang[1], Xiao-min Yu[1], Yi-min Wang[1], Bin Cao[1], Chen Wang[2], for Beijing Network for Adult Community-Acquired Pneumonia (BNACAP)

[1]Beijing Chao-Yang Hospital, Capital Medical University, Beijing Institute of Respiratory Medicine, Beijing, China; [2]Beijing Hospital, Ministry of Health, Beijing Institute of Respiratory Medicine, Beijing, China

Objective: to describe the clinical and etiological data collected from adolescents and adults with radiographically confirmed CAP in whom causative agents were sought in respiratory secretion samples during the acute phase of the disease.

Patients and methods In the study, 954 patients with radiographically confirmed CAP had been enrolled, whose respiratory secretion samples were tested using Seeplex RV Detection kit, urine antigen tests, PCR method, blood and sputum culture to detect 15 respiratory viruses, atypical pathogens and bacteria. The distribution of the microbial etiology in relation to age and severity score (PSI) were analyzed.

Results: mean (SD) age of the defined population was 45.24 (19.478) years (range 14-94). A total of 393 patients (41.2%) were positive for at least one pathogen: the most frequently detected was Mycoplasma pneumoniae (n=168, 17.6%), followed by influenza virus A (n=94, 9.9%), bacteria (n=47, 8.8%), human rhinovirus (n=41, 4.3%) and adenovirus (n=40, 4.2%). Co-infections were found in 75 patients (7.9%), and showed higher mortality than single infections ($p=0.032$). The frequency of Mycoplasma pneumoniae and adenovirus decreased according to the increasing age groups ($p<0.001$). Opposite trend was found in influenza virus A ($p<0.001$) and bacteria infected cases ($p=0.005$). The frequency of atypical pathogens decreased in moderate and high risk groups (7.1% and 8.9%, respectively, $p<0.001$), whereas that of main bacteria pathogen, Streptococcus pneumoniae and Klebsiella pneumoniae, increased according to risk groups ($p=0.001$). Similar trend was found in the distribution of pandemic H1N1.

Conclusions: in the defined population of CAP, M. pneumoniae, Influenza virus A, human rhinovirus, adenovirus and S. pneumoniae, were the most common pathogens isolated, while co-infection was very frequent. CAP due to M. pneumoniae is recognized by severity scoring as a low-risk condition, and always found in patients younger than 45 years old. Bacteria and pandemic H1N1 are identified as moderate- and high- risk pathogens, usually found in patients older than 45 years old. Thus, the choice of empiric treatment for CAP should be made according to local epidemiologic data.

O28

Influenza Pneumonia: A Concurrent Comparison between pH1N1 and H3N2 in the Post-Pandemic Period

Jiu Xin Qu[1], Shu Qiao Yang[1], Bin Cao[1], Xiao Min Yu[2], Ying Mei Liu[2], Chen Wang[2]

Department of Infectious Diseases and Clinical Microbiology, Beijing Chao-Yang Hospital, Beijing Institute of Respiratory Medicine, Beijing Key Laboratory of Respiratory and Pulmonary Circulation Disorders, Capital Medical University, Beijing 100020, China

Objectives: to investigate the differences of demographic data, clinical features and cytokine profile between pandemic influenza A (pH1N1) 2009 infection and seasonal influenza A (H3N2) infection in adult patients with community-acquired pneumonia during the post-pandemic period.

Methods: based on Beijing Network for Adult Community-Acquired Pneumonia (BNACAP), patients with community-acquired pneumonia (CAP) during the first flu season after 2009 pandemic(from November 2011 to March 2012) were enrolled. Identification of influenza types and subtypes was performed. Main characteristics of demographic and clinical data, and the pattern of serum cytokine were compared between patients with 2009 H1N1 or seasonal H3N2 pneumonia.

Results: a total of 88 CAP patients were studied, including 58 patients with pH1N1 infection and 30 patients with seasonal H3N2 infection. Age distribution was similar in the two groups, a shift toward old people was noticed in pH1N1 group compared to pandemic period. When compared with H3N2 group, higher serum levels of aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and a lower level of albumin (ALB) were observed in pH1N1 group ($p=0.006$, 0.018, <0.001, respectively). Hospitalization rate in pH1N1 group was also higher (81.0% vs 56.7%, $p=0.023$), and oseltamivir was more frequently prescribed(44.8% vs 0%). ARDS had been found in two patients, and were responsible for one death, both two were infected with pH1N1 virus. Comprehensive elevated serum levels of cytokines and chemokines were observed in both two groups when compared with those of normal controls. Serum levels of IL-10 and IL-12 were found to be significantly higher in pH1N1 group than H3N2 group ($p=0.005$, 0.001, respectively).

Conclusions: our findings show a shift toward older people in pH1N1 infections during the post-pandemic period compared to pandemic year of 2009. CAPs caused by pH1H1 virus seemed to be more serious than seasonal H3N2 virus.

O29

Efficacy of a Single Dose of Intravenous Immunoglobulin to Prevent Pandemic Influenza

Steven Rockman[1], Sue Lowther[2], Sarina Camuglia[1], Kirsten Vandenberg[1], Deborah Middleton[2], Darryl Maher[1]

[1]CSL Limited Parkville Australia, [2]Australian Animal Health Laboratories CSIRO Geelong Australia

Objective: intravenous immunoglobulin (IVIG) is widely used to treat immune deficiencies, autoimmune disease and chronic inflammatory conditions. Sporadic reports have suggested that IVIG may be useful in the influenza setting. The influenza pandemic of 2009 and global circulation of highly pathogenic H5N1 has sparked interest in alternative therapies for the treatment of serious influenza infection. IVIG has been demonstrated to contain cross-reactive antibodies to pandemic influenza. Further, IVIG has properties of modulating the immune response that may influence the hypercytokinaemia or "cytokine storm" which is a suspected contributing factor to mortality in the pandemic influenza setting.

Aim: was to investigate the efficacy of IVIG in two ferret models of pandemic influenza.

Methods: two models of pandemic influenza were analysed. The swine origin H1N1 pandemic of 2009 and highly pathogenic H5N1 model in ferrets. IVIG was administered as a single dose at the

time of challenge. Ferrets were assessed for weight loss, temperature, activity and viral replication over a 14 day post-challenge period.

Results: IVIG harvested prior to 2009 prevented significant viral replication (number of isolates and viral titre) of the swine origin H1N1 virus in the lung but not the upper respiratory tract. A single dose of IVIG prevented mortality and significant morbidity following challenge with a lethal dose of H5N1 virus. The level of virus replicating in the deep lung response was related to the dose of IVIG administered. The mechanism of IVIG in this setting will be discussed.

Conclusion: these studies suggest that human IVIG is effective in preventing serious influenza infection and provides an alternative treatment option requiring clinical trials.

O30

The Ethics of Research in Rapidly Evolving Infectious Disease Epidemics

NTC Binh[1 2], TT Hien[1], Michael Parker[2], Jeremy Farrar[1]

[1]Oxford University Clinical Research Unit, Wellcome Trust Major Overseas Programme, Hospital for Tropical Diseases, Viet Nam; [2]Ethox Centre, Department of Public Health, University of Oxford, Old Road Campus, Oxford, UK

Background: the world is at risk of epidemics of novel and reemerging infectious diseases. These may be national, regional or international as in the case of Nipah, African Viral Haemorrhagic Fevers, SARS and H1N1 respectively. It is crucial that vital public health and clinical research is conducted in such epidemics. Yet the conduct of heath research during rapidly evolving epidemics or disasters represents an enormous challenge. There are a number of practical challenges to undertaking such research but there are also major ethical issues to consider. However, there is very little understanding of these issues and very little empirical evidence of the views of patients, their families, society and key stakeholders. This oral presentation will review the ethical issues and present data on the views of key individuals from centers directly involved in responding to epidemics.

Objective: to collect data on ethical considerations arising in the setting of research on rapidly evolving epidemics posed by the urgent and unpredictable nature of epidemics.

Design: The study was conducted in Oxford University Clinical Research Unit (OUCRU), Viet Nam and 3 other hospitals in Viet Nam with experience of epidemics. A modified grounded theory approach has been adopted. Data are primarily collected by in-depth interview and focus group discussions. The first interview round was completed at the Hospital for Tropical Diseases (HTD) with people representing different constituents of the health system including research staff, IRB members, patients/family members and study sponsors/funders who have participated in or reviewed research projects on infectious diseases including SARS, H5N1, H1N1, dengue and Hand, Foot, Mouth disease. The interviews were audiotaped, transcribed and translated in English for analysis.

Result: through the interim analysis, 7 main themes emerged. These included issues concerning 1) the dividing line between public health needs, medical practice and research, 2) vulnerability of research participants and family members, 3) research information provision and factors influencing decisions of research participants/family members, 4) challenges faced by IRBs and factors which might influence their review and oversight in the setting, 5) dynamics of research collaboration, 6) multiple commitments of investigators and staff due to existing and emergency workloads, and 7) the role of the media and wider society during such rapidly evolving epidemics.

Conclusion: the issues and types of considerations and their relative importance were raised and/or valued differently between key stakeholder groups due to their role and experience in research participation. Some of these issues which were raised relate to all health related research. However, some very important considerations were unique to the setting of rapidly evolving epidemics. It is inevitable that epidemics of emerging and reemerging infectious diseases will occur in the future and there is a clear need to undertake crucial research. It is therefore imperative that we understand the challenges and ethical issues surrounding such research. It is desirable that research into these ethical challenges takes place in the inter-epidemic period in order to better prepare for the next epidemic.

Wednesday 31st October - Concurrent Workshop 3b

Consequences of antiviral resistance mutations, fitness, animal models

Chairs: *Jilong Chen, Chinese Academy of Sciences, Beijing, China & Elena Govorkova, St Jude Children's Research Hospital, Memphis, USA*

O31. Identification of Novel Host Factors Involved in Regulating Influenza Virus Infection and Replication
Jilong Chen

O32. Impact of Genetic Variations of the Neuraminidase of the H5N1 and Pandemic H1N1pdm09 Viruses on their Enzymatic Activity and Sensitivity to Neuraminidase Inhibitors
Sylvie van der Werf

O33. Using Ferrets to Assess the Fitness of the Oseltamivir-Resistant A(H1N1)pdm09 viruses Responsible for a Cluster of Community Cases in Australia in 2011
Aeron Hurt

O34. H1N1 2009 Pandemic Influenza Virus: Resistance of the 1223R Neuraminidase Mutant Explained by Kinetic and Structural Analysis
Erhard van der Vries

O35. Evaluation of Pandemic Influenza A/H1N1 Virus Mutations Conferring Resistance to Zanamivir
Andrés Pizzorno

O36. T-705 Suppresses Influenza A virus Infectivity via Lethal Mutagenesis
Tatiana Baranovich

Abstracts

O31

Identification of Novel Host Factors involved in Regulating Influenza Virus Infection and Replication

Song Wang, Jing Ouyang, Xiaojuan Chi, Yuhai Chen, Haitao Wei, Jilong Chen

CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences (CAS), Beijing 100101, China

In an attempt to provide insights into the mechanisms by which the host cell interacts with influenza A virus, cDNA microarray and long non-coding RNA (lncRNA) microarray were used to determine the differentially expressed protein-coding and non-coding genes in A549 human alveolar epithelial cells in response to infection with A/WSN/33 influenza virus (H1N1). All the genes whose expressions were altered by at least 2-fold were clustered and displayed. The protein-coding cDNA microarray analysis revealed 337 up-regulated genes and 1024 down-regulated genes following the viral infection. Many genes are involved in innate immunity, inflammatory response and intracellular transport. The long non-coding RNA microarray analysis revealed that 494 lncRNAs displayed up-regulated and 413 lncRNAs showed down-regulated in A549 cells infected with influenza A virus compared with uninfected cells. We further investigated the functional involvement of selected protein-coding and non-coding genes in regulating influenza virus infection and replication. Interestingly, expression of ARHGAP21, a Cdc42-specific GAP, was found to be greatly down-regulated by influenza A virus infection. We found that expression of constitutively active Cdc42 or depletion of ARHGAP21 had profound effect on the transport of neuraminidase (NA) to the plasma membranes. Importantly, silencing Cdc42 reduced influenza A virus replication, whereas silencing ARHGAP21 increased the virus replication. Together, our results reveal that ARHGAP21 and Cdc42-based signaling regulates the NA transport and thereby impacts virus replication. In addition, eukaryotic initiation factor 4B (eIF4B) was also down-regulated by the virus infection. Knockdown or overexpression of eIF4B in A549 cells significantly affected influenza virus replication. Further functional analysis demonstrated that eIF4B is required for efficient expression of host antiviral proteins such as ISG15, IFIT2, IFITM1 and Mx1. Furthermore, we identified a differentially expressed lncRNA named as IVDR8. Our experiments demonstrated that IVDR8 plays a key role in influenza virus infection and host defense.

O32

Impact of Genetic Variations of the Neuraminidase of the H5N1 and Pandemic H1N1pdm09 Viruses on their Enzymatic Activity and Sensitivity to Neuraminidase Inhibitors

G. Abou-Jaoudé[1,2,3], MC. Vaney[2,4], and S. van der Werf[1,2,3]

[1]Institut Pasteur, Unité de Génétique Moléculaire des Virus à ARN, Département de Virologie, France; [2]CNRS, URA3015, France; [3]Université Paris Diderot Sorbonne Paris Cité, Paris, France; [4]Institut Pasteur, Unité de Recherche de Virologie Structurale, Département de Virologie, France

The aim of our study was to determine whether genetic variations of the neuraminidase (NA) of the pandemic H1N1pdm09 (N1pdm09) and H5N1 (H5-N1) viruses could counteract the deleterious effects of the H275Y mutation in the NA, that confers high level resistance to oseltamivir (Tamiflu®) and lead to better fitness and transmission of resistant viruses, as seen for seasonal influenza H1N1 viruses.

Alignment of neuraminidase sequences of seasonal H1N1, H5N1 and pandemic H1N1pdm09 viruses along with the available crystal structures, emphasized the importance of positions 222 and 344 for which the impact on the NA activity of seasonal H1N1 viruses was previously shown (Rameix-Welti et al.Antivir.Ther.2011;16(4):597-603). The sequences of representative NAs were cloned into an expression vector and substitutions at positions 222 (N to K/Q/R) and 344 (Y/N to Y/D/N) were

introduced alone or in combination with the H275Y substitution. The transiently expressed proteins were assayed for their enzymatic properties and sensitivities to neuraminidase inhibitors (NAIs).

Substitutions at position 222 had no significant effect on the affinity for the MUNANA substrate and on the sensitivity to NAIs of the N1pm09 and H5-N1 for the sensitive protein. However, in the presence of the H275Y mutation, substitutions at position 222 increased the mutants' affinity for the substrate and decreased their sensitivities to oseltamivir. In contrast, whether in the presence or absence of the H275Y mutation, substitutions at position 344 increased or decreased the Km of the NA depending on the residue and the background; sensitivity to NAIs of N1pm09 changed according to the variations of Km, but not that of H5-N1. Using molecular modeling on structural data, we attempt to understand the different behavior of NAs derived from different genetic backgrounds.

These studies should help determine whether such genetic variations could lead to increased fitness and transmissibility of viruses resistant to NAIs.

O33

Using Ferrets to Assess the Fitness of the Oseltamivir-Resistant A(H1N1)pdm09 Viruses Responsible for a Cluster of Community Cases in Australia in 2011

AC Hurt[1], JM Butler[1], J McCaw[2], L Xue[1], T Guarnaccia[1], A Kelso[1], IG Barr[1]

[1]WHO Collaborating Centre for Reference and Research on Influenza, VIDRL, Melbourne, Australia; and [2]Melbourne School of Population Health, The University of Melbourne, Australia

Oseltamivir (Tamiflu) is the most commonly used antiviral drug for the treatment of influenza infection in humans. In 2007-2008, oseltamivir-resistant seasonal A(H1N1) influenza viruses with a H275Y neuraminidase mutation spread globally. Prior to this event, widespread transmission of oseltamivir-resistant viruses was thought to be unlikely as the mutations that caused resistance were also thought to compromise viral 'fitness'. Studies have since shown that other compensatory mutations in the NA of the seasonal A(H1N1) in 2007/2008 may have enabled this virus to overcome the detrimental effect of the H275Y resistance mutation and retain the ability to spread. Since the emergence of the pandemic 2009 H1N1 (A(H1N1)pdm09) influenza viruses, oseltamivir-resistant A(H1N1)pdm09 viruses with the H275Y neuraminidase mutation have been detected in treated patients but transmission of these viruses has been rare. However in the Newcastle region of Australia in 2011, oseltamivir-resistant H275Y A(H1N1)pdm09 variant viruses caused a cluster of influenza cases among patients within the community not undergoing oseltamivir treatment. We assessed the relative fitness of these viruses using a competitive mixtures model in ferrets. Ferrets were infected with a series of deliberately prepared mixtures of a Newcastle H275Y oseltamivir-resistant virus and a closely related Newcastle oseltamivir-sensitive A(H1N1)pdm09 virus. Additional naive ferrets were co-housed with infected ferrets to analyse the relative contact transmissibility of each virus. The relative proportions of oseltamivir-resistant and -sensitive virus in each ferret were assessed over time by molecular analysis of daily nasal wash samples. Mathematical analysis of the results demonstrated that the Newcastle H275Y oseltamivir-resistant A(H1N1)pdm09 viruses had equivalent or possibly enhanced replication and transmissibility to naive ferrets, compared to the very similar oseltamivir-sensitive virus. Further work is being conducted to identify the viral mutations which may permit the acquisition of oseltamivir resistance without compromising viral fitness. Such studies are critical to help determine the risk of oseltamivir-resistant H275Y A(H1N1)pdm09 viruses spreading globally.

O34

H1N1 2009 Pandemic Influenza Virus: Resistance of the I223R Neuraminidase Mutant Explained by Kinetic and Structural Analysis

Erhard van der Vries[1], Patrick Collins[2], Sebastien Vachieri[2], Xiaoli Xiong[2], Junfeng Liu[2], Philip Walker[2], Lesley Haire[2], Alan Hay[2], Martin Schutten[1], Albert Osterhaus[1], Steve Martin[2], Charles Boucher[1], John Skehel[2], Steve Gamblin[2]

[1]Erasmus Medical Centre, Department of Virology, Rotterdam, the Netherlands and [2]National Institute for Medical Research, The Ridgeway, Mill Hill, United Kingdom

Two classes of antiviral drugs, neuraminidase inhibitors and adamantanes are approved for prophylaxis and therapy against influenza virus infections. A major concern is that antiviral resistant viruses emerge and spread in the human population. Recently, a novel neuraminidase I223R mutation was identified in this subtype. So far, the resistance mechanism of this change is unknown. Here, the enzymatic properties of the I223R mutant, the most frequently observed resistance mutation, H275Y, and the double mutant I223R/H275Y were compared and the results interpreted in relation to the structures of wild type and I223R mutant neuraminidases determined by X-ray crystallography. Interestingly, in contrast to a pandemic H1N1 neuraminidase structure published recently, the active site of the I223R mutant adopted an open conformation in the absence of an active site ligand. Relative to wild type, KM values for MUNANA increased only 2-fold for the single mutant and up to 8-fold for the double mutant. Oseltamivir inhibition constants (KI) increased 48-fold in the single I223R mutant and 7500- fold in the double mutant, in both cases the change was largely accounted for by an increased dissociation rate constant for oseltamivir. These observations were linked to shrinkage of a hydrophobic pocket in the active site, as a result of the I223R change and the interaction of R223 with S247 which adopts a different rotamer in the mutant. In the wild type, this hydrophobic pocket normally facilitates binding of the pentoxyl substituent of oseltamivir. In contrast, due to the smaller, and polar, glycerol substituent in zanamivir, resistance to this inhibitor is much lower. This should be taken into account in the development of novel neuraminidase inhibitors targeting the hydrophobic pocket. In addition, the observation here that the 2009 pandemic neuraminidase contains the “open” 150-cavity makes this cavity a suitable target for new neuraminidase inhibitors.

O35

Evaluation of Pandemic Influenza A/H1N1 Virus Mutations Conferring Resistance to Zanamivir

A Pizzorno[1], Y Abed[1], C Rhéaume[1], X Bouhy[1] and G Boivin[1]

[1]Laval University and CHUQ, Québec, Canada

Background: the neuraminidase inhibitors (NAIs) oseltamivir and zanamivir play a central role in the control of influenza epidemics and pandemics. During the 2009 influenza pandemic (A/H1N1pdm09), oseltamivir-resistant variants were infrequently found but were shown to maintain their fitness and contact transmissibility in animal models. The aim of this study was to investigate the effect of mutations conferring resistance to zanamivir in the A/H1N1pdm09 background.

Methods: reverse genetics was used to rescue a recombinant A/H1N1pdm09 wild-type (WT) virus as well as two NA mutants (E119G and Q136K) reported to be resistant to zanamivir in different influenza A subtypes. The susceptibility profiles to NAIs and the enzymatic parameters Km and Vmax were determined by fluorometric-based assays. Replicative capacity was evaluated by yield assays on ST6Gall-MDCK cells. Virulence was evaluated in BALB/c mice and contact transmissibility in ferrets.

Results: both E119G and Q136K mutants remained susceptible to oseltamivir but showed a high level of resistance to zanamivir, with 832- and 749-fold increases in IC50 values, respectively, compared to the WT virus. The E119G mutation significantly reduced the affinity (9-fold increase in

Km vs the WT) but not the relative enzymatic activity. The Q136K mutation increased the affinity (5-fold decrease in Km vs the WT) with a reduction on relative enzymatic activity (8% Vmax ratio vs the WT). In vitro, viral titers of both mutant viruses were reduced in the first 36h p.i. compared to the WT although similar peak viral titers were attained for the three viruses at 48h p.i. In mice, the E119G and Q136K mutants generated significantly reduced lung viral titers on day 3 (3.4x106 and 4.1x107 vs 8.8x107 PFU/ml) and 5 (3.0x105 and 8.6x105 vs 5.8x107 PFU/ml) p.i. compared to the WT, respectively. All index and contact ferrets of the three groups seroconverted for A/Quebec/144147/2009. The E119G mutation rapidly reverted to the WT in index and contact ferrets. The Q136K mutation was conserved in ferrets yet nasal wash viral titers from the Q136K contact group on days 2-4 p.i. were significantly lower compared to WT.

Conclusions: our results demonstrate that zanamivir-resistance mutations compromise viral fitness and transmissibility in A(H1N1)pdm09 viruses. This may help to explain the very low frequency of these mutations, particularly in the N1 subtype.

O36

T-705 Suppresses Influenza A Virus Infectivity via Lethal Mutagenesis

T Baranovich[1], S-S Wong[1], J Armstrong[1], H Marjuki1, RJ Webby[1], RG Webster[1,2], EA Govorkova[1]

[1]Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN 38105-2794; [2]Department of Pathology, University of Tennessee, Memphis, TN 38105

Objectives: the nucleoside analog T-705 is a promising investigational anti-influenza agent. It has demonstrated in vitro and in vivo activity against influenza viruses through mechanisms which are not completely understood. Here, we studied the hypothesis that T-705 enhances the influenza virus mutation rate, forcing the virus beyond the tolerable mutation frequency and creating a nonviable virus population. This mechanism, known as lethal mutagenesis, is well documented for many RNA viruses but not for influenza viruses.

Methods: T-705's antiviral activity was evaluated in MDCK cells during multiple sequential passages with seasonal influenza A H1N1 viruses at a low multiplicity of infection (MOI = 0.001 PFU/cell) and with 2009 pandemic H1N1 viruses at a high MOI (MOI = 10 PFU/cell). Antiviral activity was assessed in MDCK cells by measuring the reduction of virus infectivity and the inhibition of viral polymerase activity. Relative virus infectivity was calculated by comparing virus titers determined by performing plaque assays and RNA titers determined by performing quantitative RT-PCR assays. Mutant spectrum complexity was determined by performing a Sanger sequence analysis.

Results: we demonstrated that persistent infection with influenza A H1N1 viruses during the first few passages in MDCK cells inoculated with low or high virus doses and then treated with T-705 led to a progressive decline of viral infectivity and extinction (i.e., loss of infectivity) of virus progeny in vitro. The results of phenotypic assays based on cell viability and polymerase activity confirmed that T-705–resistant variants were not selected. The relative infectivity of viral RNA synthesized in the presence of T-705 was less than that of mock-supplemented controls. Sequence analysis of the influenza virus variants passaged in the presence of T-705 revealed significant increases in transversion mutations, indicating that drug-induced error-prone replication occurred.

Conclusions: our data support the hypothesis that lethal mutagenesis is at least one of the antiviral mechanisms of T-705's activity against influenza A viruses. Thus, T-705 could provide a new approach to treating influenza virus infections and preventing or decreasing the development of drug resistance during the course of treatment.

Wednesday 31st October

Symposium Session 5: Future Directions
Chairs: Alan Hay & Tran Tinh Hien

Influenza Evolution: Can We Predict What's Next?
Jesse Bloom – Fred Hutchinson Cancer Research Center, Seattle, USA

Influenza poses a constant threat to public health efforts in large part because the virus's rapid evolution can quickly render antiviral drugs and vaccines ineffective. I will discuss strategies that might eventually be applied to better predict future influenza evolution. I will begin by describing the experimental deconstruction of an historical influenza evolutionary pathway. I will then discuss how next-generation sequencing and computational techniques can be used to understand some of the molecular constraints that shaped the historical viral evolution. Finally, I will discuss the prospects for using similar approaches to forecast future viral evolution.

Antivirals in 2009 Pandemic – Lessons and Implications for Future Strategies
Maria Zambon – UK Health Protection Agency, London, UK

Antiviral policy decisions during the pandemic 2009-10 were largely guided by:

1. Identification of priority groups
2. The role of drug resistance
3. Feasibility of disease containment vs mitigation strategies
4. Availability of stockpiles

There was significant diversity amongst the national antiviral use policies. Factors contributing to diversity included the structure of national health systems and influence of antiviral use polices for seasonal influenza.

The impact of the use of antivirals, particularly neuraminidase inhibitors (NAIs) on clinical outcomes during the 2009-10 pandemic has not been firmly established. A systematic approach to evaluating national polices used for antiviral distribution during the pandemic may provide insights about optimum use of antivirals at a population level. A review of available clinical outcome data from observational analyses in several different countries with an analysis of gaps in evidence will be presented, to assist updating pandemic plans, and promote consideration of optimum use of antivirals during pandemics of influenza.

New Research Paradigms in Response to Emerging Infectious Threats
Jeremy Farrar –The Hospital for Tropical Diseases, Ho Chi Minh City, Viet Nam

A key lesson from a series of recent outbreaks of emerging pathogens of potential global public health importance including SARS-CoV, highly pathogenic avian influenza A (H5N1) virus, Viral Hemorrhagic Fevers, Nipah virus and the 2009 H1N1 virus pandemic was that mounting clinical research in response to a rapidly emerging infectious disease is extremely challenging and often delayed. During these events, critical clinical management and pathogenesis insights came mostly from sites that were already undertaking related clinical studies including those on inter-pandemic influenza or from established national or regional research networks such as the Canadian and Australia/New Zealand Critical Care Trials Group. Even from these groups only a very small number of patients were enrolled in randomized controlled trials of therapeutic interventions. There was little cross-border coordination, unlike that which now exists in epidemiology, virology and genomics. The clinical research response was cumbersome and slow despite years of global preparations for a potentially devastating influenza pandemic of avian origin or the next SARS-like outbreak. Although observational registries were mobilized, initiatives to launch randomized controlled trials or more sophisticated biologic studies generally missed the initial waves of the 2009 H1N1 pandemic and in many cases failed to enroll sufficient numbers of patients across the entire clinical spectrum of

disease into studies, even during subsequent waves. During the 2009 H1N1 virus pandemic the efforts to prepare for a respiratory disease outbreak allowed a reasonably rapid and coordinated response on epidemiologic and diagnostic aspects of disease but failed in the timely conduct of clinical research aimed at improving patient management or understanding pathogenesis. The failure to have coordinated, comparable data on clinical management and pathogenesis of 2009 H1N1 virus infection meant that we missed the opportunity to improve patient outcomes. Indeed this has been a problem in almost all epidemics over the last decade (Nipah, SARS, H5N1, and in outbreaks of VHF) with very little research aimed at improving clinical management or understanding pathogenesis. This has demonstrated that unless something is done to change the barriers faced in 2009, the next influenza (or other) epidemic will result in a similar missed opportunity to save lives. There is a need to establish a sustainable consortium of clinical research groups with broad geographic coverage (including low resource settings), cross-border coordination, commitment to open access, and capacity to conduct complementary high-quality, hospital-based pathogenesis and clinical management studies and the flexibility to respond immediately to rapidly emergent threats. We need a new paradigm for clinical research in the context of rapidly emerging public health threats and one appropriate to the sorts of challenges we will face in the 21st Century.

Poster Abstracts

P1

Post-exposure Prophylaxis for Influenza in Pediatric Wards: Oseltamivir or Zanamivir after a Rapid Antigen Detection

M Shinjoh[1] & N Sugaya[2]

[1] Keio University, Tokyo, Japan; [2] Keiyu Hospital, Kanagawa, Japan

Post-exposure prophylaxis (PEP) using neuraminidase inhibitors against exposure to influenza virus has been well studied in household settings but not in nosocomial settings in pediatric wards. We have used oseltamivir or zanamivir as PEP in our pediatric wards. All influenza cases were diagnosed by the influenza rapid diagnostic test.

Results: between 2003 and 2011, there were 20 nosocomial introductions of influenza (10 were A, 9 were B, and 1 was undetermined). The index cases consisted of 17 inpatients, 2 parents, and 1 medical staff member. The 17 inpatients had been admitted to the hospital for reasons other than infectious disease and they developed influenza after hospitalization. Among the 81 contacts, 28 (35%) were exposed to influenza A, and 52 (64%) were exposed to influenza B. The rate of secondary infection among contacts not given PEP was 29% (5/17), and the rate among contacts given PEP was significantly lower at 3% (2/63) ($p = 0.004$). The two infected contacts who had been given PEP were both influenza B cases, and both had received oseltamivir. The contacts who received PEP within 24 hours (59), for influenza A (23), and those who received zanamivir (15) did not develop influenza. No adverse events were reported.

Conclusions: PEP using oseltamivir or zanamivir for unexpected occurrences of nosocomial influenza in pediatric wards is safe and effective. The influenza rapid diagnostic test that we used was helpful for detecting nosocomial influenza in children.

P2

Efficacy of Laninamivir Octanoate (CS-8958) in Contact Transmission of Influenza Viruses in Guinea Pig

M Yamashita, M Kakuta, M Tanaka, S Tobiume and S Kubo

Biological Res. Labs., Daiichi Sankyo Co. Ltd. Japan

Objectives Laninamivir octanoate (CS-8958) is a long acting neuraminidase (NA) inhibitor which completes influenza treatment by a single inhalation and has been commercially available in Japan as the brand name, Inavir®. Efficacy of CS-8958 has been investigated in mice and ferrets with a direct infection of influenza viruses. In this study, the efficacy of CS-8958 and other NA inhibitors was investigated in a contact transmission model of guinea pigs.

Methods: a guinea pig (Hartley, female) was infected with 500 pfu of A/Nagasaki/I01/2009v,(H1N1) pdm09 subtype (donor) and housed with an uninfected guinea pig (acceptor) the next day (Day 0) to cause contact infection from a donor to an acceptor. Sets of these pairs were created. The acceptors were treated with NA inhibitors under various experimental conditions. Virus titers in the nasal wash of the donor were measured on Day 1 to confirm virus infection and those of acceptors on Day 3, 4 and every other day afterwards to investigate the efficacy. Lower limit of detection was 101.70 pfu/mL.

Results: 1.Transmission model: In donors, the viruses grew to near 107pfu/mL at the peak of Day 1 and then quickly eliminated. In acceptors, the viruses grew to 106-7 pfu/mL at Day 3-4 and were then eliminated.
2. Administration at contact: Acceptors were administered 33 μg/kg of CS-8958 intranasally once, 33 μg/kg of zanamivir intranasally once daily for 5 days and 100 mg/kg of oseltamivir orally once daily for 5 days. CS-8958 and zanamivir delayed virus loads and reduced about 1/1000 of virus loads in acceptors at the peak of Day 6. Oseltamivir did not delay the virus load and was reduced by only 1/5 at the peak of Days 3-4. 3. Administration before contact: Acceptors were treated once with 190 μg/kg of CS-8958 8, 5 or 4 days before contact. Virus loads were delayed in all treated groups and no viruses were detected in 2 out

of 4 acceptors of the group of - 4 days administration.

Conclusion: in the contact transmission model of guinea pigs, a single treatment of CS-8958 was efficacious both in administration at contact and before contact.

P3

Preliminary Results of the Epidemiology of Human Cases of Influenza A/H5N1 Virus in Vietnam from 2003-2012

Nguyen Thi Thu Yen[1], Tran Nhu Duong[1], Nguyen Hai Tuan[1], Pham Duc Tho[1], Nguyen Phuong Thanh[1], Nguyen Bien Thuy[1], Tran Ngoc Huu[2], Bui Trong Chien[3], David T. Dennis[4], Do Thuy Trang[5], Bryan K. Kapella[5], James C. Kile[5], Nguyen Tran Hien[1]

[1]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam, [2]Pasteur Institute, Ho Chi Minh City, Vietnam, [3]Pasteur Institute, Nha Trang, Vietnam, [4]DUKE-NUS Graduate Medical School, Singapore, [5]Influenza Program, U.S. Centers for Disease Control and Prevention, Hanoi, Vietnam

Background: with avian influenza A/H5N1 virus (A/H5N1) endemic in poultry in the country, Vietnam currently has the third highest number of human cases of A/H5N1 in the world. A/H5N1 continues to pose a serious threat to both public health and animal health. This study proposes to provide epidemiological, laboratory, and clinical characteristics of 123 confirmed A/H5N1 cases, and to identify potential health implications associated with temporal gaps in seeking healthcare services.

Methods: this is a retrospective review of A/H5N1 case documents from regional official reports, provincial preventive medicine centres, and hospital medical records. All A/H5N1 cases were confirmed by reverse transcriptase polymerase chain reaction at the National Influenza Center in Vietnam. Epidemiological analysis was performed to describe characteristics of reported cases.

Results: from 2003-2012, Vietnam experienced only two years with no confirmed human cases of A/H5N1, 2006 and 2011. Of 63 provinces/cities of the country, 41 reported cases during the other eight years. The median age of the 123 cases was 23 years, (IQR 11-35). The mortality rate was 49.6%. Potential exposure history included close contact with sick or dead poultry in 45% of cases, poultry flocks in 54% of case households, 51% of cases living in a current poultry outbreak area, and 54% had traveled to a poultry raising area. Age and delay of hospital admission were significantly associated with mortality. Cases ≤ 18 years old were more likely to die than cases > 18 years, (OR 2.1; 95% CI 1.0-4.6; p=0.036). Of 99 persons who were ill > 48 hours before hospitalization, 54 (54.5%) died compared with 7 (29.2%) of 24 persons hospitalized ≤48 hours after illness onset, (OR 2.9; 95% CI 1.1-7.6; p=0.03). Early Tamiflu treatment during the first two days after illness onset date was associated with lower mortality compared to treatment of more than two days, (OR 9.1; 95% CI 1.1-80.1; p= 0.02).

Conclusions: fifty percent mortality was found in confirmed human cases of influenza A/H5N1 virus in Vietnam from 2003-2012. A delay in hospital admission was associated with higher mortality. Tamiflu treatment during within the first two days from onset decreased mortality from A/H5N1 virus. We conclude that early hospital admission as well as early treatment by Tamiflu is life-saving for cases infected with influenza A/H5N1 virus.

P4

New Therapeutic Agents Against Influenza Viruses Including the Oseltamivir-Resistant Strains

Jim-Min Fang[1,2], Jiun-Jie Shie[2], Yih-Shyun E. Cheng[2] and Chi-Huey Wong[2]

[1] National Taiwan University, Taiwan, ROC, [2]The Genomics Research Center, Taiwan, ROC

The most effective therapies for the treatment of influenza infections involve administration of oseltamivir (Tamiflu) and zanamivir (Relenza). Both oseltamivir and zanamivir target the viral neuraminidases. These neuraminidase inhibitors are designed to have (oxa)cyclohexene scaffolds to mimic the intermediate of oxonium-like geometry in the enzymatic cleavage of N-acetylneuraminic acid (Neu5Ac, also known as sialic acid), the outmost saccharide on the cell surface glycoprotein for binding with the active site of viral neuraminidase. However, the oseltamivir-resistant strains of influenza viruses have

evolved due to the extensive use of oseltamivir. Though the resistance to zanamivir is still rare, we may also face the problem in treatment of the zanamivir-resistant viral infection. Therefore, new anti-influenza agents are in urgent need, especially global pandemics of influenza may happen in near future.

In the past years, we have explored the phosphonate congeners of oseltamivir and zanamivir, namely tamiphosphor and zanaphosphor, which are more potent against the neuraminidases of avian and human influenza viruses, including the oseltamivir-resistant strains. The phosphonate group is generally used as a bioisostere of carboxylate in drug design. In comparison with carboxylic acid, the phosphonic acid has higher acidity and stronger electrostatic interactions with the guanidine group. The enhanced affinity of these new anti-influenza agents is attributable to the strong electrostatic interactions of the phosphonate group with the three arginine residues (Arg118, Arg292 and Arg371) in neuraminidase. In this conference, we shall present the biologic studies and pharmaceutical development of tamiphosphor, zanaphosphor and analogs.

P5

Avian Immunoglobulin IgY for Control of Influenza and Influenza-Facilitated Pneumococcal Transmission

HJ Park[1], PE Kilgore[1], C Czerkinsky[1], TM Tumpey[2], JM Katz[2], D Briles[3], OL Wijburg[4], and HH Nguyen[1]

[1]International Vaccine Institute, Seoul, Korea, [2]Centers for Disease Control and Prevention, Atlanta, GA. USA, [3]Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL. USA, and [4]Department of Microbiology and Immunology, University of Melbourne, Melbourne, VIC. Australia

Infection with influenza type A virus causes epidemic outbreaks of acute respiratory disease that can spread as pandemics affecting millions of people. Vaccines are the best option to control the disease. However, not all vaccinated individuals respond to vaccines and the protective vaccines are not always available when they are most needed. Passive immunization (the transfer of specific immunoglobulins/Abs to a previously non-immune recipient host) could offer an alternative strategy to prevent and treat influenza, and an additional therapeutic option to antiviral drugs that are limited by widespread drug resistance among influenza virus strains. We have developed an approach using avian immunoglobulin IgY, obtained from egg yolk of immunized hens, for prevention and treatment of influenza. We found that intranasal administration of influenza virus-specific IgY in mice before or after lethal infection with influenza virus prevents the disease or significantly reduces viral replication resulting in complete recovery from the disease, respectively. Importantly, treatment with the influenza virus specific IgY prevented transmission of influenza-facilitated pneumococcal infection that is responsible for excess morbidity and mortality during influenza outbreaks. The findings suggest an affordable and effective approach for control of influenza, and influenza-facilitated pneumococcal infection and transmission.

P6

Burden of Influenza-Related Severe Acute Respiratory Infection at Three District Hospitals in Vietnam, March 2011 to July 2012

Nguyen Hai Tuan[1], Nguyen Thi Thu Yen[1], Tran Nhu Duong[1], Pham Duc Tho[1], Nguyen Phuong Thanh[1], Nguyen Bien Thuy[1], Le Thi Quynh Mai[1], Tran Ngoc Huu[2], Nguyen Thanh Long[2], Bui Trong Chien[3], Trinh Xuan Mai[3], David T. Dennis[4], Do Thuy Trang[5], Bryan K. Kapella[5], James C. Kile[5], Nguyen Tran Hien[1]

[1]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam, [2]Pasteur Institute, Ho Chi Minh City, Vietnam, [3]Pasteur Institute, Nha Trang, Vietnam, [4]DUKE-NUS Graduate Medical School, Singapore, [5]Influenza Program, U.S. Centers for Disease Control and Prevention, Hanoi, Vietnam

Background: the burden of disease due to influenza viruses is not well characterized for hospitalized severe acute respiratory infection (SARI) patients in Vietnam. There is little recognition of the clinical and economic impact, only limited hospital control policies in place, and no strategy for seasonal influenza vaccination. Our study proposes to provide initial data on the burden of human influenza illness in terms of morbidity, mortality and socioeconomic impact at the District hospital level in Vietnam.

Methods: a modified WHO case definition of SARI was used. Adults and children meeting the definition were enrolled at three selected district hospitals in north, central, and south Vietnam. Nasal and throat swabs were collected for influenza virus detection by real-time reverse transcription polymerase chain reaction. A survey was conducted to identify direct and indirect costs, and loss of work days or school absenteeism, were estimated to evaluate the burden of SARI on the patient and family.

Results: from March 2011 through July 2012, of 35,228 hospitalized patients at three district hospitals, 4,775 (13.5%) were identified as SARI. Of 1,185 SARI cases tested, 173 (14.6%) were positive for influenza viruses, including A/H3N2 (44.3%), B (42.2%), pA/H1N1 2009 (12.7%) and A/H3N2 & B (0.6%). Both normal and low white blood cell counts were found in influenza related SARI patients. Of influenza related SARI patients, 18.5% required treatment in ICU. There were no SARI deaths at the three district hospitals. The median hospital stay was five days (IQR 3-6). Of 1,169 survey respondents, 13 (1.1%) reported receiving an influenza vaccination during the previous 12 months. Socioeconomic impact included direct and indirect costs per patient of 1,328,906 VND ($US 63), an average of 6 work days lost and 4 school days lost, and care giver 5 days lost.

Conclusions: we found no deaths due to SARI for any cause in this study. The most frequent influenza viruses identified were A/H3N2 and B. A low rate of influenza vaccination was found in the study population. The costs due to SARI case hospitalization are important to patients with limited resources (per capita monthly income = 1,387,000 VND ($66)), even for only one week of the treatment. This study at District-level hospitals may have under-estimated influenza related SARI burden and impact in Vietnam. Patients with more severe illness may go directly to higher level hospitals, such as Provincial or National, which would likely increase the costs. The study indicates that the burden and socio-economic impact of influenza related SARI is present but low at district level hospitals in Vietnam, and suggests that SARI burden may be better identified at provincial-level hospitals or higher.

P7

Molecular Characterization of A/H1N1pdm/09 in the North, Central and Highland Vietnam, 2009-2010

Hoang Vu Mai Phuong[1], Nguyen Le Khanh Hang[1], Nguyen Thi Kim Phuong[2], Nguyen Co Thach[1], Nguyen Ngoc Linh[1], Le Thi Quynh Mai[1]

[1]National Institute of Hygiene and Epidemiology; [2]Military Hospital 108

Background: the first case of A/H1N1pdm/09 in Vietnam was determined on May 31, 2009, and the first A/H1N1pdm/09 detected by influenza sentinel surveillance network on July 9, 2009, then reported in all sentinels in September 2009. The pandemic H1N1 was predominant to other seasonal influenza viruses during 2009 – 2010 and continuing circulates up to date. In this study, the molecular characterization of A/H1N1pdm/09 viruses circulated in the North and Central of Vietnam 2009-2010 is analyzed in order to monitor genetic mutation related to antigenic changing as well as antiviral resistance in its evolution.

Methods: throat swab sample was collected from suspected cases following the NIS criteria. Typing and subtyping using Onestep RT-PCR Qiagen kit. Isolates from RT-PCR positive samples reached sufficient HA titer (>8) on MDCK cells were selected. Six segments (PB1, PB2, HA, NA, M, NS) were amplified and sequenced (ABI 3130). Phylogenetic trees were analyzed using maximum likelihood methods.

Results: total 43 representatives virus strains were selected for three regions [Northern (31/43), Central (5/43) and Highland (7/43)] in Vietnam. During 2009-2010, the HA gen was diverged in to 2 groups (I & II), and genetic different between group II was found, it may introduced for 4 sub-group (IIA, IIB, IIC, IID).The maximum likelihood tree of HA showing the alternate appearance of virus from Vietnam and those from China, Thailand and Taiwan. The mutations related to changing in receptor binding have not been found.

The NA gene is clustered in same group that corresponding with group 2 of HA gene. No mutation related to antiviral resistance was found.

All A/H1N1pdm09 isolates showed mutation referred to amantadine resistance on M gene (S31N). Two other amino acids changed on M protein indicate the conservative characterization of M gene.
PB1, PB2 and NS gene showing a similar phylogenetic tree.

Conclusion: total 43 A/H1N1pdm/09 virus strains circulated during 2009-2010 in the North, Central and Highland Vietnam was molecular characterized. HA gen was diverged in to 2 groups; NA gene is clustered in one group corresponding with group 2 of HA gene. No mutation related to changing in receptor binding and antiviral resistance was found.

P8

The Higher Mortality Rate of ARDS Associated with Viral Infection - From the Prospective Study Cases of PICU in NHP-Hanoi

Shoji Kawachi[1, 2], Noriko Nakajima[1], Tatsuya Okamoto[1, 2], Hiroyuki Nunoi[4], Luong Thi San[3], Nguyen Trung Thuy[3], Nguyen Thanh Liem[3], Kazuo Suzuki[5]

[1]National Institute of Infectious Diseases, Tokyo, Japan; [2]National Center for Global Health and Medicine, Tokyo, Japan; [3]National Hospital of Pediatrics, Hanoi, Vietnam; [4]University of Miyazaki, Miyazaki, Japan and [5]University of Teikyo, Tokyo, Japan

Background: clinically, ARDS associated with H5N1 avian influenza virus (H5N1) infection is so severe compared as general ARDS that we named it Fulminant-ARDS (FARDS) (Kawachi, et al. JID 2009). It is an urgent mission to elucidate the mechanism of FARDS and to develop a therapeutic strategy.

Methods: we have been co-operating the prospective study for FARDS with National Hospital of Pediatrics–Hanoi (NHP-Hanoi) from October 2007. The entry criteria are as follows; 1. The patients who admitted the PICU-NHP with ARDS under intra-pulmonary reason (pneumonia), and needed mechanical ventilation. 2. The patients aged over one month. 3. P/F ratio (PaO2/FIO2 ratio) ≤ 100 mmHg in arterial blood gas analysis during the stay in the PICU. Serum, tracheal lavage fluid and/or nasopharyngeal aspirate samples were collected on admission. Viral genomes were examined by PCR or RT-PCR method with TLF/NPA samples of each patient. In 55/84 patients we tried 2 gram /kg gamma globulin therapy after informed consent. All patients were tracheal intubated and mechanical respiratory support was performed basically according to the AECC strategy (1998).

Results: eighty-four patients were diagnosed as FARDS matched in the criteria of prospective study. In 30 patients among 84, the following viruses were detected; 8 cases: Influenza viruses: H5N1 (3), A(H1N1)pdm09 (4), H3N2 (1) and 22 cases: Rhinovirus(11), Adenovirus (3), CMV (4), RSV (3), Measles (1). In the other 54 patients, viral genomes were under detection limit. . No differences were observed in gender, age or body weight between viral genome positive and negative groups (viral genome positive: M:F=12:18, age 0.33 years, weight 4.50 kg; viral genome negative: M:F=20:34, age 0.25 years, weight 4.65 kg.) There are significant differences only in survival time (days) (viral genome positive: 24.85 vs. viral genome negative: 42.25) and survival probability (p=0.079 by log-lank test, p=0.0129 by Wilcoxon test).

Conclusions: in Vietnam, several viral infections occurred severe ARDS and the mortality rate of ARDS associated with viral infection was much higher than the others (Viral: 67%, non-Viral 31%, p = 0.0019 by Mann-Whitney's U test). These results reinforce the importance of further research on the etiology of viral infection induced FARDS, especially H5N1. Our study will provide the crucial clinical information for development of the strategies of future therapeutic options.

P9

A Comparison of the Clinical Presentation and Outcomes of Severe Influenza Infections with Different Virus Subtypes

Win MK[1], Lee LK1, Gan V[1], Leo YS[2]

[1]Department of Clinical Epidemiology, [2]Department of Infectious Diseases, Tan Tock Seng Hospital, Singapore

Objective: since February 2011, the Department of Clinical Epidemiology at Tan Tock Seng Hospital initiated surveillance of patients admitted with a positive influenza test by reverse-transcriptase polymerase chain reaction (RT-PCR) as part of a nationally mandated programme to monitor severe influenza cases at all hospitals.

Methods: we prospectively tracked all influenza PCR positive patients admitted from 1st Feb 2011 to 29th Feb 2012. Data were collected by case note review and all samples were subtyped.

Results: a total of 761 patients with influenza were admitted within the 13-month period, with 467 (61.4%) positive for influenza A/H3, 220 (28.9%) influenza B, 46 (6%) influenza A/H1N1-2009 and 28 (3.7%) influenza A subtype undetermined (A/ND) respectively. Of these, 53 (7%) were severely ill patients (51% with A/H3; 23% with B; 19% with A/H1N1; 7% with A/ND respectively) required Intensive Care and/or died. The median age of these severely ill patients with influenza were 74 for A/H3; 65 for influenza B; 57 for A/H1N1 and 66 for A/ND (p=0.089). Of these patients, 23% had chronic respiratory problems. Fifty-five percent had more than two underlying medical conditions. Most common diagnoses at admission were respiratory problems (36%) followed by sepsis (32%). Twenty-nine patients (55%) required mechanical ventilation. The difference in the proportion of severely ill patients aged 65 years or older with A/H3 (21/27; 78%), B (7/12; 58%), A/H1N1 (3/10; 30%) and A/ND (2/4; 50%) was statistically significant (p=0.044). There were no significant differences between the influenza subtypes with regards to gender, race, co-morbidities, the reasons for admission, need for mechanical ventilation, length of stay, or death. Of these 53 patients, 25 died (47%). There were no significant differences between those who died or survived in terms of gender, race, underlying medical conditions, the reasons for admission, flu subtypes and length of stay. However, age 65 years or older was an independent predictor (Adjusted odds ratio, 7.0; 95%CI, 1.9-25.8; p=0.003) of death after adjusting for gender, race and flu subtypes.

Conclusions: we documented co-circulation of the different sub-types of influenza virus in tropical Singapore. There were no distinct characteristic differences among the various sub-types. However, severely ill patients aged 65 years or older was an independent predictor of fatality.

P10

Epidemiology of Severe Influenza Illness in a Singapore Hospital

R. Sridhar[1], GK. Goh[1], I. Venkatachalam[1]

[1]Epidemiology Unit, Department of Medicine, National University Hospital, Singapore

Objectives: influenza surveillance in Singapore is a part of the emergency preparedness for SARS affected countries recommended by the World Health Organization. Being in close proximity to countries where the highly pathogenic avian influenza is endemic in poultry and human cases are reported sporadically, an influenza pandemic in Singapore is a significant threat and hence the need for ongoing surveillance.

Methods: data collection for all laboratory confirmed cases of influenza is part of a broader infectious diseases surveillance carried out by the Epidemiology Unit of the National University Hospital, a 1000 bed tertiary teaching hospital. Data, obtained from the hospital information systems and laboratory, on inpatients with influenza between January 2011 and July 2012 were analyzed using the statistical software STATA 12. Admission to the intensive care unit (ICU) was a surrogate marker of illness severity. Logistic regression was used for risk estimation of association with severe influenza. The variables included in the final multivariable logistic regression model were based on pre-existing literature and the Akaike criteria for model selection.

Results: 180 inpatients were diagnosed with influenza during the study period. 93 (51%) were female and median age was 30 years (range: 0.2 to 82 years). 28 (16%) patients were admitted to ICU. They were older (median age 46 years versus 23 years, p=<0.01) and more likely to be female (20 (71%) versus 73 (48%), p=0.02). Influenza A was the dominant strain in the ICU cohort (27 (96%) versus 120 (78%), p=0.02). H1N1 2009 influenza was equally distributed in the two groups (14 (50%) versus 60 (39%), p=0.20). In the ICU cohort, 13 (7%) patients required ventilatory support and 2 (7%) died. Death was more common amongst patients admitted to ICU (2 (7%) versus 0 (0%), p=0.02). On the multivariable regression model, age (OR: 1.02, 95% CI 1.01-1.04, p=0.01) and female sex (OR: 1.16, 95% CI 1.00-6.23, p=0.05) were

significant risk factors for severe infection while influenza A was not (OR: 6.65, 95% CI 0.81-49.62, p=0.08) (H-L goodness of fit=0.31).

Conclusions: seasonal influenza causes significant morbidity and mortality particularly amongst the elderly and female population. Vaccination of high risk groups is an important public health intervention in the management of this illness.

P11

High Mortality Associated With Pandemic Influenza A(H1N1)pdm09 in Children in Pakistan

Asad Ali[a], Fatima Aziz[a], Nida Akhtar[a], Shahida Qureshi[a], Kathryn Edwards[b], Anita Zaidi[a]

[a]Department of Paediatrics and Child Health, Aga Khan University, Karachi, Pakistan
[b]Division of Pediatric Infectious Diseases, Vanderbilt Vaccine Research Program, Vanderbilt University, USA

Objective: during the 2009-10 Influenza A(H1N1)pdm09 pandemic season, Pakistan reported only 262 laboratory confirmed cases with 29 deaths (all ages) as a result of this virus. This is likely an under- estimation, given the inadequate surveillance system and limited diagnostic facilities to detect this virus in both public and private sector facilities. In the first of its kind study, we sought to determine the frequency and case fatality rate of Influenza A(H1N1)pdm09 infections in children hospitalized with acute respiratory illness at a tertiary care hospital in Karachi, Pakistan.

Methods: children less than 5 years old admitted with acute respiratory illnesses to the Aga Khan University Hospital, Karachi from 17thAugust 2009 to 16thSeptember 2011 were enrolled and tested for influenza A(H1N1)pdm09 using real-time reverse transcriptase polymerase chain reaction (RT–PCR).

Findings: of the 812 children enrolled, A(H1N1)pdm09 virus was detected in 27 (3.3%) children. The proportion of A(H1N1)pdm09 positive cases varied from 0% to 15% in different months. The admission diagnosis of children with A(H1N1)pdm09 included pneumonia (48%), bronchiolitis (17%), upper respiratory infection (11%), asthma exacerbation (7%), and one case each (3%) of febrile seizure, urticaria, myocarditis, hemolytic uremic syndrome, and suspected Barter syndrome. Case fatality rate for children with A(H1N1)pdm09 was 15%. Children with A(H1N1)pdm09 were 5 times more likely to be admitted or transferred to the intensive care unit (ICU) (95% CI 1.8-13.6), 5.5 times more likely to be intubated (95% CI 2.0-15.0) and 12.9 times more likely to die (95% CI 4.2-39.3) as compared to children testing negative for A(H1N1)pdm09.

Conclusion: A(H1N1)pdm09 infections were associated with high rates of complications, including death in children in Karachi, Pakistan. Given the high case fatality rate and a< 5 year old population of > 18 million in Pakistan, it is likely that even with the relatively low attack rate, A(H1N1)pdm09 led to much higher number of pediatric deaths than what has been reported.

P12

Virological Surveillance of Influenza Like Illness and Severe Acute Respiratory Infection in Cameroon, 2007-2012

R Njouom[1] for the Cameroon Network of Laboratories for influenza surveillance

[1]National Influenza Centre, Centre Pasteur of Cameroon, Yaounde, Cameroon

Objective: to detect and characterize influenza viruses circulating in Cameroon through a sentinel surveillance system for ILI (influenza like illness) and SARI (Severe Acute Respiratory Infection) program in five years since November 2007.

Methods: all ILI and SARI cases were considered eligible for enrolment. A standardized questionnaire to record patients' demographic characteristics and medical history was used. Nasopharyngeal swabs were collected from all enrolled cases in different surveillance sentinel sites and then sent to the laboratory for biological confirmation. Detection and subtyping of influenza viruses were performed by real time RT-PCR using the CDC protocol. Patient information and laboratory results were recorded in a central database (MS Access®) situated at the CPC. Statistical significance was assessed at $p<0.05$ for all parameters.

Results: we collected and tested a total of 2731 respiratory specimens from November 2007 to June 2012. Of these, 2557 (93.6%) were from ILI patients, and 174 (6.4%) were from SARI patients which were obtained during a short period (October 2011 to June 2012). Among ILI patients, 558 (21.8%) tested positive. Overall, influenza viruses were detected in 34.8%, 29.1%, 26.8%, 24.8%, 20.7%, 3.1% ILI cases in late 2007, 2008, 2009, 2010, 2011 and early 2012, respectively. Of the 174 SARI cases, only 12 (6.9%) tested influenza positive and the majority of patients were less than 5 years of age (75%). During the same period of surveillance, the difference between ILI and SARI system for influenza detection was significant ($p < 0.05$) with highest detection among ILI than SARI patients. During this period, influenza infections were observed mainly during rainy season from September to November. However, in 2008 influenza activity was observed throughout the year and in 2009-2010, some influenza activities were observed out of this period due to the pandemic context.

Conclusion: we document that influenza is responsible for both mild and severe respiratory illnesses in Cameroon. We were able to identify severe influenza infections among hospitalized patients paralleling the same seasonality as observed within the ILI surveillance. To our surprise, few cases of influenza were detected among hospitalized patients with SARI. It is plausible that bacterial and/or other respiratory viral infections are involved. Further testing are on-going to clarify this situation.

P13

Establishment of Severe Acute Respiratory Infections Sentinel Surveillance System in Armenia

A.Vanyan[1], N.Bakunts[2], L.Avetisyan[3], L.Torosyan[4], Sh.Sargsyan[5], A.Hayrapetyan[6]

[1,2,3,4]State Hygiene and Anti-Epidemic Inspectorate, MOH, Armenia [5]Center for Disease Control and Prevention, MOH, Armenia, [6]National TB Control office, MOH, Armenia

Background: The State Hygiene and Anti-Epidemic Inspectorate, MOH, is responsible for infectious diseases surveillance and response, including outbreak investigations. Back in 2005 diagnostic facilities were limited at the primary level. The national laboratories did not have the diagnostic capability, the required bio-safety level facilities for influenza virus isolation and sub-typing, or the equipment to perform PCR testing.
The protocols and guidelines for the surveillance and response to an influenza outbreak were not developed. Laboratory and epidemiology capacity to collect virology and epidemiology data for influenza, including appropriate samples and viral isolates were in need to be upgraded.
Objectives: development of sentinel surveillance system and laboratory capacity for influenza.

Material and Methods: a list of projects was implemented in cooperation of WHO, World Bank, USAID and CDC aiming at development of sentinel surveillance and introduction of laboratory detection of influenza.

Results: the sentinel surveillance standard and guidance materials were developed based on the WHO and CDC guidelines, including standards of practice, recording and reporting forms.
Sentinel sites were selected in Yerevan, Lori and Syunik. In each site, virological laboratory capacity was strengthened by introducing PCR methodology. Laboratories were renovated, fully equipped with equipment and reagents, laboratory personnel were trained.
In each site medical facilities were selected, the scheme for collecting and transporting samples were developed, doctors and nurses were identified and trained according to sentinel standard, including sampling technique. Necessary primers and probes for seasonal influenza viruses were provided by CDC US and WHO.
The regular sentinel surveillance of severe acute respiratory infections started since 2009, national Influenza lab included in the WHO external QA program.
Armenia actively participates in WHO global influenza surveillance by sending aggregate influenza testing data to EURO FLU platform weekly, published on www.euroflu.org.

Conclusions: complementary activities of different projects lead to successful introducing of sentinel SARI surveillance in Armenia. It has a good representation of age groups, gender, ethnicity, socio-economic status and risk factors/medical conditions and provides up-to-date, routinely collected, standardized data on influenza. This is a sound system for monitoring severe disease, risk factors,

disease burden and providing timely data on priority groups to policy-makers.

P14

Improvement of the Information System for Influenza, ARI and SARI Surveillance in the Republic of Moldova

Dr.Radu Cojocaru Dr.Stela Gheorghita, Dr.Natalia Caterinciuc, Prof.Constantin Spinu, Biol.Veronica Eder, Dr.Igor Spinu, Dr.Petru Scoferta

National Center of Public Health, Ministry of Health, Chisinau, Republic of Moldova

Background: an influenza pandemic has greater potential than any other naturally occurring infectious disease event to cause large and rapid global and domestic increases in deaths and serious illnesses.

Methods: clinical, epidemiological and virology surveillance of influenza, acute respiratory infections (ARI) and severe acute respiratory infection (SARI) in the Moldova is made in according to criteria recommended by EuroFlu, such as: geographical spread, intensity and trend of the epidemic process, impact on health services, the dominant type/subtype of virus.

Results: during the epidemic season weeks 40/2011-30/2012 there were sporadic cases of influenza, caused mainly by influenza A(H3N2), with a low intensity of the epidemic process and minimal impact on medical services. Totally, during this season were registered 227 (6370/0000) influenza cases, which show a reduction of 20.0 times in morbidity over the same period of the previous season.
ARI morbidity ranged from 50.4 0/0000 (week 52/2011) to 179.3 0/0000 (week 11/2012), falling below the epidemic threshold (187.00/0000), peaking (201.9 0/0000) at week 12/2012. From week 13/2012 morbidity has been decreasing. This season were registered 139 964 (3929.6 0/0000) ARI cases, which represents a reduction of 1.2 times in morbidity compared with the previous season.
SARI morbidity ranged from 10.8 0/0000 (week 40/2011) to 48.30/0000 (week 3/2012) subsequently decreases successively and continuously until the end of the season. During this time had been recorded 36,932 (1036.9 0/0000) cases of SARI, that represent an increasing of 1.7 times in morbidity in comparison to the previous season.

Conclusions: improvement of the information system for influenza, ARI and SARI surveillance connected to the WHO, ECDC requirements has allowed us to monitor the epidemiological situation in these infections, appreciation of the epidemic process trend and the spread forecast with developing control and response measures according to the situation. SARI morbidity served as an argument for extending the range of population on increased risk quotas for influenza immunization for the season 2012-2013 with vaccine recommended by WHO.

Acknowledgement: CDC Cooperative Agreement Number: 1U51IP000329.

P15

Influenza A(H1N1)pdm09 and Other Respiratory Infections Among Fatal Cases in Women at Reproductive Age – Preliminary Findings

Maria de Lourdes Aguiar Oliveira[1]; Sharon Carney[1]; Roger Rohloff[3]; Renato Medeiros[1]; Valdiza Valente[2]; Valeria Saraceni[2]; Glaucia Paranhos-Baccala[4]; Marilda M. Siqueira[1]

[1]Laboratory of Respiratory Viruses, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil; [2]Health Secretariat from Rio de Janeiro, Brazil; [3]Municipal Hospital Fernando Magalhães, Rio de Janeiro, Brazil; [4]Emergent Pathogens Laboratory, Merieux Foundation, France

Acute respiratory infections during pregnancy are an issue of Public Health concern, due to the higher chance of clinical progression to severe disease/death and neonatal complications. In this study, we investigated the epidemiological and virological features of fatal cases among pregnant and non-pregnant women from Rio de Janeiro (June/2009-june/2011), information still scarce in Brazil. The study population was composed of 741 women at reproductive age (15-44 years) from which, 38 progressed to death. Demographic/clinical/epidemiological information was assessed by the Epidemiological Surveillance team, using a nationally standardized questionnaire. After nucleic acid extraction, influenza and other respiratory pathogens were detected by real time RT-PCR (FTD Respiratory Pathogens 21plus, Fast-Track Diagnostics). Descriptive

and bivariate analyses (chi-square/Fisher's exact test and t-test for means) and multiple logistic regressions (taking into account variables of epidemiological relevance/plausibility) were carried out and considered significant when p<0.05. Influenza A(H1N1)pdm09 was detected in 23 (60.5%) and 300 (42.7%) from fatal and non-fatal cases, respectively (p=.024). From these 23 fatal cases, 13 women were pregnant. Among confirmed Influenza A(H1N1)pdm09 cases, those who died presented lower per capita income (p<.001), higher frequencies of dyspnea (p=.003), hospitalization (p<.001), co-morbidities (p=.025) and a higher interval between the 1st. symptoms and hospitalization (p<.001). The last, a risk determinant for deceases among infected pregnant women (n=5.1±3.9 vs. 2.5±3.2 days, p=.012). Among the infected non-pregnant women, the presence of any co-morbidity was identified as an independent risk factor for death (AOR=18.5). From 38 fatal cases, 6 samples remained negative for all pathogens. Influenza A(H1N1)pdm09(n=5), influenza A(n=2); H.influenzae(n=4); S.aureus(n=2), M.pneumoniae(n=1) and S.pneumoniae(n=1) were the solely pathogen detected in 15 women. Multiple infections were detected in 17 cases, mostly associated with detection of virus+bacteria (n=12). These preliminary findings reinforce the lethal role of Flu infections - especially among those with underlying disease - and highlight the usefulness of a syndromic approach for elucidation of SARI/fatal cases.

Financial support: Science and Technology Department (DECIT), MoH and National Counsel of Technological and Scientific Development (CNPq), Brazil.

P16

Epidemiological Patterns of Influenza A(H1N1)pdm09 Infections Among Women of Reproductive Age from Rio de Janeiro, 2009-2011

Maria de Lourdes Aguiar Oliveira[1]; Renato Rodrigues Medeiros[1]; Valdiza Valente[2]; Valeria Saraceni[2]; Roger Rohloff[3]; Sharon Marie Carney[1]; Marilda Mendonça Siqueira[1]

[1]Laboratory of Respiratory Viruses, Oswaldo Cruz Foundation, Fiocruz, Rio de Janeiro (RJ); [2]Health Secretariat from Rio de Janeiro, RJ; [3]Municipal Hospital Fernando Magalhães, RJ, Brazil

Influenza infections are a major public health issue, due to their relevant morbimortality and economical impact. Although pregnant women are under a higher risk for severe outcomes and death, this scenario is still poorly explored in Brazil. In this investigation, we assessed the clinical and epidemiological features of Influenza A(H1N1)pdm09 infections among 845 women of reproductive age (15-44 years) from Rio de Janeiro municipality, from June/2009-June/2011.

Demographic/clinical/epidemiological information was assessed by the Epidemiological Surveillance team, using a nationally standardized questionnaire. After RNA extraction, Influenza A detection was carried out using CDC real time RT-PCR protocol. Descriptive/bivariate analyses (chi-square/Fisher's exact test and t-test for means) and multiple logistic regressions (taking into account variables of epidemiological relevance/plausibility) were performed and considered significant when p<0.05. Influenza A(H1N1)pdm09 was detected in 43.1% (n=326; 185 pregnant and 141 non-pregnant women), with a total of 41 deceases (4.4% vs. 5.7%, in each group, respectively). The epidemic peak occurred at epidemiological week 30/2009, with a substantial decline after that - only 1 suspected case was confirmed in 2010 (in a non-pregnant women). Infected pregnant women were significantly younger (25.6±6.3 vs. 29.3±8.8; p<.001) and presented significantly higher frequencies of dyspnea (65.9% vs. 51.4%, p=.006), SARI (56.9% vs. 45.7%, p=.030) and hospitalization (82.7% vs. 47.4%, p<.001), suggesting a more severe clinical presentation and a higher demand for health services (OR=5.3; 95%CI 3.1-8.8), when compared to their counterparts. After multiple logistic regressions, Influenza A(H1N1)pdm09 infection among pregnant was independently associated with the progression to a fatal outcome (AdjOR=3.7; 1.1-12.3); tabagism (AdjOR=3.1; 1.2-7.6) and presence of fever (AdjOR=2.2;1.1-4.4). Moreover, the chance of viral infection inversely decreased with age (AdjOR=0.96; 0.93-0.99). Our findings reinforce the impact of Influenza infections during pregnancy and highlight the benefits of vaccination for this exposure category. This information is pivotal to tailor health policies directed to prevention/control of Influenza infections, in the scope of the National Program for Influenza Surveillance.

Financial support: Science and Technology Department (DECIT), MoH and National

Counsel of Technological and Scientific Development (CNPq), Brazil.

P17

The Magnitude of the 2009 Pandemic H1N1 Influenza Virus Infection Among Schoolchildren in Areas with Various Population Densities in Taiwan

Wei Liu[1], Pui-I Ho[1], Zheng-Rong Tiger Li[1], Shiou-Pin Lin[1], Chwan-Chuen King[1]

[1]Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan, Republic of China

In March of 2009, fatal Influenza human cases had alarmed in Mexico. Within a short period, the 2009 pandemic influenza A (H1N1) virus [pdmH1N1] spread in many countries worldwide. Since the pdmH1N1 flu virus is a re-assortment influenza virus, most people have no pre-existing antibodies against it. Therefore, it offers the best opportunity to investigate the factor related to the infection, transmission and severity of the epidemic due to the clear antibody background titer. Moreover, many studies indicated that school children played an important role in spreading the pdmH1N1 virus with higher titers of virus and longer period of shedding virus during infection. In addition, school as a high population density place might also facilitate transmission of the virus. However, there were few studies supported whether different population density might lead to the different severity of epidemic. Accordingly, we would like to address this issue by sero-epidemiology studies among schoolchildren.

To explore the relationship between the population density and severity of the flu epidemic, we conducted a sero-epidemiology study by choosing schools in Taipei and Kaohsiung City to take blood from 765 and 901 schoolchildren, respectively and testing for their serotiters of anti-influenza hemaggultination inhibition (HI) antibody. To investigate the other potential confounding factors, we obtained the students' demographic characteristics, activity at schools and health related information through questionnaires. Student's t and chi-square tests were used to analyze the data. Furthermore, we compared the data between two flu season (2009-2010 and 2011-2012) to investigate possible changes in the anti-influenza HI antibody prevalence.

The results showed that the schoolchildren in Taipei had significantly higher seroprotection rate of anti-pdmH1N1 HI antibody (serotiters >1:40) than those in Kaohsiung [42.61% (326/765) vs. 23.16% (104/449) ,$p<0.05$], and Taipei a capital city in Taiwan has more than seven folds of population density comparing to Kaohsiung. Besides, our preliminary results of seroprotection rate in 2011-2012 in Taipei was higher than those in 2009-2010 flu season. [69.40% (161/232) vs. 42.61% (326/765)].

These results provide scientific evidence of the relationship between population density and the severity of flu epidemic and an overview of the prevalence of seroprotection against pdmH1N1 virus among schoolchildren. This would help government make more effective policies in vaccine allocation and disease prevention for next pandemic with emerging novel influenza viruses.

P18

Clinical, Epidemiologic and Virologic Characteristics of 2009 Pandemic Influenza A (H1N1) Cases in Taiwanese Patients Located in Different Climate Zone and International Perspective

Kuan-Ying Chu[1], Chuan-Liang Kao[1], Ta-Chien Chan[2], Chia-Kun Jasper Chang[1], Yea-Huei Shen[5], Shu-Fang Chuang[1,3], Chu-Han Tsai[1], Luan-Yin Chang[2] , Li-Min Huang[2], Ping-Ing Lee[2], and Chwan-Chuen King[3]*

[1]Dept of Clinical Laboratory Sciences & Medical Biotechnology,[2]Dept of Pediatrics, College of Medicine, [3]Institute of Epidemiology and Preventive Med, College of Public Health, National Taiwan University (NTU),[4]Dept. of Laboratory Medicine, NTU Hospital, [5]Dept. of Internal Medicine, Yuan General Hospital, Taiwan

Meteorological factors are very important to facilitate efficient transmission of influenza viruses. In general, outbreaks of seasonal influenza in Taiwan start from autumn and peak in winter season of every year. And it is interested to understand whether the emerging 2009 H1N1 influenza virus (pdm H1N1/09) would behave the same as seasonal influenza viruses. The specific aims of this study were: (1) to investigate whether different daily

meteorological factors would be associated with different patterns of ILI cases in two metropolitans with various climate zones (subtropical vs tropical); (2) to evaluate clinical and epidemiological characteristics of the laboratory-confirmed pdm H1N1/09 influenza cases in Taipei and Kaohsiung Cities, and (3) to examine the survival of the pdm H1N1/09 virus at different temperatures.
Epidemic curve of the pdm H1N1/09 showed two waves occurred in both Taipei and Kaohsiung in 2009. The ILI patients who showed positive results on the Quidel OuickVue Influenza A+B rapid test were further confirmed by RT-PCR. Using daily laboratory-confirmed influenza cases, we found these cases had strong correlation with mean temperature, maximum temperature, water pressure and relative humidity with 1-2 lag days.
However, the influenza transmission would be affected the existence of live virus particles. Therefore, we chose the human seasonal H1N1 influenza virus and the pdm H1N1/09 virus to observe the survival curve. the pdm H1N1/09 virus may keep more live virus particles at room temperature and can spread fast in the summer season in areas of tropical zones.

P19

Hospital-based Influenza Surveillance in Korea: Hospital-based Influenza Morbidity & Mortality (HIMM) Study Group

Joon Young Song[1,2], Hee Jin Cheong[1,2], Jin Soo Lee[3], Seong-Heon Wie[4], Young Keun Kim[5], Won Suk Choi[1,2], Jacob Lee[6], Hye Won Jeong[7], Woo Joo Kim[1,2]

[1]Division of Infectious Diseases, Department of Internal Medicine, Seoul, Korea,[2]Asian Pacific Influenza Institute (APII), Seoul, Korea,[3] Division of Infectious Diseases, Department of Internal Medicine, Inha University School of Medicine,Incheon, Korea,[4] Catholic University Medical College, St. Vincent's Hospital3, Suwon, Korea,[5] Division of Infectious Diseases, Department of Internal Medicine, Yonsei University, Wonju College of Medicine, Wonju, Korea, [6] Division of Infectious Diseases, Department of Internal Medicine, Hallym University College of Medicine, Chuncheon, Korea,[7] Division of Infectious Diseases, Department of Internal Medicine, Chungbuk National University College of Medicine, Cheongju, Korea

Background: influenza epidemic is repeated annually with variation in size and severity. For the early detection and management of influenza outbreak, effective surveillance system is essentially required.

Methods: hospital-based Influenza Morbidity & Mortality (HIMM), a hospital-based influenza surveillance system was established to monitor the trend of influenza epidemic and its severity, which was composed of two surveillance system: emergency room (ER)-based and inpatients-based surveillance. ER influenza-like illness (ILI) index was defined as ILI cases per 1,000 ER-visiting subjects, and its change was monitored weekly. Number of laboratory-confirmed cases and distribution of influenza types were also estimated serially. Inpatient-based surveillance included monitoring for the hospitalization, complication and mortality.

Results: ER ILI index correlated well with the number of laboratory-confirmed influenza, and it showed bimodal peak at week 4 (179.2/1,000 ER visits) and weeks13-14 (169.6/1,000 ER visits). Influenza A was predominant during first epidemic peak, while influenza B was exclusively isolated during second peak after week 10 of 2012. In 2011-2012 influenza season, mean admission rate of ER-ILI patients was 16.3% (weeks 52-20) without any increase over the epidemic periods. Among the hospitalized patients with influenza, 33.6%(41 among 122 patients) were accompanied by complications, and pneumonia (28.7%, 35 among 122 patients) was the most common. Most fatal cases were caused by influenza A (96.2%) later than first epidemic peak.

Conclusions: HIMM was effective to monitor the trend of circulating influenza activity and its severity at the same time. In 2011-2012 season, influenza epidemic persisted over ≥5 month periods with bimodal peak of influenza A and B in sequence. Overall, 2011-2012 seasonal influenza was mild, particularly regarding influenza B.

P20

Hospital-based Influenza Morbidity and Mortality: Correlation with Korean Influenza Surveillance Scheme (KISS)

Yu Bin Seo[1,2], Hee Jin Cheong[1,2,] Jin Soo Lee[3], Seong-Heon Wie[4], Young Keun Kim[5], Won Suk Choi[1,2,] Jacob Lee[6], Hye Won Jeong[7], Woo Joo Kim[1,2]

[1]Division of Infectious Diseases, Department of Internal Medicine, Seoul, Korea,[2] Asian Pacific Influenza Institute (APII), Seoul, Korea,[3] Division of Infectious Diseases, Department of Internal Medicine, Inha University School of Medicine, Incheon, Korea,[4] Catholic University Medical College, St. Vincent's Hospital[3], Suwon, Korea,[5] Division of Infectious Diseases, Department of Internal Medicine, Yonsei University, Wonju College of Medicine, Wonju, Korea,[6] Division of Infectious Diseases, Department of Internal Medicine, Hallym University College of Medicine, Chuncheon, Korea,[7] Division of Infectious Diseases, Department of Internal Medicine, Chungbuk National University College of Medicine, Cheongju, Korea

Background: established in 2011, the Hospital-based Influenza Morbidity and Mortality (HIMM) surveillance system, which consists of seven tertiary-care teaching hospitals, initiated an emergency-room-(ER) based influenza surveillance system in Korea. During the 2011–2012 influenza season, this study assessed the correlation between data generated from HIMM surveillance and the Korean national influenza surveillance system, an integrated clinical and laboratory surveillance network involving public health centers and private clinics called the Korean Influenza Surveillance Scheme (KISS).

Methods: this study was conducted over a 37-week period (from the fourth week of September 2011 through the fourth week of June 2012). We collected influenza-like illness (ILI) indices on a weekly basis from the HIMM surveillance system, for which an index was defined as ILI cases per 1,000 ER visits. The index for the KISS was defined as ILI cases per 1,000 visits at public and private health facilities located in the nation. We compared the correlation of data collected through the HIMM surveillance system and the KISS using cross-correlation analysis.

Results: the ER-ILI index generated from the HIMM surveillance system was correlated with the national ILI index from the KISS (correlation coefficient = 0.887, p-value<0.001). A cross-correlation analysis showed the highest correlation that resulted in a 0-week lag, demonstrating that no substantial lag existed in the HIMM surveillance system. Further, when compared by region, a high data correlation was found in the regions in which the HIMM hospitals were located—Seoul, Gyeonggi, Gangwon, Incheon, and Chungbuk.

Conclusions: the ER-based influenza surveillance system operated by the seven tertiary-care teaching hospitals in Korea shows a strong correlation with the national influenza surveillance system. This high correlation validates its use as a complementary tool.

P21

Multi-site Influenza Surveillance in India: 2009-2012

M S Chadha[1],V A Potdar[1],S Broor[2],C P Gunasekaran[3],M Chawla-Sarkar[4],D Biswas[5], S Shrikhande[6], B Anukumar[7], P Koul[8], A C Mishra[1]

[1]National Institute of Virology, Pune, [2]All India Institute of Medical Sciences, New Delhi, [3]King Institute of Preventive Medicine & Research, Chennai, [4]National Institute of Cholera and Enteric Diseases, Kolkata, [5]Regional Medical Research Centre, Dibrugarh, [6]Indira Gandhi Government Medical College, Nagpur, [7]National Institute of Virology, Kerala Unit, Alleppy, [8]SheriKashmir Institute of Medical Sciences, Srinagar

Objectives: identification and characterization of influenza strains and determination of influenza seasonality in different geographical areas of India.

Methods: epidemiologic data and throat swabs were collected from patients with influenza like illness. Virus isolation was carried out in Madin-Darby canine kidney cells and strains identified by hemagglutination inhibition assay. After June 2009, real-time RT-PCR was used for diagnosis. HA and NA genes were sequenced for phlylogenetic analyses and determination of markers for drug resistance. Meteorological Data were collected.

Results: from January 2009 to May 2009, 140 of 3679 (3.8%) cases yielded isolates. Influenza positivity by r RT-PCR from June 2009 to August 15th 2012 was found to be 15.9% (2549/16015). In 2009, influenza A(H3N2) and p(H1N1), in 2010 p(H1N1) and Type B, in year 2011 influenza A(H3N2) and Type B, in 2012 p(H1N1) and Type B respectively were predominantly circulating.

P22

Establishment of a Sentinel Surveillance System for Severe Influenza – Experiences in England

NL Boddington[1],R Maini[1], R Pebody[1] on behalf of the Health Protection Agency UK Severe Influenza Surveillance System Steering Group
[1]Health Protection Agency, London, UK

Background: the UK Severe Influenza Surveillance System (USISS) is a newly established scheme to collect surveillance data on hospitalised influenza cases from a sentinel network of NHS Acute hospital Trusts in England. The system was developed following post-2009 pandemic recommendations. The system aims to monitor the impact of seasonal influenza on the population and describe the epidemiology of severe disease in time, place and person.

Objectives: to evaluate this new surveillance system.

Methods: the system was evaluated according to the US Centers for Disease Control and Prevention Framework for Evaluating Public Health Surveillance Systems. Quantitative data sources used included data from an independent mandatory ICU surveillance scheme. Qualitative sources included online questionnaires and semi-structured interviews with participating trusts and HPA scientists.

Results: a sentinel group of trusts was recruited for the 2011/12 influenza season using stratified random sampling. The group recruited was largely representative of trusts by region, size and trust type. On average 30 of 36 trusts reported each week. Information collected included aggregate numbers of all hospitalised confirmed influenza cases and detailed epidemiological data including clinical risk factors and antiviral use for all ICU/HDU admissions. Completion rates for these variables were high (80.4% and 76.1% respectively).

The peak number of hospitalisations during the 2011/12 season corresponded with the peak of influenza like illness consultations (per 100,000) as reported by a GP sentinel surveillance system. Most ICU cases were reported to both the mandatory and sentinel, although a number of additional cases were reported to the mandatory scheme only.

75% (n=27) of participating trusts responded to the user feedback questionnaire. 63.0% of users found the system simple and easy to use and 72.0% agreed the level of data requested was reasonable.

Reports to trusts were distributed within a week of reporting, with a time interval between admission and reporting of 3-10 days.

Conclusions: the USISS sentinel system effectively signalled changes in influenza activity over the 2011/12 season. The system had high acceptability amongst users and provided timely and useful information to describe the epidemiology of severe influenza in England.

This system could be used as a model for other countries wishing to establish a system for monitoring severe disease.

P23

Viruses from Fatal Cases of Pandemic Influenza in the First, Second and Third Wave: The Contribution of Viral Evolution

M Galiano[1], PM Agapow[2], C Thompson[1], HK Green[2], R Pebody[2], J Ellis[1], M Zambon[1]

[1]HPA Microbiology Services; [2]HPA Health Protection Services, London, UK

Three waves of activity caused by the 2009 pandemic A(H1N1) virus occurred in the UK over the period April 2009 to March 2011, with reports of increased severe disease in 2010/11. Although the majority of infections were mild, more than 400 confirmed deaths were reported during the first and second wave of the pandemic and more than 500 confirmed deaths during the winter of 2010/11 (third wave).

Objectives: the aims of this study were to determine whether viruses from fatal cases were genetically different from mild community cases and to assess the possible impact of

these genetic changes in the increased severity observed in the third wave through phylogenetic analysis and growth curves.

Methods: we sequenced whole genomes (n=92) of A(H1N1)pdm09 viruses isolated from fatal and mild cases in the UK during the three waves of A(H1N1)pdm09 activity. The concatenated full length coding regions were used to construct a time-calibrated phylogeny using Bayesian methods. Virus replication was evaluated in human airway epithelium cells using representative viruses from each wave. Results: whole-genome sequencing and phylogenetic analysis showed that viruses from fatal cases were not different from mild cases, with no statistical correlation between phylogenetic clustering and clinical outcome and no mutations significantly associated with fatal cases. Two main genetic groups circulated during the third wave, with several signature changes along their genome, mostly in polymerase and haemagglutinin genes. These findings correlate with a sudden increase in the relative genetic diversity of pandemic viruses. Despite this, no significant antigenic drift was detected. Genome-wide changes observed in A(H1N1)pdm09 viruses from the third wave might have improved virus fitness and consequently have an impact on virulence. We compared the in vitro replicative ability of representative viruses from the first, second and third wave of pandemic in human airway epithelium.

Conclusions: these findings are discussed in relation to the evolution of the pandemic virus and within the epidemiological context of fatal cases during the three waves of A(H1N1)pdm09 activity in the UK.

P24

Evolution of Highly Pathogenic Avian Influenza H5N1 Viruses Isolated from Humans in Vietnam, 2003–2011

Nguyen Le Khanh Hang[1], Le Quynh Mai[1], Hoang Vu Mai Phuong[1], Vuong Duc Cuong[1], Nguyen Co Thach[1], Le thi Thanh[1], Pham Thi Hien[1]

[1]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam

Vietnam has been one of the most severely infected countries for HPAI viruses A/H5N1 (CFR of 49.6%). The molecular characterization of HPAI viruses A/H5N1 circulated in Vietnam 2003-2011 is analyzed to identify mutations associated with antigenic drift and shift, antiviral resistance, receptor susceptibility.

Method: human H5N1 isolates from 2003-2011 were characterized by high homology to contemporary poultry isolates from Vietnam in all genes using Sanger sequencing (ABI 3130). Phylogenetic trees were analyzed using Maximum Likelihood (ML) and Bayesian MCMC methods.

Result: phylogenetic analysis of the hemagglutinin (HA) genes of H5N1 viruses isolated revealed multiple introductions of genetically divergent viruses. However, predominant of antigentically are recognized with clade 1 from 2003 to 2005, clade 2.3.4 HA genes (genotypes V and Z) from 2005 to 2010, and recently named H5N1 clade 2.3.2 has become predominant over previously circulating clades since 2010.Only viruses belonging to two clades (1 and 2.3.4) have been recognized as causing human infections in this period. Compared to avian genes, the PB2 gene from a majority of H5N1 isolates from humans had a glutamate to lysine substitution that has been previously associated with enhanced replication of influenza virus in mammalian MDCK cell and animal models. Other mutations previously correlated with reduced susceptibility to antiviral drugs that inhibit neuraminidase or block M2 ion channels were detected on NA and M genes of clade 1 as well as clade 2.3.4 H5N1 viruses. Compared to the clade 2.3.4 H5N1 vaccine candidate virus, A/Anhui/1/2005, the recent isolates from Vietnam showed 5-7 amino acid substitutions in HA1, suggesting the need to monitor the antigenic properties of these viruses.

Conclusion: genotypes of A/H5N1 virus in Vietnam were continued predominance of genotypes Z and V strains. All of the human viruses isolated between 2003 and 2010 belonged to clade 1 and 2.3.4, whiles animal viruses clustered into clade 1, 2.3.2 and 2.3.4. PB2 gene from a majority of H5N1 isolates from humans had a glutamate (E) to lysine (K) substitution that has been previously associated with enhanced replication of influenza virus in mammalian MDCK cell and animal models.

P25

Infection Characteristics of Avian Influenza Virus A/H9 in Shanghai, China between 2008 and 2010

Wang qianli[1], JU Liwen[1], JIANG Lufang[1], JIANG Qingwu[1], Liu Peihong[2], Zhou Jinping[2]

[1]Fudan University, Shanghai, China [2]Animal Disease Prevention and Control Center, Shanghai, China

Background: Avian influenza viruses (AIV) play a key role in the emergence of pandemic strains. H9N2 are currently widespread in chickens, quail, and other poultry in Asia and have caused cases of influenza in humans. To evaluate the potential threat of H9N2 AIV, we analyzed the serological HAI antibody and prevalence of the H9N2 in human and poultry in Shanghai, China during 2008 to 2010. Methods: 2798 human serum samples were obtained from general and occupationally exposure population contacting with avian. The serum samples were detected for HAI antibodies of H9 subtype by HI assays. 1450 nasal and throat swabs specimens were collected from the influenza-like illnesses in sentinel hospital and outbreak, inoculated into MDCK cells to isolate influenza viruses and identified by RT-PCR. 9297 tracheal and cloacal swabs of specimens were collected from poultry in the live-poultry markets, inoculated into chicken embryo to isolate AIVs and identified by RT-PCR; 7 HA segments of AIVs H9 were sequenced to analyze the genetic variation.

Results: the average antibody positive rate was 4.19% (82/1958) of the general population to H9 antigen with HI titers ≥1:20, while 29.64% (249/840) of the occupationally exposure population contacting with avian. 938 influenza viruses were isolated from nasal and throat swabs of the influenza-like illnesses, including H3N2, H1N1 and B. 239 AIVs were isolated from tracheal and cloacal swabs, positive rate is 2.57% (239/9297), all the subtype were H9N2. Genetic analysis revealed that 7 isolates had an RSSR↓GLF motif at the cleavage site of HA, representing low pathogenicity in chicken. The mutation at position 226 (Q-L) at the receptor-binding site was detected.

Conclusion: the results suggesting that avian-to-human transmission of H9N2 AIV was existed, occupationally exposure population contacting with avian had a subclinical infection of H9N2 AIV, but human-to-human transmission was difficult. The mutation at position 226 (Q-L) at the receptor-binding site is thought to be one of the amino acid changes that must occur before AIV can replicate efficiently in humans. But the low pathogenic of the virus cleavage site may limit viral proliferation in human airway epithelial, so H9N2 had not isolated from throat swabs.

P26

Canine Influenza Virus H3N2: Emergence, Transmission and Recombination

Daesub Song[1,2]

[1]Viral Infectious Diseases Research Center, Korea Research Institute of Bioscience and Biotechnology, [2]University of Science and Technology

Interspecies transmission is a crucial feature in the ecology and epidemiology of influenza virus. Transmission of avian influenza virus to a new mammalian species is of great concern, because it potentially allows the virus to adapt to a new mammalian host, cross new species barriers, and acquire pandemic potential. Infection of an entire avian influenza virus to an unrelated mammalian species is a rare event. Recently, avian influenza virus, subtype H3N2, was first isolated from serial cases of severe respiratory disease in dogs exhibiting severe respiratory disease, and transmission among dogs was demonstrated by experimental reproduction of disease. We also demonstrated that dogs have large amount of avian influenza virus binding receptor in canine tracheal, bronchial, and bronchiolar epithelial cells, which suggests potential for direct transmission of avian influenza virus (H3N2) from poultry to dogs.

In this study, we aimed at presenting a novel AI virus causing clinical manifestation in dogs and establishing intraspecies transmission, and genetic characteristics different from equine influenza virus, subtype H3N8 or low pathogenic avian influenza virus, subtype H3N2. Especially, during recent canine influenza surveillance in South Korea, a novel canine influenza virus (CIV) H3N1 which was a putative reassortant between pandemic H1N1 2009 and H3N2 canine influenza viruses was isolated. Genetic analysis for 8 genomes of the influenza virus revealed that novel H3N1

isolate presented high similarities (99.1-99.8%) with pandemic influenza H1N1 except for the hemagglutinin (HA) gene. The HA gene nucleotide sequences of the novel CIV H3N1 was similar (99%) to that of CIV H3N2 isolated in Korea and China. Through the inoculation of the novel CIV H3N1 virus, the novel isolate could induce the clinical signs including fever, cough, and nasal discharges and also presented gross lesions in lungs and histopathologic changes. And experimental infection of seasonal H3N2 influenza virus and pandemic 2009 H1N1 virus in dogs were performed for the validation of roles of dogs as an intermediate vector of influenza viruses.

P27

Detection of Protein-Bound 3-Nitrotyrosin in Plasma from Pediatric Patient with Fulminant ARDS and Avian Influenza Virus Infection

Okamoto T[1,2], Kawachi S[2], Thuy TB Phung[3], Sato Y[4], Nakajima N[4], Nunoi H[5], Sawa T[1], Fujii S[1], San T Luong[3], Liem T Nguyen[3], Suzuki K[6], and Akaike T[1]

[1]Department of Microbiology, Kumamoto University, Japan; [2]National Center for Global Health and Medicine, Japan; [3]National Hospital of Pediatrics, Vietnam; [4]National Institute of Infectious Diseases, Japan, [5]University of Miyazaki, Japan and [6]Safety Control Department, Teikyo University, Japan

Purpose: nitric oxide (NO) and reactive oxygen species (ROS) have been suggested to be involved in the pathogenesis of various diseases 3-Nitrotyrosine (3-NT) produced by NO/ROS is considered as a biomarker for oxidative stress. In fact, we have identified the extensive and NO-dependent formation of 3-NT in the lung of influenza virus-infected mice. Acute respiratory distress syndrome (ARDS) is characterized as an inflammatory lung disease, and associates with enhanced formation of NO and ROS.

Immunohistochemical analyses suggested the formation of 3-NT in the lungs of patients with ARDS. However, biochemical and quantitative aspects of 3-NT formation in ARDS patients remains poorly understood. In this study, we investigated the levels of plasma protein-bound 3-NT from patient with fulminant ARDS by using a reverse phase-HPLC coupled with electrochemical detector (ECD).

Methods: we analyzed plasma samples from 40 patients with influenza-negative ARDS (non IFV-ARDS group) and from 7 patients with influenza-positive ARDS (IFV-ARDS group). Two patients with highly pathogenic avian influenza A (H5N1)-positive, 4 patients with swine pandemic influenza A (AH1pdm)-positive, and 1 patient with seasonal influenza A (H3N2)-positive were enrolled. Plasma samples obtained from 25 patients without ARDS were used as control (non ARDS group). Plasma proteins were precipitated with nitrite-free ethanol/acetate buffer followed by digestion with pronase. 3-NT separated with the HPLC was first reduced at -800 mV to form 3-aminotyrosine, followed by oxidation with second electrode (+200 mV) to detect 3-NT electrochemically. Detection limit of this system was approximately 1 nM for 3-NT. 3-NT levels were standardized by tyrosine levels, determined by ultraviolet detector, connected after ECD.

Results: patients in the IFV-ARDS group had significantly higher 3-NT levels (0.350 μmol/mol) than did those in the non-ARDS group (0.210, $p = 0.046$). 3-NT levels were also significantly higher in the non IFV-ARDS group (0.270, $p = 0.039$) than in the non-ARDS group. Interestingly, the difference was not significant, however, survived patients had higher 3-NT levels than those in non-survivor, and also 3-NT levels was higher in patients without multiple organ failure (MOF) compared with patient with MOF, suggesting a protective role of NO and ROS in the pathogenesis of ARDS.

Conclusions: by this method, we could successfully detect 3-NT from human plasma of ARDS patients. This method is convenient, specific and sensitive for 3-NT quantification applicable for clinical specimens and hence may help further understanding of pathological roles of NO/ROS formation in ARDS**.**

P28

Pathological Study of Formalin-Fixed Paraffin-Embedded Lung Tissues with H5N1 Influenza Infection in Vietnam

Noriko Nakajima[1], Ngo Van Tin[2] Yuko Sato[1,] Hoang Ngoc Thach[2,] Harutaka Katano[1], Pho Hong Diep[2], Toshio Kumasaka[3], Nguyen Trung Thuy[2], Hideki Hasegawa[1],Luong Thi San[2], Shoji Kawachi[4], Nguyen Thanh Liem[2], Kazuo Suzuki[5] and Tetsutaro Sata[1,6]

[1]National Institute of Infectious Diseases, Tokyo, Japan; [2]National Hospital of Pediatrics, Hanoi, Vietnam; [3]Japanese Red Cross Medical Center, Tokyo, Japan; [4]National Center for Global Health and Medicine, Tokyo, Japan; [5]University Hospital, School of Medicine, and General Medical Education Center, Teikyo University, Tokyo, Japan and [6]Toyama Institute of Health, Toyama, Japan

Objective: necropsied or autopsied tissues from 5 fatal cases with highly pathogenic avian H5N1 influenza virus (H5N1) infection were analyzed pathologically to reveal virus distribution and the expression levels of cytokines and chemokines in lungs.

Method: formalin-fixed paraffin-embedded (FFPE) lung tissues of 5 fatal cases were analyzed with several methods as follows: Immunohistochemistry for H5N1 NP antigen, proinflammatory cytokines and chemokines, *in situ* hybridization for H5N1-genomic RNA and mRNA, Double immunofluorescence staining with cell marker proteins, Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) methods for H5N1-RNA, cytokine and chemokine mRNA.

Result: main histopathological findings showed diffuse alveolar damage in the lungs. Myeloperoxidase-positive and/or CD68 (cloneKP-1)-positive neutrophils and monocytes/macrophages infiltrated remarkably in the alveolar septa and alveolar spaces. H5N1 mainly infected alveolar epithelial cells and monocytes/macrophages in lungs. H5N1 mRNA was detected in epithelial cells using *in situ* hybridization. The expression levels of TNF-α, IL-6, IL-8, RANTES and IP-10 correlated with H5N1 RNA copy numbers detected in the same lung region. Double immunofluorescence staining revealed that TNF-α, IL-6, IL-8 and IP-10 were expressed in epithelial cells and/or monocytes/macrophages. In particular, IL-6 was also expressed in endothelial cells. The dissemination of H5N1 beyond respiratory organs was not confirmed in the cases in this study. Further investigation of as many autopsied cases as possible is necessary to elucidate the pathogenesis of H5N1 infection in humans.

P29

Contribution of Influenza NS1 and Myeloperoxidase to Enchanced Sequential Cytokine-Chemokine Induction

Thuy Thi Bich Phung[1,2], Ryuichi Sugamata[8], Kazuko Uno[4], Yasuaki Aratani[5], Keiko Ozato[6], Shoji Kawachi[7], Liem Thanh Nguyen[7], Toshinori Nakayama[8], Kazuo Suzuki[9]

[1]Research Biomolecular for Infectious Disease Department, National Hospital of Pediatrics, [2]Basic Medical Research Department, Research Institute for Child Health, Hanoi, Vietnam; [3]Surgeon Department, National Hospital of Pediatrics; [4]Division of Basic Research, IFN & Host Defence Research Laboratory, Louis Pasteur Center for Medical Research, Kyoto, [5] Department of Genome System Science, Graduate School of Nanobioscience, Yokohama City University, Yokohama, [7]Department of Anesthesiology, National Center for Global Health and Medicine, Tokyo, Japan; and [6] Laboratory of Molecular Growth Regulation, Programs in Genomics of Differentiation, National Institute of Child Health and Human Development, National Institutes of Health, Baltimore, MD, USA; [8]Department of Immunology, Chiba University Graduate School of Medicine, Chiba, Safety Control Department, University Hospital, School of Medicine, General Medical Education Center, Teikyo University[9]

Background: Influenza virus infection causes both recurrent annual epidemics and more serious pandemics that spread rapidly. When the influenza A viruses transmit to epithelial cells, the viruses replicate in epithelial cells, macrophages and leukocytes respond to produce chemokines and cytokines. In addition, myeloperoxidase (MPO) in neutrophils is also associated cellular damage with influenza viral infection. H5N1 infection with acute respiratory distress syndrome (ARDS) starts with high fever, then proceeds to serious respiratory failure.

Methods: in our study, firstly, we focused on the response of cytokines, chemokines and MPO activity in ARDS pediatrics patients infected with H5N1. Secondly, we examined cytokines/chemokines production in A549 epithelial cells infected with influenza A/H1N1 virus (PR-8) or nonstructural protein 1 (NS1) plasmid in vitro.

Results: first, in the plasma, levels of IL-12p40 and TNFR2 in the H5N1-positive group were significantly higher than in the H5N1-negative. In the NPA, the concentration of sIL-6R in the H5N1-positive group was significantly higher than in the H5N1-negative group. In addition, MPO activity in plasma was significantly higher in the H5N1-positive group. These results suggest that sIL-6R may be a major contributor in the nasal space associated with lung injury induced with H5N1 infection. Moreover, IL-12p40 and TNFR2 may be produced from the pulmonary space or endothelial cells to circulate in the blood and MPO may be released into the blood from activated neutrophils to make the lung injury. Second, in vitro study suggests that TNF-α and RANTES were predominantly produced from the A549 epithelial cells infected with PR-8 virus. siTNF-α down-regulated RANTES expression and secretion of RANTES, IL-8, and MCP-1. In addition, siRANTES suppressed interferon (IFN)-γ expression and the secretion of RANTES, IL-8, and MCP-1. Furthermore, administration of TNF-α and RANTES promoted elevated secretion of RANTES, IL-8, and MCP-1 production without the infection, strongly suggesting that TNF-□ may regulate RANTES production followed by increase of IL-6, IL-8, and MCP-1 and IFNs levels in the initial step. In the next step, the cells transfected with viral NS1 plasmid showed production of a large amount of IL-8 and MCP-1 in the presence of the H_2O_2-MPO system, suggesting that NS1 of PR-8 may induce a “cytokine storm” from the epithelial cells when an H_2O_2-MPO system exists. These findings are similar to the result that MPO promotes the development of lung neutrophilia and indirectly influences subsequent chemokine and cytokine production in the lung.

Conclusions: influenza virus infection induces cytokine storm produced in lung epithelial cells, which are associated with MPO and NS1 of influenza virus when the H_2O_2-MPO system acts.

P30

Influenza A(H1N1)pdm09 Virus as the Cause of Lethal Pneumonia in Russia, 2009-2011: Epidemiological, Biological, and Genetic Properties

EI Burtseva[1], VV Lavrischeva[1], ES Shevchenko[1], EV Silujanova[1], TA Oskerko[1], SV Trushakova[1], NV Breslav[1], VT Ivanova[1], MY Schelkanov, IT Fedyakina[1], AG Prilipov[1], SV Alhovsky[1], LV Kolobukhina[1], YN Homyakov[2], DK Lvov[1]

[1]D.I. Ivanovsky Institute of Virology Ministry of Health of the Russian Federation, Moscow, Russia; [2]FGUZ “Plague Control Center” Rospotrebnadzor, Moscow, Russia

The autopsy materials (bronchi, trachea, and lungs) from 161 patients who succumbed to lethal pneumonia caused by influenza A(H1N1)pdm09 virus during 2009-2011 were examined. Most of the cases were registrated in European part of Russia (87%). The mean patient age was 41.2 years (from 3 to 77 years); 52% of the patients were women; 8% were pregnant. Many patients (34.4%) had chronic diseases, mainly metabolic (11.5%), cardiac (8.2%), and renal (9.4%). The mean duration of the illness was 10.5 days (from 2 to 26 days). We used RT-PCR, virus isolation on MDCK and embrionated eggs (EE), Hemagglutiniation Inhibition, sequencing, and in-cell ELISA to study the properties of A(H1N1)pdm09 viruses. 29 strains of A(H1N1)pdm09 were isolated: 23 – on EE (79%), and 5 – on MDCK (21%). Most isolates were characterized as A/California/7/2009-like, the virus used as the influenza A(H1N1) component of the 2009-11 influenza vaccines for the Northern hemisphere. One virus (3.4%) showed reduced titers with antiserum produced against the reference virus. All viruses tested were susceptible to the arbidol and neuraminidase inhibitors, oseltamivir and zanamivir, respectively, and were rimantadine-resistant. We detected mutations in HA1 receptor-binding site in 87 samples [39% of total; 29/42 (69%) samples collected in 2009-2010, and 5/45 (11%) samples collected in 2010-2011] and in 88.5% of 26 strains isolated from these materials. D222G and Q223R were the most common mutations found in 69% and 30.8% of the strains accordingly. Only in 11 of the 61 samples (18%) revealed co-infection of influenza A(H1N1)pdm09 virus and other pathogens, including BoV (1 case), adenovirus (2 cases), parainfluenza (1 case),

rhinoviruses (6 cases) and Streptococcus (1 case). Our investigations were supported by the Centers for Disease Control and Prevention, Atlanta, USA, CoAg: U51IPOOO527-02.

P31

Factors Associated with Severity in Hospitalized Adult Patients with Influenza in Korea, 2011-2012; HIMM (Hospital-based Influenza Morbidity & Mortality) Surveillance

Kyung-Wook Hong[1], Hee Jin Cheong[1], Won Suk Choi[1], Jacob Lee[2], Seong Heon Wie[3], Jin-Soo Lee[4], Young Keun Kim[5], Hye Won Jeong[6] and Woo Joo Kim[1]

[1]Korea University, Seoul; [2]Hallym University, Seoul; [3]The Catholic University of Korea, Seoul; [4]Inha University, Incheon; [5]Yonsei University Wonju College of Medicine, Wonju and [6]Chungbuk National University, Cheongju, Republic of Korea

Objectives: influenza is a representative acute respiratory illness, which causes complications in a small portion. The objectives were to figure out the clinical manifestations of laboratory-confirmed hospitalized adult influenza patients using complications as a determinant of severity.

Methods: retrospective case-control study was done in adult influenza patients who admitted in 7 tertiary care university hospitals in Korea from October 2011 to May 2012. A complicated case was defined as influenza with other organ dysfunction beyond upper respiratory tract, which includes pneumonia, acute renal failure, acute bronchiolitis, ARDS, asthma or COPD aggravation, encephalitis, heart failure aggravation and rhabdomyolysis.

Results: there were 39(31.7%) complicated cases among 123 hospitalized adult patients with influenza. The most common complication was pneumonia (70.6%), followed by acute renal failure (9.8%) and acute bronchiolitis (5.9%). In univariate analysis, factors associated with complications were old age (median, 57 vs. 74, P = 0.005), male sex (P = 0.043), diabetes (P = 0.0003), chronic cardiovascular disease (P = 0.0086), neuromuscular disease (P = 0.025) and having 2 or more chronic medical diseases (P < 0.0001). Complicated patients showed tachypnea (P < 0.0001), crackle (P = 0.001), increased ESR (P = 0.022) and CRP (P = 0.0009), lower oxygen saturation (P = 0.004) more frequently. Multivariate logistic regression analysis revealed that old age (OR 0.97; 95% CI 0.94-0.99; P = 0.015) and having 2 or more chronic medical diseases (OR 11.36; 95% CI 1.87-68.97; P = 0.008) were risk factors for complicated influenza. Influenza virus type (A vs. B) was not associated with risk for complication. Thirty-seven complicated patients (90.2%) and 77 uncomplicated patients (95%) received antiviral treatment (P = 0.441). Complicated patients received antibiotics more frequently (P < 0.0001) and showed longer hospital stay (P = 0.0178). Influenza-related deaths occurred in 4(3.2%) of 123 patients.

Conclusions: the prognosis of hospitalized adult patients with influenza during 2011 to 2012 season in Korea was not serious. Our results demonstrate that having 2 or more chronic medical diseases is a strong risk factor for complicated influenza, therefore early intervention and careful observation is mandatory for high risk group with multiple chronic medical diseases.

P32

Different Mechanisms of Apoptosis by Influenza A and B Virus

LA Santos[1,2], S Solá[2], V Correia[1], M Gíria[2], CMP Rodrigues[2], H Rebelo de Andrade[1,2]

[1]Instituto Nacional de Saúde, Dr. Ricardo Jorge, Lisboa, Portugal; [2]Faculdade de Farmácia da Universidade de Lisboa, Lisboa, Portugal

Background and aims: the ability of Influenza A (infA) viruses to counteract and manipulate the host response to infection is well acknowledged. In addition, the activation of the PI3K/Akt survival pathway by the infA NS1 protein has been described as one of the strategies to delay the apoptotic response of the infected cell. As influenza B (infB) viruses differ genetically and phenotypically from infA viruses, namely at the NS protein level, we aimed to compare general apoptosis and survival pathways induced by each influenza type.

Methods: MDCK-SIAT1 cells were infected with infA(H1N1)pdm09 virus A/Portugal/82/2009 (APT82) and infB virus B/Lisboa/08/2006 (BLx08). Activities of caspase-3 -7 -8 and -9 were measured at

several time points post-infection (hpi). Total levels of Akt, pAkt, NF-□B, I□B, p53 and □-actin were also examined by Western blot.

Results: our results indicate that the apoptosis process induced by BLx08 was associated with activation of both intrinsic, caspase-9-dependent and extrinsic, caspase-8-dependent pathways as early as at 8hpi. In contrast, APT82-induced apoptosis only involved the activation of the intrinsic pathway, and occurred at 32hpi.

Surprisingly, our data show that the activation of the survival pathway PI3K/Akt was significantly increased upon BLx08 infection when compared with APT82 infection. In fact, increased levels of pAkt were observed at the same time of caspase activation in the early phase of BLx08 infection. This, however, did not result in increased downstream NF□B activation, since its inhibitor I□B was also markedly upregulated.

Increased p53 levels associated with APT82 infection may also explain the delayed apoptosis response in infA, assessed by caspase activity, as it may require transcriptional activation that it is deregulated and directed to viral replication.

Conclusion: InfB and infA infection differs in time and levels of activation of apoptosis and survival signaling pathways.PI3K/Akt activation in infB is not sufficient to inhibit apoptosis. Further studies will clarify this difference and shed light into the use of cellular mechanisms as new ways to fight influenza.

Acknowledgements: LA Santos, V Correia and M Gíria were recipients of PhD fellowships from FCT (SFRH/BD/62676/2009, SFRH/BD/48532/2008 and SFRH/BD/65211/2009, respectively).

P33

Hemagglutinin Receptor Binding Preferences of Recent Influenza A Viruses in Taiwan

Ya-Fang Wang[1,5], Chuan-Fa Chan[2], Chia-Yu Chi[1,3], Hsuan-Chen Wang[1], Ih-Jan Su[1,4], Jen-Ren Wang[1,2,4,5]

[1]Division of Infectious Disease and Vaccinology, National Health Research Institutes; [2]Departments of Medical Laboratory Science and Biotechnology, [3]Departments of Pediatrics, [4]Departments of Pathology, [5]Center of Infectious Disease and Signaling Research, Medical College, National Cheng Kung University, Taiwan

Influenza virus infection is initiated by virus attachment to cell-surface sialoside receptor via influenza hemagglutinin (HA). The carbohydrate binding specificities have been found to be different among avian and human influenza A viruses and may affect the tissue tropism and transmission. In this study, we explored the carbohydrate binding specificity of seasonal H3N2, H1N1 influenza and the 2009 pandemic H1N1 strains that isolated from Taiwan during 1999 to 2010 by a high-throughput carbohydrate solution array. The results showed that seasonal H1N1 accepted not only α-2,6 sialylated glycans but also α-2,3 sialylated glycans. However, some of the 2009 pandemic H1N1 viruses were predominantly bound to α-2,3 sialylated glycans and the viruses isolated in 2010 had changed the binding profiles from 2009, revealing the virus adaptation in human population. These results indicated a distinct carbohydrate-binding repertoire between seasonal H1N1 and the 2009 pandemic H1N1 viruses. Furthermore, thirty-six H3N2 clinical isolates were explored successfully and the binding patterns could be further classified into three groups. Group 1 viruses bound predominantly to α-2,6 linked glycans and group 2 viruses preferentially bound not only to α-2,6 sialylated glycans but also other α-2,3 linked sialyl glycans. Interestingly, although belong to same H3N2 genotype, group 2 isolates revealed receptor specificity broader than group 1 isolates. There were other three virus strains bound specifically to α-2,3 linked sialyl glycans, but weak or no interaction with α-2,6 linked sialyl glycans. These results revealed an unexpected diversity in receptor binding specificities among recent H3N2 viruses. In addition, the data also revealed the

occurrences of carbohydrate binding patterns of H3N2 every 1-2 years and continuous change patterns which were similar to the phylogenetic analysis. The viruses isolated from 1999 and 2003 bound to dual type glycans were more likely to cause severe diseases than those isolated from 2001, 2002 and 2006 which bound only to α-2,6 sialyl glycans. This study provided not only systematically analysis of receptor binding specificities for influenza A clinical isolates, but also useful information to monitor the antigenic shift and vaccine seeds selection of influenza viruses.

P34

Influenza A H1N1 Pathogenesis and Transmissibility are Structurally Correlated to the Salt Bridge between the HA1 110-helix and HA2 B-loop

ME Cueno, K Imai and K Ochiai

Nihon University School of Dentistry, Tokyo, Japan

Background: hemagglutinin (HA) receptor-binding plays an important role in the viral life cycle. Alterations in the receptor-binding domain was proposed to be attributed to the salt bridge between the HA1 110-helix and HA2 B-loop. However, this was not fully elucidated. In this study, we identified HA2 residues that can affect HA1-HA2 salt bridge formation and, likewise, correlated these residues to alterations in HA receptor-binding properties.

Methods: throughout this study, H1N1 HA amino acid sequences of human, swine and avian strains from 1918-2012 were collected from the NCBI Web site and a total of 2,830 amino acid sequences were analyzed. We designated subscript 1 and 2 to refer to HA1 and HA2, respectively. We compared all influenza strains obtained from 1918-2012 and identified interspecies similar amino acid residues that affect the HA1-HA2 salt bridge. Similarly, we compared the HA2 B-loops from all influenza strains obtained from 1976-2011 and, likewise, identified interspecies differences in the HA2 B-loop that may likewise affect the HA1-HA2 salt bridge. We used the Phyre 2 server to predict the protein conformation and J-mol software for structural analyses.

Results: we found that from 1918-2012, there was interspecies similarity in residues 145_2 and 146_2 whereas from 1976-2011 there was interspecies difference in residue 88_2 among the human, swine and avian strains. In establishing interspecies similarity, we found that Asn is the preferred amino acid in both residues 145_2 and 146_2 that would favor HA1-HA2 salt bridge formation. In addition, amino acid substitution in either or both residues alter the formation of the putative salt bridge which consequentially affects HA receptor-binding properties. In establishing interspecies difference, we found that interspecies amino acid residue 88_2 differs in the HA2 B-loop of the human ($K88_2$), swine ($H88_2$) and avian ($N88_2$) strains. Moreover, we established that variations in the amino acid in residue 88_2 could likewise affect the HA receptor-binding properties.

Conclusion: we propose that in the H1N1 subtype, the amino acid in residue 88_2 and the $N145_2$-$N146_2$ residue influence the HA1-HA2 salt bridge formation. Furthermore, we established that variations in residue 88_2 and the 145_2-146_2 residue alters HA receptor-binding properties.

P35

Avian, Human and Swine H2 Influenza Viruses are Pathogenic in Mammalian Models but Remain Susceptible to Current Antivirals

Jeremy Jones[1], Scott Krauss[1], Tatiana Baranovich[1], Bindumadhav Marathe[1], Elena Govorkova[1], Robert Webster[1]

[1]St. Jude Children's Research Hospital, Memphis, TN, USA

Introduction/Objectives: the H2N2 pandemic of 1957-1958 caused significant morbidity and mortality worldwide. Absent from humans since 1969, the H2 subtype continues to circulate in birds, and recent swine isolates possess characteristics of mammalian adaptation. Individuals under the age of 50 lack immunity to the H2 antigen. Thus, continued H2N2 surveillance and biological characterization is critical to mitigate their impact should they re-emerge in humans. Here we assess the risk of avian, human and swine H2 viruses isolated from 1957-2008 by evaluating pathogenicity, virulence and transmission in multiple mammalian models,

as well as susceptibility to current antiviral drugs.

Methods: twenty-seven avian, human and swine H2 viruses were assessed for replication in differentiated normal human bronchial epithelial (NHBE) cells, morbidity and mortality in DBA2/J mice and contact and/or aerosol transmission in ferrets. Fifty-nine H2 viruses were tested for susceptibility to adamantanes and neuraminidase inhibitors (NAIs). Susceptibility to adamantanes was assessed by sequence analysis of M2 protein, and to NAIs (oseltamivir, zanamivir) by fluorescence-based neuraminidase enzyme inhibition assay.

Results: in NHBE cells, 17 (73%) avian and all human and swine isolates replicated productively (>3 log10TCID50/ml). In mice, 10 (42%) avian all human and swine viruses induced morbidity (>5% weight loss). Replication in mouse lungs was observed with 80% of avian viruses and with all human and swine viruses. Three avian and multiple swine and human viruses induced mortality in mice. In ferrets, 5 of 9 avian viruses tested replicated in the upper respiratory tract (URT) and 3 exhibited direct contact transmission. Human and swine viruses replicated in the ferret URT, but transmission was observed only with swine infections. In all viruses tested, M2 gene sequence analysis did not reveal residue changes conferring resistance to adamantanes. All viruses tested were highly susceptible to NAIs with IC50 values in the nanomolar range (0.1-6.0 nM).

Conclusions: the data presented here are a thorough risk analysis of H2 viruses isolated from multiple species over 6 decades. The ability of avian and swine viruses to replicate and transmit in mammalian models elevates their risk to humans. However, all viruses remain highly susceptible to FDA-approved antiviral drugs, suggesting their usefulness as a control strategy should H2 viruses re-emerge in humans.

P36

Human Infection Potential of H6 Avian Influenza Virus

CH Wang[1], RH Juang[2], JL He[2], YT Chen[1]

[1]School of Veterinary Medicine, National Taiwan University, Taipei 10617, Taiwan
[2]Department of Biochemical Science and Technology, National Taiwan University, Taipei 10617, Taiwan

Transmissions of avian influenza viruses (AIV) from poultry to humans have raised fears of an impending influenza pandemic. Several low pathogenic virus strains have been isolated in Taiwan. Similarly, migratory bird transfer in seasons from Southeastern countries and China to Taiwan, which might be dangerous in carrying infectious virus. It is important to analyze the potential adaptation for the virus to infect human. By comparing the sequences of HA of the H6N1 viruses, we found that amino acid changes on HA1 were higher than those on HA2. The antigenic changes have occurred on the globular head of HA molecule. By hemagglutinination inhibition (HI) assays, monoclonal antibodies elicited from one H6N1 virus showed different HI titers with those H6N1 viruses. Amino acid substitutions on the HAs of viruses isolated from chicken were confined to the HA1 mostly, indicating that viruses were circulating in the presence of antibody selection pressure in chicken flocks in Taiwan. Although the 228th amino acid of HA protein changed from G to S, the 627th amino acid was still E, restriction infection in chickens. Both H6N1 2838V and 2838N AIV strains contain 167N glycosylation site in the HA1 near head region, but it is absence in H5N2 3233 AIV. In addition, mAbs recognized peptide itself but not carbohydrate. By using Qproteome GlycoArray Kit, sialic acid showed the highest binding with H6N1 (strain 2838). This indicated that the glycan of the H6N1 was terminated with sialic acid instead of others. By using the DIG Glycan Differentiation Kit, H6N1 bound Maackia amurensis agglutinin but not Sambucus nigra agglutinin. So the virus recognized 2-3 linkage instead of 2-6 linkage. From the data of gene sequence analysis, virus characterization, and glycan binding ability, we propose that the present AIVs in Taiwan from chickens might not transmit to humans. However, it is necessary to detect the amino acids of the residue 627th in PB2 and 225th residue in HA to determine the cross-species ability of further AIVs isolated.

P37

Detection of Antiviral-Resistant Influenza Viruses in Japan from 2008-2009 to 2011-2012 Influenza Seasons

E Takashita[1], M Ejima[1],S Fujisaki[1], N Kishida[1], H Xu[1], M Imai[1], N Kim[1], A Sato[1], H Sugawara[1], R Itoh[1], T Doi[1], M Tashiro[1] and T Odagiri[1]

[1]National Institute of Infectious Diseases, Tokyo, Japan

Objectives: in Japan, four neuraminidase inhibitors (NAIs), oseltamivir, peramivir, zanamivir and laninamivir, are approved for chemotherapy against influenza and prescribed with the highest frequency in the world. Therefore, Japan is at high risk for emergence of antiviral-resistant influenza viruses. We have monitored antiviral-resistant viruses since 2008-2009 influenza season by both phenotypic and genotypic assays.

Methods: approximately 5-10% of total isolates in Japan were randomly selected and subjected to NAI susceptibility assays, NA gene sequencing and/or real time RT-PCR allelic discrimination to detect H275Y mutation in NA gene. The susceptibilities of viruses to NAIs were examined by chemiluminescent (CL) assay and/or fluorescent (FL) assay and expressed as the drug concentrations required to inhibit NA activity by 50% (IC50).

Results: as of July 2012, we detected 157 (1.3%) oseltamivir- and peramivir-resistant A(H1N1)pdm09 viruses possessing H275Y mutation and 1 (0.3%) oseltamivir- and peramivir-resistant A(H3N2) viruses possessing R292K mutation. Out of 157 H275Y mutant A(H1N1)pdm09 viruses, 28 (18%) were mixed isolates of 275H and 275Y (275H/Y) viruses. By CL assay, 275H/Y viruses with a minor composition of the H275Y mutation exhibited indistinguishable susceptibility to oseltamivir and peramivir from the sensitive isolates with 275H, whereas by FL assay, they were clearly discriminated between 275H/Y and 275H isolates. The detection rates of H275Y mutant A(H1N1)pdm09 viruses were 0.5% during the 2008-2009 season, 1.1% during the 2009-2010 season, and 2.0% during the 2010-2011 season. Although the H275Y mutant viruses did not spread in community, a number of person-to-person transmission cases were confirmed in hospitals and a facility for the handicapped. Furthermore, the percentage of H275Y mutant viruses from the cases with no known exposure to NAIs increased from 20% during the 2008-2009 season to 44% during the 2010-2011 season.

Conclusions: sustained monitoring of antiviral-resistant viruses in national level is important and a combination of CL and FL assay systems is useful to precisely evaluate susceptibility of viruses to NAIs.

This study was carried out by collaboration with the influenza virus surveillance group of Japan.

P38

The NA-Fluor™, NA-XTD™ and NA-Star® Influenza Neuraminidase Assays: Phenotypic Assays for Neuraminidase Inhibitor Resistance Quantitation

MA Gee, A Mihali, RR Juo, J Salhaney, B D'Eon, and A Chiulli

Life Technologies, 35 Wiggins Avenue, Bedford, MA 01730, USA

Objectives: the NA-Fluor™, NA-XTD™ and NA-Star® Influenza Neuraminidase Assays are functional, phenotypic enzyme assays for direct quantitation of neuraminidase inhibitor (NI) sensitivity of influenza virus isolates, enabling detection of any resistance mutation. These assays were designed to provide researchers with reliable and consistent results through standardized reagents and protocols, choice of detection technology, compatibility with simple instrumentation, flexible assay format and high sensitivity. Additional applications include discovery of new NI compounds, and for cell-based assays as an easier alternative to microneutralization assays.

Results: the fluorescent NA-Fluor™ assay uses MUNANA substrate that is quality controlled for purity and low background signal. Assay reagents and protocol were optimized to provide an economical, standardized and easy-to-use assay kit that generates IC50 data directly comparable to data obtained with NISN MUNANA protocols. The NA-Fluor™ assay signal is extremely stable, enabling read-time flexibility and high-throughput capability, or is performed with a real-time kinetic read-out.

The chemiluminescent NA-XTD™ assay provides the next-generation NA-XTD™ substrate, with assay reagents and microplates. Like the chemiluminescent NA-Star® assay, the NA-XTD™ assay provides highly sensitive neuraminidase quantitation with a wide assay dynamic range, providing similar IC50 values over a broad range of virus concentration. The NA-XTD™ assay provides extended-glow light emission that enables accurate IC50 determination up to several hours.

IC50 determination has been performed with A/H1N1, A/H3N2, B and NI-sensitive/resistant (H275Y) influenzas. NA-XTD™ and NA-Star® assay IC50 values are nearly identical and are typically slightly lower than NA-Fluor and other MUNANA-based assay values. The NA-Fluor™ and NA-XTD™ assays are also used to quantitate influenza NA activity directly in cell-based virus cultures to monitor viral growth or inhibition in the presence of inhibitory compounds or antibodies.

Conclusions: phenotypic neuraminidase assays are important in conjunction with sequence-based screening for global NI resistance monitoring. These assays provide convenient, flexible and reliable influenza neuraminidase activity quantitation for NI-sensitivity and cell-based virus quantitation assays.

For Research Use Only. Not intended for any human or animal therapeutic or diagnostic use.

P39

Development of a H275Y Real Time Allelic Discriminating PCR and CE Marked Control Material for Influenza A(H1N1)2009

B Vipond[1], M Guiver[2], A Lackenby[3], J Ellis[3], A Bermingham[3], M Zambon[3], D Carrington[1] and P Muir[1]

[1]HPA, Microbiology Services Bristol, UK, [2]HPA, Microbiology Services, Manchester, UK, [3]HPA, Microbiology Services Colindale, London, UK

The H275Y substitution in A(H1N1)pdm09 virus, causing oseltamivir resistance, has been detected sporadically since June 2009. Oseltamivir treated immunocompromised patients are at higher risk of developing resistance and require close monitoring. Furthermore, an increasing number of detections of the H275Y substitution in specimens are from untreated cases from hospital and community sources. Rapid, sensitive screening methodologies are critical for effective surveillance and clinical management, particularly in high risk patients.

We developed a real-time PCR assay to detect the H275Y substitution in A(H1N1)pdm2009 viruses, which includes 2 allelic discriminating probes and the additional benefit of a third control probe binding to a non-influenza sequence. Two positive run controls were designed to facilitate assay standardization and quality assurance. These CE marked synthetic RNA transcripts include the PCR primer sequence, control non-influenza sequence and specific probe sequence for either the H275 or Y275 variant. These transcripts are positive controls for RNA extraction, reverse transcription and PCR, but also eliminate the need to include 275Y containing samples as controls in the PCR, removing a potential contamination risk with false detection of resistance in a test clinical specimen. Discrimination of true H275 or Y275 in specimens from contamination with the positive control is given by signal from the third probe in the PCR.

All primers and probes were screened to provide absolute discrimination between the single nucleotide variants encoding the H275Y polymorphism. The assay was established in one step and two step formats to comply with the workflow of diagnostic laboratories in the HPA Microbiology Services network. Validation was achieved using a blinded panel of specimens containing oseltamivir sensitive, resistant and mixed genotype viruses, and comparison with the 'gold-standard' results of pyrosequencing.

The assay and controls have been in use for two influenza seasons (2010-2012) by seven laboratories across England, with more than 2000 specimens tested. All Y275 detections were confirmed by pyrosequencing indicating specificity and sensitivity of the real-time PCR. Dissemination of the assay and controls to regional laboratories increased national capacity for surveillance, and allowed effective monitoring of treated patients, with delivery of results in a clinically relevant time frame.

P40

Susceptibility to Neuraminidase Inhibitor Oseltamivir of Human H3N2 Influenza Viruses Circulated in South Korea in 2009-2011

Sehee Park[1,2], Jin Il Kim[1,2], Ilseob Lee[1,2], Saem Shin[1,2], Hye-Eun Hyun[1,2], Joon-Yong Bae[1,2], Min-Woong Hwang[1,2], Yongkwan Won[1,2], Sangmoo Lee[1,2], Jun Heo[1,2], Eun-Joo Lim[3], Won-Seok Seok[3], Hee Jin Cheong[4], Joon Young Song[4], Woo Joo Kim[4] and Man-Seong Park[1,2§]

[1]Department of Microbiology, [2]Center for Medical Science Research, College of Medicine, Hallym University, Chuncheon, Gangwon-do, South Korea; [3]Infectious Disease Investigation Division, Gangwon Institute of Health and Environment, Chuncheon, Gangwon-do, South Korea; [4]Division of Infectious Diseases, Korea University Guro Hospital, College of Medicine, Korea University, Seoul, South Korea

Nasopharyngeal specimens which were collected from patients in South Korea during the 2009-2011 influenza seasons are passaged in Madin-Darby canine kidney (MDCK) cells to isolate influenza viruses. The total of 380 influenza viruses were identified by immunofluorescence assay and by sequence analysis. Of the total 380 viruses, most viruses were subtyped as 2009 pandemic H1N1 strains, and only one (0.26%) was categorized as an influenza B virus. The remaining 39 (10.26%) viruses were identified as H3N2 strains. Whereas the IC50 values of H3N2 viruses were ranged within those of oseltamivir-sensitive strains, some of H3N2 viruses exhibited unusual sensitivity in a plaque reduction assay. These H3N2 viruses were found to have novel mutations in the neuraminidase gene.

In addition, one of these H3N2 viruses retained an additional mutation and resulted in the unique property to oseltamivir when characterized by plaque reduction assay in MDCK cells. Considered together, these novel mutations might account for the exclusive characteristics of selected H3N2 viruses observed in a plaque reduction assay.

P41

Evaluation and Characterization of Influenza Antiviral Drug Resistance in Portugal: Major Results and Achievements of a 5-Year Study

V Correia[1], LA Santos[1], M Gíria[2], H Rebelo-de-Andrade[1,2]

[1]Instituto Nacional de Saúde Dr.Ricardo Jorge,Lisbon,Portugal; [2]Faculdade de Farmácia da Universidade de Lisboa, Lisbon, Portugal

In 2007 started to be carried out for the first time in Portugal a study focused on influenza antiviral drug resistance. Three main objectives were established:(1)to determine the antiviral profile of influenza viruses to oseltamivir, zanamivir and amantadine;(2)to determine and monitor the baseline level of susceptibility along winter seasons and for each influenza sub(type);(3)to analyse and characterize the whole genome of viruses that showed phenotypic levels of inhibition to neuraminidase inhibitors(NAIs).

NAIs profile was determined phenotypically, using a fluorescence MUNUNA assay, and genotypically by NA and HA sequencing. A total of 340 seasonal viruses(117 A(H3N2),93 A(H1N1),130 B) were tested for oseltamivir and of 297(112 A(H3N2),68 A(H1N1),117B) for zanamivir. Additionally, 142 A(H1N1)pdm09 viruses were evaluated for both NAIs. Whole genome sequencing was performed in 27 of the A(H1N1)pdm09 viruses. Amantadine profile was determined through M2 pyrosequencing or conventional sequencing in a total of 205 seasonal A viruses (138 A (H3N2),84 A(H1N1)) and of 117 A(H1N1)pdm09 viruses.

Main results are:

- Resistance to oseltamivir in 27 A(H1N1) seasonal viruses(29%,N=93) from 2007/2008 and 2008/2009 and in one A(H1N1)pdm09 virus(0.7%,N=142) from 2010/2011. These viruses exhibited a highly reduced level of inhibition to oseltamivir by phenotypic analysis (170-650 IC_{50} fold-change) and NA H275Y mutation;

- One suspected case of clinical resistance to oseltamivir with a mixed population of H275Y viruses (73,8%H275,26.2%Y275);
- No resistance to zanamivir;

- Dual reduced susceptibility to oseltamivir and zanamivir in one B virus (0,85%,N=117) and in two A(H1N1)pdm09 viruses(1,41%,N=142). These viruses exhibited a 2-4 IC_{50} fold-change level of inhibition to both NAIs. A mixed population of D197N viruses was found in the B virus (56%D197,44%N197) and the two A(H1N1)pdm09 viruses shared NA I223V and PB2 V480I mutations;

- Resistance to amantadine in 49 A(H3N2) viruses(35,5%,N=138) from 2005/2006 to 2008/2009(46 S31N,3 S31N+V27A), and in all A(H1N1)pdm09 viruses(S31N).

This 5-year study allowed to establish a technical platform for influenza antiviral drug resistance evaluation, to timely detect the emergence of resistant viruses, to acquire know-how on the natural variation of virus susceptibility, and to contribute for the management of cases suspected of clinical resistance. Additionally, it allowed the gathering of a large amount of data that will be used in more advanced studies, focused on evolutionary analysis and on detailed characterization of specific mutations.

P42

Antiviral Drug profile of Seasonal Influenza Viruses Circulating in India: 2004-2011

Potdar VA[1], Dakhave MR[1], Kulkarni PB[1], Tikhe SA[1], Broor S[2], Gunashekaran P[3], Chawla-Sarkar M[4], Abraham A[5], Bishwas D[6], Patil KN[1], Kadam AA[1], Kode SS[1], Mishra AC[1] , Chadha MS[1]

[1]National Institute of Virology, Pune, India , [2]All India Institute of Medical Sciences, New Delhi, [3]King Institute of Preventive Medicine & Research, Chennai, [4]National Institute of Cholera and Enteric Diseases, Kolkata, [5]Christian Medical College, Vellore, [6]Regional Medical Research Centre, Dibrugarh

Background: recent influenza antiviral resistance studies in South East Asia, Europe and the United States reveal alarming increases in both adamantane and neuraminidase inhibitor (NAIs) resistance.

Objective: to evaluate antiviral resistance of Influenza viruses isolated from various parts of India.
Methods: influenza viruses, isolated from 2004to 2011 were analyzed. Matrix gene (M2) of 206 influenza A/H1N1 and 371 A/H3N2 viruses for amantadine resistance and Neuraminidase gene of 206 A/H1N1, 272 A/H3N2 and 326 Type B for oseltamivir resistance were sequenced. Pandemic (H1N1) (n= 493) isolates were tested for H274Y mutation by allelic discrimination real time RT-PCR. Randomly selected resistant and sensitive influenza A/ H1N1 and A/H3N2 viruses were confirmed by phenotypic assay.

Results: serine to asparagine (S3IN) mutation was detected in six isolates of 2007-2008. One dual-resistant A/H1N1 was detected for the first time in India with leucine to phenylalanine (L26F) mutation in M2 gene and H274Y mutation in NA gene. A/H3N2 viruses showed an incremental trend in resistance to amantadine from 22.5% in 2005 to 100% in 2008 onwards with S3IN mutation. 50 /61 A/H1N1 viruses tested in 2008-2009 were oseltamivir resistant with H274Y mutation, while all A/H3N2, pandemic A/H1N1 and Type B isolates remained sensitive. Genetic results were also confirmed by phenotypic analysis of randomly selected 53 resistant A/ H1N1 and 40 sensitive A/H3N2 isolates.

Conclusions: emergence of influenza strains resistant to amantadine and oseltamivir in spite of negligible usage of antivirals emphasizes the need for monitoring antiviral resistance as part of National Influenza Program.

P43

Highly Pathogenic Avian Influenza A(H5N1) Viruses Isolated from Poultry in Vietnam during 2009–2011: Antiviral Susceptibility Profiles and Detection of Naturally Occurring Oseltamivir-Resistant Clade 2.3.2.1 Virus

HT Nguyen[1,2], T Nguyen[3], VP Mishin[1], K Sleeman[1], J Jones[1], M Poh[1], A Creanga[1], AI Klimov[1], CT Davis[1], and LV Gubareva[1]

[1]Influenza Division, Centers for Disease Control and Prevention, [2]Battelle Memorial Institute, Atlanta, GA, USA; and [3]Department of Animal Health, National Center for Veterinary Diagnostics, Hanoi, Vietnam

Since 2003, highly pathogenic avian influenza A (H5N1) viruses have spread in bird populations in several countries worldwide resulting in sporadic human infection and occasionally death. H5N1 viruses continue to

evolve in poultry, thus presenting challenges for the development of effective vaccines and antiviral medications. Oseltamivir, an oral neuraminidase (NA) inhibitor (NAI), is the most commonly used medication for treatment and chemoprophylaxis of influenza infections. Resistance to oseltamivir, natural or acquired, is one of major public health concerns. As part of enhanced surveillance efforts, viruses collected from wild birds and poultry in Vietnam from 2009-2011, have been sequenced and analyzed for established molecular markers of resistance. Moreover, the susceptibility profiles of these viruses to approved and investigational NAIs were determined in fluorescent NA inhibition assay. Susceptibilities to other anti-influenza drugs were assessed using the virus yield reduction assay in MDCK cells. Overall, the H5N1 viruses (n=102) from this study were classified into three HA clades: 1.1, 2.3.2, and 2.3.4. Oseltamivir susceptible viruses belonging to the two latter clades exhibited a mean IC50 greater than that of the former by ~44 and ~6 times, respectively. The increased oseltamivir susceptibility of clade 1 viruses was previously linked to the presence of the Y253H mutation. In accord with these findings, a clade 1.1 virus with a revertant mutation, H253Y, showed 10-fold reduction in oseltamivir susceptibility compared to other viruses of the same clade. One virus from clade 1.1 contained multiple unique substitutions outside of the NA active site and exhibited a ~95- and ~37-fold reduction in susceptibility to zanamivir and laninamivir, respectively, yet had only a moderate decrease in susceptibility to oseltamivir (~14-fold).Three viruses from clade 2.3.4.2 possessed the I223T change in the NA active site and showed a 6-8-fold reduction in oseltamivir susceptibility compared to the closest matching susceptible virus. Susceptibility of these three viruses to peramivir was not affected (≤3-fold). Of note, a single virus, A/duck/Vietnam/NCVD-664/2010, carrying the H275Y mutation, a known oseltamivir resistance marker, was detected among the recently emergent 2.3.2.1 viruses. In laboratory tests, this virus was resistant to two NAIs, oseltamivir and peramivir, but susceptible to zanamivir and laninamivir, as well as M2 blockers and the investigational drugs, DAS181 and favipiravir. The H275Y substitution did not appear to impair the NA functionality of this virus or its replication in cell culture. The naturally occurring oseltamivir-resistant H275Y virus will be tested in a ferret model to assess its replicative potential and drug susceptibility in vivo. In conclusion, the results of this study highlight the importance of monitoring viruses for naturally occurring substitutions that may alter H5N1 drug susceptibility and in vitro drug susceptibility profiles. This is especially important in countries like Vietnam, where zoonotic H5N1 viruses are genetically divergent and continuously evolving. The identification of both known and novel molecular markers, particularly for newer NAIs, for which resistance markers are not well established, is critical in order to understand how virus evolution may lead to resistance.

P44

Evaluation of a New Immunochromatographic Assay for Rapid Identification of Influenza A, B, and A(H1N1)2009 Viruses

Keiko Mitamura[1], Chiharu Kawakami[2], Hideaki Shimizu[3] and Norio Sugaya[4]

[1]Eiju General Hospital, Tokyo, Japan, [2]Yohohama City Institute of Health, Yokohama, Japan, [3]Kawasaki City Institute of Public Health, Kawasaki, Japan and [4]Keiyu Hospital, Yokohama, Japan

Objectives: accurate and rapid diagnosis is very important for appropriate use of antivirals and infection control. We evaluated Clealine Influenza A/B/(H1N1)2009(Alere Medical Co., Ltd. Tokyo, Japan), a new multi-line immunochromatographic assay for rapid detection of antigens of influenza A(FluA), B(FluB), and A(H1N1)2009 viruses.

Methods: vaccine strains and clinical isolates were used for evaluation of detection limit of Clealine. Nasopharyngeal aspirate specimens, nasopharyngeal swab specimens and self-blown nasal discharge specimens were collected from children and adults with influenza like illness for clinical evaluation. The performances of Clealine were compared with multiplex RT-PCR and other rapid antigen test (Espline Influenza A&B-N), and the sensitivity and specificity of RAT using multiplex RT-PCR as a reference standard were determined.

Results: clealine detected FluA, FluB, and A(H1N1)2009 viruses with detection limit of 4.6×103 to 7.5×104 pfu/assay. The sensitivity and specificity of Clealine in nasopharyngeal aspirate specimens was as follows: FluA=94.5% and 100%, FluB=96.8% and 100%, and A(H1N1)2009=97.3% and 99.1%, respectively. Self-blown nasal discharge

specimens were easy to collect but the sensitivities were lower in self-blown discharge specimens than in nasopharyngeal specimens.

Conclusions: these finding suggest that Clealine may have high sensitivity and specificity, so may be useful in early diagnosis and treatment of influenza in the clinical setting.

P45

Post-Marketing Assessment of Neuropsychiatric Adverse Events in Influenza Patients Treated with Oseltamivir: An Updated Review

S Toovey[1], R Dutkowski[2], M Wollenhaupt[1]

[1]F. Hoffmann-La Roche Ltd, Basel, Switzerland; [2]Hoffmann-La Roche, Inc., Nutley, NJ, USA

Our 2008 review concluded that the risk of neuropsychiatric adverse events (NPAEs) in influenza patients was not increased by oseltamivir exposure, and did not identify any mechanism by which oseltamivir or its metabolites could cause or worsen such events. We have reviewed more recent information on this topic. Between 16 September 2007 and 15 May 2010, 1,805 spontaneously-reported NPAEs were identified in 1,330 patients receiving oseltamivir: 767 (42.5%) from Japan, 296 (16.4%) from the USA, and 742 (41.1%) from other countries. NPAEs were more common in children: 1,072 (59.4%) events were in those aged □16 years. The majority of NPAEs occurred within 48 hours of treatment initiation (953 events; 52.8%). Nearly half of the events were serious in nature (838; 46.4%). The three largest categories of events were abnormal behavior (457 events; 25.3%), miscellaneous psychiatric events (370; 20.5%) and delusions/perceptual disturbances (316 events; 17.5%). A total of 1,545 events (85.6%) in eight different categories were considered to be delirium or delirium-like. Twenty-eight suicide-related events were reported. A US healthcare claims database analysis showed that the risk of NPAEs in 7,798 oseltamivir-treated patients was no higher than in 10,411 patients not on antivirals. A study on oseltamivir and abnormal behavior in Japan was less conclusive. NPAE frequency in oseltamivir-exposed Japanese and Taiwanese children with influenza was the same as in unexposed children with influenza. New analysis of the UK General Practice Research Database (GPRD) showed that the relative adjusted risk of NPAEs in influenza patients was 2.18 times higher than in the general population. Other epidemiology studies reported occurrence of encephalitis and similar disorders in influenza patients independently of oseltamivir exposure. These new data support the findings of the original assessment. There is evidence that influenza-related encephalopathies are caused by influenza-induced inflammatory responses, but more work is needed to confirm the underlying mechanisms.

Notes

Speakers' Biographies

Jesse Bloom

Fred Hutchinson Cancer Research Center, Seattle, USA

Jesse D Bloom, Ph.D. is an Assistant Member in the Division of Basic Sciences and the Computational Biology Program at the Fred Hutchinson Cancer Research Center. He uses a combination of computational and experimental approaches to study the molecular evolution of proteins and viruses, with a special emphasis on influenza. Dr. Bloom's research is especially focused on understanding the underlying physical constraints that shape viral evolutionary pathways.

Prior to beginning a faculty position at the Fred Hutchinson Center in 2011, Dr. Bloom performed postdoctoral research with Dr. David Baltimore at the California Institute of Technology. He also received his Ph.D. in Chemistry from the California Institute of Technology, where he studied the directed evolution of proteins with Dr. Frances Arnold. He also has an MPhil in Theoretical Chemistry from Cambridge University and a B.S. in Biological Chemistry from the University of Chicago.

Guy Boivin

Laval University, Quebec, Canada

Dr. Guy Boivin is a medical microbiologist/virologist and an infectious disease specialist working at the "Québec City University Hospital Center" (CHUQ-CHUL) in Canada. Dr. Boivin is also a professor of microbiology-immunology at Laval University and a senior researcher in virology at the "Research Center in Infectious Diseases" of the same University.

Dr. Boivin holds a MD from Laval University, a master (MSc) degree in microbiology from University of Montréal and a 3-year specialized research training (Fellowship) in Molecular Virology from University of Minnesota.

Dr. Boivin is currently the holder of the Canada research chair on Emerging Viruses and Antiviral Resistance (2006-13). He also holds numerous research grants from governmental health organizations (Canadian Institutes of Health Research, Quebec Health Research Foundation) and private companies. His main research interests concern the diagnosis, pathogenesis, prevention and treatment of viral diseases caused by herpesviruses (mainly cytomegalovirus and herpes simplex virus) and respiratory viruses (mainly influenza virus, human metapneumovirus and human respiratory syncytial virus) and the mechanisms of resistance to antiviral drugs.

Dr. Boivin is member of several distinguished societies including the American Society for Microbiology, the American Society of Transplantation, the Canadian Association of Medical Microbiology and Infectious Diseases, the Canadian Society for Clinical Investigation and the Quebec AIDS and Respiratory Diseases Networks.

Finally, Dr. Boivin has published more than 220 peer-reviewed manuscripts and presented more than 60 invited conferences and 230 communication abstracts since 1993. He is on the Editorial board of the Journal of Infectious Diseases, in which he has published more than 36 articles.

Philippe Buchy

Institut Pasteur in Cambodia, South East Asia

Philippe Buchy is a medical doctor, specialist in Clinical Pathology and holds a PhD in virology. He was director of a clinical biology laboratory of the Majunga Teaching Hospital in Madagascar in 1995 and then joined the Institut Pasteur International Network. He worked as Scientific Advisor at the Institut Pasteur in Nha Trang (Vietnam) in 1999 where he was in charge of the development of the clinical biology department and of the virology laboratory. Since September 2004, he is head of the Virology Division at the Institut Pasteur in Cambodia, Director of the WHO National Influenza Centre, the National Reference Centre for arboviruses and of the National Reference Laboratory for rabies. His particular areas of interest are: influenza viruses including H5N1 virus, respiratory viruses, arboviruses, neurotropic viruses, emerging and zoonotic viruses. He is author of over 90 papers in international journals and of several book chapters.

Bin Cao

Capital Medical University, Beijing, China

Dr Cao's research interests include pneumonia and influenza. He has more than 70 papers published in international and national journals. His recent publication includes an article in the Ann Intern Med (August 2011) entitled "Oseltamivir Compared With the Chinese Traditional Therapy Maxingshigan–Yinqiaosan in the Treatment of H1N1 Influenza: A Randomized Trial" and New England Journal of Medicine (December 2009) entitled "Clinical Features of the Initial Cases of 2009 Pandemic Influenza A (H1N1) Virus Infection in China."

Dr Cao acts as editorial board member of the Chinese Journal of Mycology, Chinese Journal for Clinicians and Chinese Journal of Respiratory & Critical Care Medicine. He also serves as committee member of Chinese Medical Society, Beijing Infectious Disease Branch as well as member of Respiratory & Infectious Disease branch under the Chinese Society of Respiratory Disease. He had the honor of the 14th Mao Yisheng Beijing Youth Science and Technology Award in 2011. He received grants from Chinese National Natural Science Foundation (2008 and 2010) and Ministry of Education of "New Century Excellent Talents Support Program" (2010).

Menno de Jong

Academic Medical Center, Amsterdam, The Netherlands

After Medical School at the University of Amsterdam (1991), and PhD research on HIV treatment (1996), Menno de Jong specialized in Clinical Microbiology at the Academic Medical Center, University of Amsterdam (2000). He worked as consultant clinical microbiologist at the Leiden University Medical Center (2000-2001) and the Academic Medical Center, University of Amsterdam (2001-2003). In 2003, he moved to Ho Chi Minh City, Vietnam, where he set up and headed the Laboratory of Virology at the Oxford University Clinical Research Unit (OUCRU), Hospital for Tropical Diseases, and where he became involved in clinical and virological research on avian and human influenza. Whilst remaining senior faculty member at OUCRU, Menno has moved back to Amsterdam in August 2008, where he was appointed professor in Clinical Virology and Head of the Department of Medical Microbiology at the Academic Medical Center, University of Amsterdam.

Jane Deng

University of California at Los Angeles (UCLA), USA

Dr. Jane Deng is an assistant professor in the Division of Pulmonary and Critical Care Medicine at the University of California, Los Angeles School of Medicine. She received her M.D. degree from University of California, San Francisco, and did her internal medicine residency and pulmonary and critical care medicine fellowship at the University of Michigan. Her laboratory investigates pulmonary host defense against influenza, Streptococcus pneumoniae, and other respiratory pathogens. Her work has revealed that activation of the type I interferon pathway during influenza and other viral infections suppresses antibacterial immune responses in the lung. The underlying mechanisms by which viral infections results in enhanced susceptibility to bacterial infections remains a major focus of her laboratory.

Jeremy Farrar

The Hospital for Tropical Diseases, Ho Chi Minh City, Viet Nam

Jeremy trained in medicine in London, Edinburgh, Melbourne and Oxford with a PHD from Oxford University and UCSF. Since 1996 he has been the Director of the Clinical Research Unit Vietnam, a Professor of Medicine at Oxford University, Hon Professor of International Health LSHTM, Global Scholar Princeton University and Adjunct Professor NUS. His interests are in integrated health research across a range of infectious diseases. He was awarded the Ho Chi Minh Medal from the Government of Vietnam and the OBE for Tropical Medicine. He is also the Director of the SEAICRN a multilateral partnership involving Indonesia, Thailand, Vietnam, NIAID, Wellcome Trust, and led the setting up of ISARIC (www.wellcome.ac.uk/News/Media-office/Press-releases/2011/WTVM053638). The Hospital where he is based is involved in the direct management of several thousand patients each year with a catchment population of 40 million people. He has almost 400 publications.

Elena Govorkova

St Jude Children's Research Hospital, Memphis, USA

Dr. Govorkova is currently a Laboratory Director in the Department of Infectious Diseases at St. Jude Children's Research Hospital, Memphis TN, USA and is a leading scientist in the NIAID Center of Excellence for Influenza Research and Surveillance. She received a Medical degree from the Medical Academy in Moscow, Russia, and a PhD from the D.I. Ivanovsky Institute of Virology in Moscow, Russia. Dr. Govorkova's research interests are primarily focused on evaluation of antiviral drugs against highly pathogenic influenza viruses on enzymatic and cellular levels, as well as in animal models.

Her work has involved investigation of advantages/disadvantages of combinations of antiviral drugs that target different viral proteins and have different mechanisms of action on the reduction of influenza virus replication in vitro, protection in animals, and emergence and fitness of resistant variants. She is the author of over 80 original research publications and review papers in peer-reviewed journals, in addition to patents and awards. Dr. Govorkova is a member of a number of professional societies and is a member of the Editorial Board of the Journal of Antiviral Research. She is actively sharing her expertise to mentor and train graduate students and postdoctoral fellows.

Larisa Gubareva

Centers for Disease Control and Prevention, Atlanta, USA

Dr. Larisa Gubareva is the Lead of the Molecular Epidemiology Team in the Virus Surveillance and Diagnosis Branch, Influenza Division, at the Centers for Disease Control and Prevention in Atlanta, GA, USA. The Team actively participates in influenza virus surveillance and assessment of influenza virus susceptibility to FDA-approved and investigational antiviral agents. The Team also monitors influenza drug resistance among community isolates/specimens, offers antiviral testing for clinical care use in the US, and provides training and technical expertise to partners in global influenza virus surveillance.

Dr. Gubareva has been an influenza virologist for over 20 years. In 2006, she joined the Influenza Division at the CDC. Her research and public health activities focus on understanding the mechanisms of drug resistance, methods for drug resistance detection and assessment of drug resistant viruses' replicative fitness. In recent years, she has served as a technical advisor for the WHO on the Influenza Antiviral Resistance.

Dr. Gubareva is an author/co-author of over 100 peer-reviewed papers and textbook chapters. She also serves on the editorial boards of the journals, Antimicrobial Agents and Chemotherapy, Antiviral Research, and Antiviral Chemistry & Chemotherapy. Dr. Gubareva is internationally recognized for her expertise in influenza antiviral research, as reflected by her various collaborative partners.

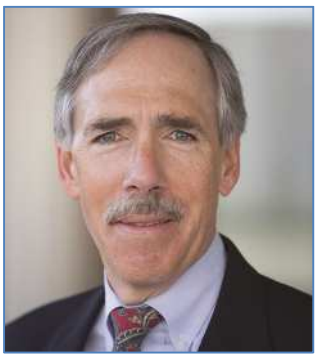
Alan Hay

National Institute for Medical Research, London, UK.

Alan Hay studied Biochemistry at the University of Aberdeen, where he first became involved in the study of viruses during his PhD research. Following two years of post-doctoral research at Duke University, North Carolina, he became a member of MRC scientific staff at the National Institute for Medical research (NIMR) in London, till his retirement in 2009.

His research interests have been principally concerned with various aspects of the biochemistry of influenza viruses and their replication, in particular: the mechanisms of transcription and replication of the virus genome; the antiviral action of the anti-influenza drug amantadine and the proton channel function of the target M2 protein; and the molecular bases of resistance to antiviral drugs against the M2 and NA proteins.

As Director of the WHO Collaborating Centre for Reference and Research on Influenza at NIMR from 1993, his interests also encompassed the epidemiology and evolution of human and animal influenza viruses, in particular in relation to current changes in human viruses and the compositions of influenza vaccines, and the emergence of novel human viruses with the potential to cause a pandemic.



Frederick Hayden

University of Virginia School of Medicine, Charlottesville, USA

Dr. Hayden is Stuart S. Richardson Professor of Clinical Virology and Professor of Medicine and Pathology at the University of Virginia School of Medicine in Charlottesville, Virginia, USA. During 2006-2008 he served as a medical officer in the Global Influenza Programme at the World Health Organization, Geneva, Switzerland, and since September 2008, has been serving as influenza research coordinator at the Wellcome Trust, London.

Dr. Hayden received his medical degree from Stanford University School of Medicine in 1973 and completed his clinical training in internal medicine and infectious diseases at Strong Memorial Hospital, University of Rochester, New York, USA. He joined the faculty of the University Of Virginia School Of Medicine in 1978 and became Richardson Professor in 1990. His principal research interests have been on the respiratory viral infections with a particular focus on the development and application of antiviral agents for influenza and rhinovirus infections.

Dr. Hayden is a Fellow of the Infectious Diseases Society of America, American Academy of Microbiology, American Society for Clinical Investigation, and Association of American Physicians.

Nguyen Tran Hien

National Institute of Hygiene and Epidemiology, Hanoi, Vietnam

Assoc. Prof. is Director of the National Institute of Hygiene and Epidemiology (NIHE), a leading scientific research institute in Vietnam in the area of epidemiology, medical microbiology, immunology and molecular biology, vaccine and biological product development.

He graduated from Hanoi Medical College, Vietnam in 1978. He had two years (1986-1988) Postgraduate training on Epidemiology and Clinical Immunology at the National Institute for Lung Diseases and Tuberculosis, Berlin, Germany. He finished the Master Course on Health Development (Master of Public Health), at the Royal Tropical Institute, Amsterdam, The Netherlands (1992-1993). Then in 1996, he finished a Postdoctoral Course on HIV/AIDS Epidemiology, at University of California, Los Angeles, USA. In 2002, he got PhD Degree at Freedom University, Amsterdam, The Netherlands. Since 2004 he is the Chairman of the National Sub-Committee of HIV Surveillance Vietnam; Vice-Chairman, National Committee of Prevention and Control of Human Avian Influenza and Pandemic (since 2006). He is also the Chairman, Department of Epidemiology, and Director of Center for HIV/AIDS Research and Training of Hanoi Medical University; and President, Vietnam Association of Preventive Medicine

By working in HIV/AIDS area for over 24 years, he has much experience in the HIV/AIDS surveillance, prevention and control in Vietnam. In the last 7 years, he devotes a lot of time for research, control and prevention of emerging and re-emerging infectious diseases such as avian influenza H5N1, Pandemic influenza A/H1N1/09, Cholera, Dengue, Foot-Hand-Mouth and Rabies in Vietnam.

Over 20 students have successfully completed their master or doctorate courses with the supervision of Assoc. Prof. Nguyen Tran Hien. He published more than 100 articles in Vietnam and International scientific journals. He is also author or co-author of over 44 text books.

Tran Tinh Hien

Oxford University Clinical Research Unit, Ho Chi Minh City, Viet Nam

Prof. Tran Tinh Hien is the director of Oxford University Clinical Research Unit (OUCRU) in Viet Nam. He received his medical degree from the University of Medicine in Saigon, Vietnam in 1978, and his PhD from Oxford University Clinical Research Unit and the Open University UK in 2004. He was appointed Professor of Tropical Diseases at Oxford University, in 2010 and has been a Fellow of the Royal College of Physicians (Edinburgh) since 2004. He was a member of several committees and advisory groups, including the Clinical Committee on the National Programme for the Control of Avian Influenza Virus Infection in Humans in Vietnam. He has worked as Principal Investigator for various projects on malaria, typhoid fever, Dengue haemorrhagic fever and has involved in dealing with SARS, H5N1 outbreaks and the pandemic H1N1/2009 at the Hospital for Tropical Diseases in Ho Chi Minh City in Viet Nam. Prof. Hien was also awarded McKay Medal from The Royal Society of Tropical Medicine & Hygiene in 2010 and Honorary Membership of The American Society of Tropical Medicine in 2011.

Peter Horby

Oxford University Clinical Research Unit, Hanoi, Viet Nam

Peter trained in adult medicine, infectious diseases and public health in the UK and Australia. He has previously held positions as International Research Fellow at the National Centre for Immunisation Research in Sydney; Consultant Epidemiologist with the UK Health Protection Agency; head of Communicable Disease Surveillance and Response in the Vietnam WHO Country Office; and Director of the Oxford University Clinical Research Unit at the National Hospital for Tropical Diseases, Hanoi, Vietnam. Peter is currently Senior Clinical Research Fellow at the Oxford University Clinical Research Unit in Vietnam, and Adjunct Associate Professor in the Department of Infectious Diseases, Yong Loo Lin School of Medicine, National University of Singapore. His interests are in the emergence and control of infectious diseases, including avian and seasonal influenza, community acquired pneumonia, and dengue.

David Hui

The Chinese University of Hong Kong, SAR China

Prof Hui is the Stanley Ho Professor of Respiratory Medicine and a Chair Professor at the Dept of Medicine & Therapeutics, The Chinese University of Hong Kong. He is also the Director of the Stanley Ho Centre for Emerging Infectious Diseases, CUHK.

He graduated from the University of New South Wales in Australia in 1985 and undertook postgraduate training in Respiratory Medicine and Sleep Medicine in Sydney. During the major outbreak of SARS in 2003 and the H1N1 influenza pandemic in 2009, he was heavily involved in the clinical management of severe cases. In Feb 2004, he attended the early human cases of H5N1 in Vietnam as a WHO advisor.

Dr Hui has published over 200 peer-reviewed journal articles and 21 book chapters since joining the CUHK in 1998. His research interests include clinical management of emerging respiratory infections, the safety of respiratory therapy in the post SARS era, and medical ward environmental airflow in preventing nosocomial infections.

Michael Ison

Northwestern University, Chicago, USA

Dr. Michael Ison obtained is medical degree at the University of South Florida College of Medicine. He then completed his Internal Medicine Residency and General Internal Medicine Fellowship at Oregon Health Sciences University in Portland, Oregon. He then obtained his Master of Science in Health Evaluation Sciences and did his Infectious Diseases Fellowship at the University of Virginia. During his fellowship, he was mentored by Drs. Fredrick Hayden, Larisa Gubareva, and Tom Brachiale. His research focused on the immunopathogenesis of influenza and its treatment in immunosuppressed and hospitalized patients. He developed a immunocompromised mouse model of influenza to study the development of antiviral resistance. In addition, he did studies of the pharmacokinetics and outcomes of treatment with oseltamivir, zanamivir, and rimantidine in hospitalized patients. After leaving the University of Virginia, he undertook additional training in Transplant Infectious Diseases at the Massachusetts General Hospital and Harvard Medical School under the mentorship of Dr. Jay A. Fishman. He then joined the faculty of the Divisions of Infectious Diseases and Organ Transplantation at Northwestern University Feinberg School of Medicine in 2005. He is currently the Medical Director of the Transplant & Immunocompromised Host Infectious Diseases Service, Northwestern University Comprehensive Transplant Center.

He has continued to be a leader in the respiratory viruses research arena. He has been the first author of the only two prospective interventional studies in hospitalized patients as well as a lead investigator for studies to determine how to prevent and treat influenza in immunocompromised patients. He has recently provided advice to the President's H1N1 Subcommittee, NIH, and BARDA on issues related to influenza in hospitalized and immunocompromised patients. Additionally, he is considered an expert in adenovirus infections in immunosuppressed patients.

He, too, is heavily involved in other research and leadership positions in the Transplant Infectious Diseases arena. He has been a member and is the current chair of the Advisory Committee on Blood Safety and Availability in addition to chairing the United Network for Organ Sharing's Disease Transmission Advisory Committee from 2006 – 2010. He was recently elected as one of the At Large representatives on the OPTN/UNOS Board of Directors and member of the American Society of Transplantation Board of Directors. He, too, has been involved globally as the chair of the Infectious Diseases Editorial Group for the CNT/WHO Project NOTIFY Library and the leader of the Infectious Diseases group of the World Health Organization Bologna Initiative on Global Vigilance and Surveillance.

Lance Jennings

Canterbury District Health Board, Christchurch, New Zealand

Lance Jennings is Clinical Virologist to the Canterbury District Health Board, Director of New Zealand's WHO National Measles Laboratory, Clinical Associate Professor in the Pathology Department, University of Otago, New Zealand, Fellow of the Royal College of Pathologists, London and a Founding Fellow of the Science Faculty, Royal Australasian College of Pathologists. His principal research interests include the epidemiology, diagnosis, prevention and treatment of influenza and other respiratory viral infections.

Dr Jennings has been instrumental in the development of influenza control strategies for New Zealand, including the introduction of free influenza vaccine, establishment of influenza awareness education (NISG) and pandemic planning. He is co-founder and current chairperson of the Asia Pacific Alliance for the Control of Influenza (APACI). Dr Jennings has also been a member of WHO/Western Pacific Region Office (WPRO) Avian Influenza Outbreak Response (2004) and Expert Influenza (2005) teams in Asia and has held WHO short-term consultancies on measles and influenza in Asia and Europe.

He serves on several Ministry of Health and Ministry of Agriculture Advisory Committees, is an expert reviewer for research funding agencies, and is on the editorial board of the International Society for Influenza and other Respiratory Virus Disease (ISIRV) journal Influenza and other Respiratory Viruses. His service to virology in New Zealand and internationally was recognised in 2006 with the award of the Queens Service Order.

Yoshihiro Kawaoka

University of Wisconsin-Madison and Institute of Medical Science, University of Tokyo, Japan

Dr. Yoshihiro Kawaoka obtained his education in Japan, receiving his DVM in 1978 and his Ph.D. in 1983 from Hokkaido University. Early in his career, he identified the critical determinant for high pathogenicity of avian influenza viruses; this information is now used by the USDA and the Office International des Epizooties (World Organisation for Animal Health, OIE) as a criterion for rapidly identifying lethal and non-lethal avian influenza viruses. Dr. Kawaoka also established reverse genetics, which allows the generation of 'designer' influenza viruses. This technology – coupled with his findings regarding the attenuation of deadly influenza viruses – has been used to develop candidate H5N1 influenza virus vaccines, which have proven efficacious in clinical trials. Dr. Kawaoka has also undertaken the study of the 1918 Spanish influenza virus, which killed over 40 million people. He determined that infection by the 1918 virus caused an abnormal immune response. Information uncovered by Dr. Kawaoka is used globally by public health agencies as they undertake the enormous task of influenza pandemic planning.

In addition to his work with influenza virus, Dr. Kawaoka also studies Ebola virus. Because of its extreme virulence, Ebola virus had to be studied in laboratories designated as biosafety level 4 (BSL4), the highest containment environment possible. This requirement severely hampered the progress of Ebola virus research, as few such facilities exist worldwide. Dr. Kawaoka was the first to establish a pseudotype virus system that allows the analysis of Ebola virus glycoprotein under BSL2 conditions. Recently, Dr. Kawaoka developed another system that allows the study of the entire Ebola virus replication cycle under non-BSL4 conditions.

In recognition of his work, in 2006, Dr. Kawaoka was awarded the prestigious Robert Koch Award for innovative research in the field of influenza virology.

Nelson Lee

The Chinese University of Hong Kong, SAR, China

Dr. Nelson Lee is currently the Professor and Head of Division of Infectious Diseases in the Department of Medicine and Therapeutics, Faculty of Medicine, The Chinese University of Hong Kong (CUHK). He is also the consultant and chief of the clinical infectious disease services at the university hospital, the Prince of Wales Hospital; and clinical faculty associate, Stanley Ho Centre for Emerging Infectious Diseases, School of Public Health, CUHK. Dr. Lee is the Chairman of the specialty of Infectious Diseases in The Hong Kong College of Physicians, and serves in several advisory boards in Hong Kong's health authority and professional organizations, leading research, professional training, and inform clinical practices related to infectious diseases.

Dr. Lee's major research interests include severe Influenza and other emerging infections such as Severe Acute Respiratory Syndrome, focusing on the clinical aspects: disease manifestations, diagnosis, antiviral treatment, outbreak control, viral kinetics, and immunopathogenesis, particularly among the hospitalized adults. He has published over 120 articles in international peer-reviewed journals, and received multiple academic awards, both locally and overseas, on his contributions in improving the understanding and management of these emerging infections. He has served as an associate editor or referee for more than 20 local and international medical journals.

May Li Lim

KK Women's & Children's Hospital, Singapore

Dr LIM May Li is Consultant Obstetrician & Gynaecologist and Head of Peripartum Unit at the KK Women's & Children's Hospital, Singapore. She obtained MBBS from University of Queensland, Australia in 1993. She then pursued postgraduate training in United Kingdom with attainment of MRCOG in 2000 and Certificate of Completion of Specialist Training in Obstetrics & Gynaecology in 2007. She joined KK Women's & Children's Hospital in 2008. She is actively involved in undergraduate teaching and is faculty for OBGYN clerkship for the Duke-NUS Graduate Medical School, Singapore. She leads in the obstetric emergency training workshops for junior doctors and nursing staff at her institution. Her interests are in intrapartum care, high risk pregnancy and maternal medicine. When influenza A H1N1 (2009) struck, Dr Lim was part of the team who looked at the emergency planning of O&G services and who led in the management of pregnant mothers with the infection. This led to two publications sharing her institution's experience in the BJOG and Annals Academy of Medicine, Singapore respectively.

Andrew Luks

Harborview Medical Centre, Seattle, USA

Andrew Luks is an Associate Professor in the Division of Pulmonary and Critical Care Medicine and a member of the International Respiratory and Severe Illness Center (INTERSECT) at the University of Washington. Dr. Luks' practices critical care medicine across a wide range of disciplines including the medical, trauma and neurosurgical critical care and has a strong interest in care for the patient with severe respiratory failure and refractory hypoxemia. In addition to writing the American Thoracic Society document on Salvage Therapies for Acute Respiratory Distress Syndrome Caused by H1N1 Infection and creating numerous teaching resources in pulmonary and critical care medicine, he has written extensively on the topic of medicine in austere environments and, in particular, on high altitude medicine and physiology. A leading educator at his institution, Dr. Luks directs a large program of medical student education in respiratory and critical care medicine at the University of Washington for which he has received multiple teaching awards and leads annual post-graduate seminars on respiratory physiology and hemodynamic management at the American Thoracic Society International Conference.

Jenny McKimm-Breschkin

CSIRO Material Science and Engineering, Melbourne, Australia

Dr. McKimm-Breschkin is a Chief Research Scientist and Project Leader in Virology at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Victoria, Australia. After completing a first class honours degree at Monash University, Melbourne, Victoria, in 1974 Dr. McKimm-Breschkin won a Fulbright postgraduate award to the Microbiology department at Pennsylvania State University, Hershey Medical Centre, where she obtained her PhD in virology in 1978.

She returned to Melbourne University Microbiology department as a Queen Elizabeth II Post-doctoral fellow with research focussed on the recently discovered rotaviruses. A second postdoctoral position followed at the Walter and Eliza Hall Institute of Medical Research, as a Colin Syme Junior Fellow under the supervision of Jacques F. A. Miller working in the field of cellular immunology.
She then worked for the Australian Commonwealth Health Department for two years working on animal viruses, before joining CSIRO in 1987. She was part of the team involved in the development of Relenza, providing neuraminidase proteins for X-ray crystallography studies, as well as carrying out pre-clinical resistance studies as part of the drug registration.

She has continued to work in the evolving field of drug resistance, integrating both structural and functional approaches to understanding the mechanisms of resistance. Dr McKimm-Breschkin is a member of the Australian and American Societies for Microbiology.

Le Quynh Mai

National Institute of Hygiene and Epidemiology, Hanoi, Viet Nam

Dr Mai has been beginning in basis research of Arbor viruses, with 13 years experience of virology surveillance of Dengue viruses at NIHE-Hanoi, Vietnam. She was involved to National Dengue Surveillance project in Vietnam to improve Dengue viruses surveillance system, development controllability and prevention program.

Since 2003, when SARS outbreak in Vietnam, Dr Mai has extensive experience to SARS, identification SARS infection, isolation SARS virus, study on ecology of SARS.

More recently, Dr Mai expanded her study on influenza viruses, early detection human A/H5N1 infection, identification natural links between poultry outbreak and human endemic. She is the Head of Virology department as well as Director of NIC-NIHE, Hanoi, Vietnam. NIHE's group was the first to confirm that the pathogenic avian influenza A (H5N1) caused human infections in Viet Nam and has played a key role in characterizing this influenza virus and the immunopathology association with it. Monitoring National Influenza Program activities, upgrade laboratory abilities, improving relationships to animal researchers are her work now.

Adam Meijer

National Institute for Public Health and the Environment, Bilthoven, The Netherlands

Adam Meijer graduated in 1999 at the Faculty of Medicine of the University of Utrecht, the Netherlands, on the thesis: Chlamydia pneumoniae infection and atherosclerosis, a molecular analysis. Since 2001 he has worked in the field of influenza. During 2001 he studied the effect of antiviral prophylaxis and treatment on transmission of avian influenza between chickens at the Central Institute for Animal Disease Control, Lelystad, the Netherlands. In 2002 he continued his work on influenza at the National Institute of Public Health and the Environment, Bilthoven, the Netherlands; since 2003 heading the Section Respiratory Viruses of the Virology department of the Laboratory for Infectious Diseases. Research focuses on virological surveillance of acute respiratory infections, with a special focus on antiviral susceptibility and determination of molecular determinants for virulence and transmission networks at the animal-human interface. From 2003-2008 he coordinated the virology activities of the European Influenza Surveillance Scheme and was instrumental in the establishment and coordination of the Community Network of Reference Laboratories for Human Influenza in Europe (CNRL). Since 2008 he is one of the coordinators of the CNRL under the auspices of the European Centre of Disease Control and Prevention, with a special focus on influenza antivirals. He is advisor for the influenza external quality assessment programme of QCMD, Glasgow, Scotland, has been involved in several influenza consultation meetings of the WHO (e.g. on rapid tests for influenza and on susceptibility of influenza viruses for antivirals) and recently joined the WHO GISRS working group on antiviral susceptibility.

Arnold Monto

University of Michigan, Ann Arbor, USA

Arnold S. Monto is the Thomas Francis Jr. Collegiate Professor of Epidemiology at the University of Michigan School of Public Health in Ann Arbor. The major focus of his work has been the epidemiology, prevention and treatment of acute infections in the individual and the community. Respiratory infections, in particular influenza, have been a major interest, with special reference to the evaluation of vaccines in various populations and the assessment of the value of antivirals. He has worked on these issues in tropical as well as temperate regions. He led the studies of respiratory infection in Tecumseh, MI, a landmark study of infection in the community. He has studied various approaches to influenza vaccine use, particularly to control transmission of the virus in the community and in nursing homes.

Dr. Monto is involved in assessing the efficacy of various types of influenza vaccine in prophylaxis and neuraminidase inhibitors and other compounds in prophylaxis and therapy of influenza, including implications of antiviral resistance. He now heads an observational study of effectiveness of influenza vaccines in various settings. His recent activities have included evaluation of face masks and hand hygiene in the control of influenza transmission and determination of efficacy of the traditional inactivated and live attenuated influenza vaccines. He works extensively with national and international organizations on issues related to pandemic preparedness. He has been a member of the Pulmonary Diseases Advisory Committee and the National Allergy and Infectious Disease Advisory Council of the US National Institutes of Health. He is a past president of the American Epidemiological Society and the 2009 recipient of the Alexander Fleming Award of the Infectious Diseases Society of America for lifetime achievement. He was a member of the Emergency Committee making recommendations to the World Health Organization during the past influenza pandemic.

Masashi Mizuguchi

University of Tokyo, Tokyo, Japan

Professor Masashi Mizuguchi is a pediatric neurologist and neuropathologist. He graduated from the Faculty of Medicine, University of Tokyo, Tokyo, Japan and obtained M.D. in 1980, and since then has been engaged in clinical practice and research at Tokyo Metropolitan Fuchu/Neurological Hospital, Fuchu, Japan (1981-1986), University of British Columbia, Vancouver, Canada (1989-1991), National Center of Neurology and Psychiatry, Kodaira, Japan (1993-1996) Jichi Medical University, Shimotsuke, Japan (1996-2004), and the University of Tokyo (1986-1993, 2004-present).

Peter Openshaw

Imperial College, London, UK

Peter Openshaw works on immune-mediated lung disease especially that caused by respiratory syncytial virus (RSV) and influenza. His work is focussed on discovering links between viral infections and wheezing disorders. His research interests include T cell mediated immunopathology, innate immunity, neonatal immunology and immunoregulation.

He trained in respiratory medicine and is a consultant physician at St Mary's Hospital, Paddington. He studied immunology at NIMR, Mill Hill (1985-88) and became the founding Director of Imperial's Centre for Respiratory Infection (CRI) in 2008, bringing together many of Imperial College's established leaders and groups with expertise in molecular, cellular, animal and human studies of respiratory infections.

During the 2009 flu pandemic he was part of the Flu-CIN collaboration and leads the MOSAIC consortium, studying causes of severe influenza in hospitalised patients. He is also investigating the effects of RSV infection in adult volunteers.

He is a member of the flu and RSV subcommittees of JCVI and Vice-President of the European Scientific Working Group on Influenza (ESWI).

Malik Peiris

The Chinese University of Hong Kong, SAR China

Malik Peiris is Chair in Virology, School of Public Health at The University of Hong Kong and Director of the Centre of Influenza Research. His current research focuses on the virology, evolution, pathogenesis and epidemiology of animal and human influenza and other respiratory viral infections. He has a particular interest in emerging viral infections and in virus infections at the animal-human interface and the molecular determinants that permit animal viruses to acquire transmissibility in humans and in the pathogenesis of severe viral pneumonia. In 2003, he played a key role in the discovery that a novel coronavirus was the cause of SARS. He was elected a Fellow of the Royal Society of London in 2006, awarded the Legion d'Honneur of the Republic of France in 2007 and the Silver Bauhinia Star of the Hong Kong Special Administrative Region in 2008. He serves on key international scientific advisory bodies including the WHO and FAO.

Shigeru Saito

University of Toyama, Toyama, Japan

Professor S. Saito has received his Bachelor of Medicine in 1980 from Nara Medical University, Japan, and PhD from Nara Medical University in 1985. During a postdoctoral period at Kyoto University Virus Center, he studied molecular biology and immunology, especially cytokines. Since 1990 he has served as associate Professor in the Department of Obstetrics and Gynecology, Nara Medical University. Since April 1998, he has been Professor and Chairman in the Department of Obstetrics and Gynecology, Toyama Medical and Pharmaceutical University. He is one of the Editor-in-Chief of J. Reprod. Immunol.

His research interests have centered primarily at understanding the immunology at the maternal and fetal interface with particular emphasis on the roles of cytokines and chemokines and the immune cells that produce them in reproduction and perinatal medicine.

He was a Committee Chair, Perinatal Committee of Japan Soc. Obstet. Gynecol., so he has studied the outcomes of pregnant women infected with Pandemic (H1N1) 2009 and infants exposed to oseltamivir or zanamivir. He established acute necrotizing encephalopathy as a new syndrome in 1993-1995, created a classification system of acute encephalopathy by 2007, and has recently found gene mutations and polymorphisms that predispose children to acute encephalopathy. Now he is a member of the executive committee of Japanese Society of Child Neurology and Japanese Society of Neuropathology, and is directing clinical, pathologic and genetic studies of acute encephalopathy, tuberous sclerosis and brain malformation.

Nahoko Shindo

World Health Organization, Geneva, Switzerland

Professor Shindo is Medical Officer and leads Influenza and Respiratory Disease Team of Pandemic and Epidemic Disease Department at the World Health Organization in Geneva. Her background is in medicine, infectious disease and public health. She completed medical trainings at St Thomas' Hospital, Radcliffe Infirmary, Jikei University Hospital and her Ph D in Medical Science (microbiology and cell immunology) at Jikei University School of Medicine followed by specialist training in infectious disease/public health at Infectious Disease Surveillance Centre, National Institute of Infectious Diseases (NIID) in Tokyo, which is one of five WHO Influenza Collaborating Centres.

She has worked with WHO since 2002. During her time at WHO Professor Shindo has been involved in activities such as epidemic intelligence and verification, outbreak responses and influenza pandemic preparedness. She participated in major WHO responses including SARS, avian influenza, Indian Ocean Tsunami, the deadly viral hemorrhagic fever outbreaks in Africa, and more recently, 2009 influenza pandemic.

Since January 2012, she has been leading Influenza and Respiratory Diseases Team in the department. Her responsibility also extends to cover WHO Public Health Research Agenda for Influenza, assessment of influenza disease impact and influenza pandemic preparedness. Her other area of work is clinical aspects of influenza virus infection including use of antiviral medicine. During emergencies, she serves as one of the operational staffs of WHO's Strategic Health Operations Centre and deals with severe acute respiratory disease and avian influenza outbreaks of international public health concern. She and her team also combat against highly contagious and dangerous pathogens in the field to control the outbreak.

Norio Sugaya

Keiyu Hospital, Yokohama City, Japan

Norio Sugaya, M.D., is a pediatrician and the Director of Keiyu Hospital in Yokohama City, Japan. He received his PhD from the Keio University School of Medicine for a thesis on diagnostic testing for influenza virus infection. He has been active in the area of influenza vaccines for children and the mass vaccination programs for schoolchildren for over 15 years. His international experience includes participation as a member of the Pharmacological Management of Pandemic Influenza A (H1N1) panel, held in 2009 at World Health Organization in Geneva and a member of Multinational Influenza Seasonal Mortality Study of NIH/FIC. He is also a central member of committees examining pandemic influenza under the auspices of the Japanese Association for Infectious Diseases and the Japan Pediatric Society. His current research involves the study of the effectiveness of neuraminidase inhibitors, including newly approved drugs such as laninamivir and peramivir. He is also participating the GLaMOR NIVEL-WHO project to estimate the global burden of 2009 pandemic influenza.

Tim Uyeki

National Center for Immunization and Respiratory Diseases, Atlanta, USA

Tim Uyeki is Chief Medical Officer in the Influenza Division, National Center for Immunization and Respiratory Diseases, U.S. Centers for Disease Control and Prevention (CDC). Dr. Uyeki has worked at CDC on the epidemiology, clinical aspects, prevention and control of influenza in the U.S. and worldwide since 1998. He is an Associate Clinical Professor in the Department of Pediatrics, University of California, San Francisco, and an Adjunct Associate Professor in the Hubert Department of Global Health, Rollins School of Public Health, Emory University. Dr. Uyeki has served as a consultant to WHO on clinical and epidemiological issues related to seasonal, zoonotic, and pandemic influenza, including extensive international H5N1 outbreak experience for WHO and CDC. He has also worked on Ebola and anthrax investigations, HIV/AIDS, hepatitis A, polio eradication, and was the CDC team lead for the WHO Vietnam SARS team (2003).

John Watson

Health Protection Agency, London, UK

Professor John Watson MB BS, MSc, FRCP, FFPH - is a Consultant Clinical Epidemiologist and Head of the Health Protection Agency Respiratory Diseases Department at Colindale in London. He is an Honorary Professor in the Department of Infectious and Tropical Diseases at the London School of Hygiene and Tropical Medicine and Visiting Professor in the Department of Primary Care and Population Sciences at University College London. He qualified from St Bartholomew's Hospital Medical School, London, in 1979 and subsequently trained in clinical respiratory medicine and infectious disease epidemiology before being appointed to the Health Protection Agency in 1989. His main interests include tuberculosis and acute respiratory infections (particularly influenza, legionnaires and SARS). His work has focussed on the surveillance, prevention and control of these diseases at the local, national and international levels as well as related research.

Professor Watson sits on a number of national and international committees including the National Expert Panel on New and Emerging Infections and the BCG and influenza sub-committees of the UK DH Joint Committee on Vaccination and Immunisation. He is Chairman of the International Society for Influenza and other Respiratory Virus Diseases.

Christopher Wong

Genome Institute of Singapore, Singapore

Dr Christopher Wong trained as a cancer biologist with Ruth Muschel at the University of Pennsylvania, receiving his PhD in 2001. He returned to Singapore as one of the founding scientists at the Genome Institute of Singapore (GIS), where he became an expert in microarray technology development and applications.

During the SARS outbreak, he invented the SARS Resequencing Chip, which was used for epidemiology studies of the SARS virus in Singapore, as well as to demonstrate that a subsequent case was caused by a lab accident. Subsequently, this technology was adapted for Dengue Resequencing as well as H1N1(2009) Resequencing. The H1N1(2009) Resequencing kit is still being used in Mexico for studies on H1N1.

From 2008-2011, he was concurrently Head of the Biopolis Shared Facilities, responsible for managing the shared scientific services laboratories. From 2009, he was appointed as Chief Scientific Officer at the GIS, responsible for translating GIS research discoveries in cancer genomics as infectious diseases into products which can be used for patient care in the hospitals, and for commercialization. In 2011, he founded PathGEN Dx Pte. Ltd., a spin-off company developing Infectious Disease Diagnostics products based on technology licensed from GIS.

Maria Zambon

Health Protection Agency, London, UK

Maria is Director of UK Health Protection Agency (HPA) Reference Microbiology Services. The Centre's remit includes provision of UK national microbiology reference facilities and infectious disease surveillance.

Maria is medically and scientifically qualified and has worked as a clinical virologist with R&D interests on vaccines, antivirals and surveillance. Main research interests are the diagnosis of viral infections in humans, especially RNA viruses, emerging infections and the development of new vaccines. Maria is Head of UK National Influenza Centre, and has a research group focused on influenza including antivirals and vaccines.

During 2009, Maria acted as UK national incident director in response to pandemic influenza and was responsible for multi-disciplinary team leadership to provide situation analysis, risk assessment and virus tracking evidence for UK Government decision making during the pandemic.

Since 2010 Maria has been responsible for the planning and execution of HPA microbiology support to the Olympics. This has involved development of several new diagnostic tests for detection of GI pathogens and Leptospirosis and introduction into clinical frontline UK laboratories to support public health investigations.

Author Index

Notes

Notes

Notes

Notes

Notes

Notes

Notes