Introduction
About 200 researchers and clinicians from different countries attended this symposium. 43 posters were presented during the symposium, including the one from the CML (Dr. Christophe Mérieux Laboratory, Fondation Mérieux / IPB-CAMS) located in Beijing.

Virology / Diagnostics

Virology of Parechovirus
Katja C. Wolthers
Academic Medical Center, Amsterdam, The Netherlands

* Discovered from children with diarrhea.
* Associated with gastrointestinal and respiratory infections, less with CNS symptoms.
* Not detected with EV (Enterovirus) specific molecular techniques
  Genetically distinct, reclassified as Parechovirus
  Echo 22 (EV) → HPeV1 (Par)
  Echo 23 (EV) → HPeV2a (Par), HPeV2b (Par)
* In cell cultures same CPE as for enteroviruses (HPeV1, HPeV2).
* Widespread, mainly in children (adult seroprevalence > 95%), HPeV1 frequent, HPeV2 rare.
* 14 HPeV types in “new” genus Picornavirus family.
* HPeV-1 most frequent in W-Europe
  Fever, gastrointestinal and respiratory symptoms
* HPeV-3 next most prevalent
  o Infect young infants
  o Neonatal sepsis, CNS infection
  o Bi-annual circulation, in summer
* HPeV should be included in viral diagnostics (5’ UTR PCR on CSF, stool, throat swab, blood).
* However HPeV are present in control populations, but at lower viral load (like most of respiratory viruses)
  o Case-control studies should be extended (severity scores, summer months, etc...).
Virology of Coronavirus
Kathryn Holmes
University of Colorado School of Medicine, Denver, Colorado, USA

Recent data from the study of H. Keipp Talbot and al. Which describe the pediatric burden of coronaviruses (Pediatr Infect Dis J 2009 ;28 : 682-687)

- 5% (1824 samples) were positive for HcoVs (229E OC43 or NL63).
- HcoVs were associated with
  - 67.3 URI episodes/1000 child-years (children < 5 years)
  - 11.4 LRI episodes/1000 child-years (children < 5 years)
  (12/1000 LRIs for PIV and 8/1000 for IV)

- LRI in children 6-23 months of age
  - HcoVs \approx 8 \%
  - HMPV \approx 12 \%

Spike (S) / receptor interactions in cross-species transmission of CoVs

- Recombination swaps S from unknown host
- Recombination swaps pieces of S (RBDs or RBMs)
- Mutations in S allow antigenic drift and binding to receptor homologs

CoV drug targets

- Receptor blockade
- Inhibitors of replicase, or other RNase enzymes
- Inhibitors of encoded proteases, 3-C like and papain-like
- Inhibitors of conformation changes in S protein
- Virus assembly inhibitors (Heptad repeat C peptide, fusion peptide).

Identification of Human Rhinovirus Strains Associated with Severe Respiratory Illness
Wai-Ming Lee
School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin, USA

Important features of Human Rhinovirus (HRV) Infection

- The most common respiratory viral infection
  - > 50% of common colds of 6.8 billions people each year

- Wide range of clinical outcomes
  - Common cold
  - Exacerbation of severe lower airway illness : asthma, COPD, cystic fibrosis
Bronchiolitis/pneumonia hospitalization of young children
- Asymptomatic: 20-30% of all HRV infections

- Caused by many serotypes/strains
  - 100 classical serotypes (HRV-A and HRV-B)
  - Many newly discovered HRV-C strains

HRV is one of the most common pathogens in infants

HRV infections occur year-round in infants
- Different from other results observed with older population (living in the same geographical area)

Rate of HRV-A and HRV-C infection is similar
- Together they account for > 90% of HRV infections in infants

HRV-A and HRV-C infections have similar probability of inducing severe illness, but significantly higher than HRV-B infection

The ability to induce severe illness vary with strains
- Strain HRV-C W12 is the more pathogenic
- Strain HRV-B R52 is less pathogenic.

GIS H1N1 Microarray: Accurate, low-cost, high-throughput method for biosurveillance and molecular epidemiology
**Christopher W. Wong**
Genome Institute of Singapore, Singapore

The speaker described a 12-plex microarray platform which has 2x coverage for the whole genome, with 4x-8x coverage for regions important for antibody binding, drug resistance, or virulence. The platform is paired with customized algorithms which could overcome systemic biases inherent in microarray-sequencing platforms to generate high quality sequence calls.

Details of this Flu array
- 121,928 oligos are synthetized on the array
  - 2x coverage for H1N1 genome, up to 8x coverage for selected regions
  - oligos 29-39 mer, median of 30 mer

- 8,236 control oligos
- The oligos are tiled across the genome at 1 nt resolution
- The base being re-sequenced is located in the middle, and all 4 possible bases are synthetized on the array
- Using of Nimblegen technology for the synthesis of the array
- ≈ 140,000 probes/plex
- 12 plex/chip
- maskless photolithography

**Protocol overview**

- **Day 1**
  - RT patient/cell line RNA into DNA
  - Amplification of DNA by PCR
- **Day 2**
  - end-labelling of DNA with Cy3
  - hybridization DNA on the array (6h min)
- **Day 3**: scan on 5 mm scanner, read sequence

Whole process can be also done in 2 by experienced person.

- **Analysis workflow**
  - Scanning of the microarray and generating of the image file
  - Generating of the raw signal intensities (Nimblescan)
  - Using of EvolSTAR software to call sequence bases
    - generate PDF graphical view
    - generate FASTA file
  - Tertiary analysis, such as the BII website

- **Overview of GIS algorithm**
  - Generates base calls, distinguishes high confidence from low confidence calls in FASTA output
  - Integrates best base call from multiple coverage of different genomic segments
  - Compensates for systemic hybridization bias
  - Generates graphical view for easy assessment of sequence quality, and overview of mutations found
  - Identifies flu strain which most closely resembles the sample sequenced

- **Overall performance**
  - Evaluation on > 100 samples conducted in Mexico and Singapore
  - Call rate > 99% with accuracy > 99.9%
  - The software can also highlights segments where there exists a possibility of genome reassortment.

**GIS H1N1 Surveillance Toolbox**

- GIS H1N1 8-segments RT-PCR primers mix
- GIS optimized 3rd party PCR reagents
- Nimblegen microarray reagents
- Nimblegen Nimblescan 2.5 software
- EvolSTAR : GIS proprietary analysis software (sequence calling and visualization)
**Conclusions**

- All the flu outbreak clusters in Singapore were caused by the New York variant of the H1N1 strain, and not the Mexican variant
- Advantages
  - easy to use, no sequence assembly
  - speed (< 30h)
  - sensitivity (qPCR CT< 25)
  - cost (< 500 USD / plex)
  - RT-PCR primers can be used for other sequencing technologies
- Limitations
  - Can’t get the first and last 14 bases of the sequence
  - homopolymer regions (longer than 4 bases)

**For more information:**


**Pandemic Planning and the 2009 H1N1 Pandemic**

**Pandemic Planning: Lessons learned from the virology of the H1N1 pandemic**

Nancy Cox
Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

**Having influenza vaccines even 4-6 weeks earlier likely to make big difference in disease reduction and vaccine acceptance**

**Set goals: Move detection of novel influenza viruses closer to emergence and the availability of the vaccines prior to disease occurrence**

- What we need ?
  - Sustainable multi-use respiratory disease surveillance platforms for the identification of novel virus emergence globally (pandemics emerge anywhere)
  - Library of truly HG reassortants tested and production-ready
  - Streamline methods for measuring vax Ag content, suitable for all HA protein vaccines and capable of measuring NA, M2, etc... (HPLC and MSID methods for advancement of regulatory sciences)
  - Use of adjuvants for Ag sparing and more robust immune response
- Improve vaccine capacity globally (progress but much more to do)
- Enhance partnership domestically and globally

**Global preparedness for a possible H5N1 pandemic made response to the H1N1 pandemic much easier**

Well-developed global networks (eg. GISN), relationships and partnerships allowed for a more coordinated and measured global response

Rapid information and virus sharing are essential for an effective pandemic response (must preserve rapid sharing for development of diagnostic tests and vaccines)

Better surveillance for influenza in domestic animals, essential for pandemic preparedness

**H1N1 reinforced understanding of ‘One Health Concept’**

**Tropism and Host Responses of the 2009 Pandemic H1N1 Influenza Virus in ex vivo and in vitro cultures of Human Conjunctiva and Respiratory Tract**

Renee W. Y. Chan

The University of Hong Kong, Li Ka Shing Faculty of Medicine, Queen Mary Hospital, Pokfulam, Hong Kong SAR, People’s Republic of China

**Novel pandemic influenza H1N1 (H1N1 pdm) virus of swine-origin causes mild disease but occasionally leads to acute respiratory distress syndrome and death**

It is important to understand the pathogenesis of this new disease in humans

They reported here the comparison between the virus tropism and the host response elicited by pandemic H1N1 pdm and seasonal H1N1 influenza viruses in ex vivo cultures (human conjunctiva, nasopharynx, bronchus and lung) and in vitro cultures (human nasopharyngeal, bronchial and alveolar epithelial cells)

- They found comparable replication and innate immune host responses in seasonal and pandemic H1N1 viruses
- H1N1 pdm virus differs from seasonal influenza virus in its ability to replicate in human conjunctiva, suggesting subtle differences in receptor binding profile and highlighting that the potential role of the conjunctiva as an additional route of infection with H1N1 pdm
- The greater viral replication competence in bronchial epithelium at 33°C may also contribute to the slight increase in virulence of the H1N1 pdm
- In contrast with highly pathogenic influenza H5N1 virus, H1N1 pdm does not differ from the seasonal influenza virus in its intrinsic capacity for cytokine dysregulation
Global gene expression profile revealed by microarray analysis of primary human alveolar type I-like pneumocytes infected with H1N1 pdm or seasonal H1N1 virus, showed that a number of genes down regulated by seasonal H1N1 virus were unaffected by H1N1 pdm (differences currently under investigation).

These results suggest that the H1N1 pdm virus differs in modest but subtle ways from the seasonal H1N1 virus in its intrinsic virulence for humans, findings that are in accord with the epidemiology of the pandemic to date.

Pathogenesis

New insights from studies on influenza
Yoshi Kawaoka
University of Wisconsin, Madison, Wisconsin, USA ; University of Tokyo, Japan

Highlights:

The efficient replication of the 2009 pandemic H1N1 virus in pigs explains the multiple outbreaks caused by this virus in pig population worldwide

PB2-590S and PB2-591R are responsible for the efficient replication in humans of the 2009 pandemic virus

Pandemic H1N1 influenza viruses with the 591R replicate more efficiently than one with 627K or 701N in ferrets

The 2009 pandemic influenza A viruses replicate better than seasonal viruses in the lungs of animal models

Additional mutations in PB2 are unlikely to make the 2009 pandemic virus more transmissible

Can systems and computational virology help us understand deadly influenza viruses & develop better drugs and vaccines?
Michael Katze
Washington National Primate Research Center, University of Washington, Seattle, Washington, USA

Highlights:

The 3 SOIV (Swine-Origin Influenza Virus) tested are more virulent and clinically distinct from seasonal H1N1 influenza
These 3 SOIV isolates cause different degrees of pneumonia; mild/moderate (Mex4108), moderate/severe (Ca04), severe (Mex4487)

Mex4487 showed prolonged replication in lower respiratory tract correlating with prolonged and more severe pneumonia

Ca04 has the highest potential of shedding (higher transmission?)

Due to a low probability of transmission, more virulent variants may have been lost during the pandemic and were outgrown by more transmissible low virulent strains

This supports the current situation with the SOIV pandemic

**Update of new respiratory viruses: New data**
Albert Osterhaus
Erasmus MC, Rotterdam, The Netherlands

**Worldwide impact of hMPV (retrospective study over 20 years)**
- Peak age 6-12 months
- Peak after influenza virus and RSV
- Clinical signs of hMPV and RSV are similar
- Re-infections occur frequently
- Associated with asthma
- Young children: 5 – 25% of young patients hospitalised for RTI
- Patients with underlying diseases
  - Transplant patients
  - Hematologic malignancies
  - Patients with lung disease
- Elderly people
- Normal people
  - ≈ 5% of RTI in community surveillance studies

**Viruses associated with bronchiolitis in infancy**
- RSV
- PIV
- Influenza
- hMPV
- HRV-A, HRV-B, HRV-C
- Enteroviruses
- Coronaviruses: OC43, NL63, HKU1, SARS
- Bocaviruses
- Polyomaviruses: WU, KI
- Adenoviruses
Identification and characterization of a new orthoreovirus from patients with acute respiratory infections

- Kampar virus (KamV)
- High probability that Kampar virus originated from bats and was transmitted to humans via bat droppings or contaminated fruits.

Severity of new H1N1 influenza pneumonia in ferrets approaches that of H5N1

Cats experimentally infected with pandemic H1N1 virus

Molecular screening techniques have allowed the identification of an increasing number of previously unrecognized human respiratory (and enteric) viruses: hMPV, HCoV NL63, HCoV HKU1, hBoV, KI/WU PyV

- The relative clinical impact is still largely unknown for several of them
- Many coinfections occur between these and other viruses
- Zoonotic transmission may lead to the emergence of new human viruses (flu viruses, Henipah viruses, SARS-CoV, hBoV, MelV/KamV).

Hypersusceptibility to lethal bacterial pneumonia in the post-influenza recovery period

Wilbur H. Chen
Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland, USA

- **Background**
  - Influenza-associated secondary bacterial infections are a major cause of illness and death
  - Ethiologic bacterial pathogens are those that commonly colonize the nasopharynx (Pneumococci, Staph aureus, etc…)
  - Mechanisms not completely understood
  - Existing models of infection examine co-infection with influenza and bacteria
  - However, risk of secondary infection persists after viral clearance (recovery phase).

- **Objective**
  - Develop a model of secondary infection during recovery phase (mice model).

- **Conclusions**
  - Mice in recovery from influenza remain hypersusceptible to a secondary bacterial infection
  - The secondary infection is associated with rapid dissemination of bacteria
  - Local and systemic cytokine responses are altered / blunted
Evidence for sustained impairment of the host immune response (alteration of the lung microenvironment).

**Remarks**
- Candidates leading to impaired host innate immune response:
  - Alternatively activated macrophages

- Secondary bacterial infections are common after acute viral infections (measles, RSV, parainfluenza, etc…) – Common mechanism?

**Clinical and diagnostic considerations of respiratory viral infections (satellite symposium sponsored by abbott molecular and luminex corporation)**

**Gaining insight into respiratory viral infections in hospitalized adults: lessons learned using the xtag rvp during the current influenza pandemic**

**Michael G. Ison**
Division of Infectious Diseases and Organ Transplantation Northwestern University Feinberg School of Medicine, and Transplant & Immunocompromised Host Infectious Diseases Service, Northwestern Transplant Center, Chicago, Illinois, USA

**RVI: Importance of diagnosis**
- Choice of therapy
  - Antiviral vs antibacterial
    - Resistance
    - Indirect impact on bacteria

- Isolation & institution of prophylaxis
  - Immunocompromised
  - Elderly / nursing home populations
  - Effective use of hospital isolation space

- Epidemiology & public health

**RVI: General diagnostic issues**
- Samples
  - Upper airway
    - Nasal: swab or wash
    - NP: swab
  - Lower airway
    - Transbronchial biopsy
• BAL
• Non-bronch BAL

  ▪ Non-respiratory sites

  o Serology
  o Rapid Detection Antigen
  o Direct Fluorescence Antibodies
  o Cultures
    ▪ Traditional
    ▪ Rapid (Shell vial)

  o Molecular assays
    ▪ Single agent vs multiplex

**xTAG Respiratory Virus Panel (Luminex)**

  o US FDA cleared
    ▪ Inf A (H1, H3); Inf B; RSV (A,B); PIV 1, 2, 3; hMPV; Rhinovirus; Adenovirus

  o EU CE marked
    ▪ Inf A (H1, H3); Inf B; RSV (A,B); PIV 1, 2, 3; hMPV; Rhinovirus / Enterovirus; Adenovirus; Coronavirus (NL63, 229E, OC43, HKU1, SARS)

**Northwestern Memorial Hospital: Diagnostic strategy for RVI**

  o Available diagnostic options
    ▪ Viral culture
      ▪ Available only by request
      ▪ Use R-Mix (Diagnostic Hybrids: A549 + Mv1Lu cell lines)

    ▪ Rapid Antigen Detection
      ▪ Only available in the ED
      ▪ QuickVue Influenza A+B (Quidel Corp)

    ▪ Molecular Assays
      ▪ ProFlu+ (Gen-Probe Prodesse, Inc)
      ▪ xTAG Respiratory Virus Panel (Luminex)

**Respiratory Viruses in hospitalized adults**

  o Cause of admission
    ▪ Primary infection (influenza, RSV)
    ▪ Community acquired pneumonia
    ▪ Fever
    ▪ Acute exacerbation of COPD
    ▪ Exacerbation of underlying diseases
      ▪ Congestive heart failure
• Myocardia ischemial
• Diabetes Mellitus
• Chronic kidney disease
• Neurologic disease / stroke

**Influenza: Hospitalization**
- Antiviral therapy associated with:
  - Shorter duration of virus shedding
  - Shorter hospitalisations (in some studies)

- Low mortality
  - All admitted patients (OR 0.21)
  - ICU patients (100% vs 53% survival)
  - Benefits even if started after 48 hours of symptoms

- Treat if suspect (better outcomes)
  - If you are testing for influenza, you should be treating empirically for influenza.

**RVI in hospitalized adults: Next steps**
- Need for better diagnostics
  - More rapid turn-around time
  - Greater ease in use for high volumes
  - Rapid detection of resistances

- Need for better therapeutics
  - Clearly numerous respiratory viruses are causing significant illnesses
  - Few available antivirals

- Natural history studies are needed

**Remark:** Luminex has introduced a new RVP version.
- **xTAG RVP FAST**
  - 3, 5 hours including extraction (vs 6 – 7 hours for the classical version)
  - Fewer steps (60% less than the classical one)
  - 19 viruses and subtypes detected (vs 20)
  - Bocavirus included, Adenovirus sensitivity improved, SARS and Inf A H5 removed
  - All the necessary reagents included.

**Epidemiology / Impact**
Enterovirus 71 and hand, foot and mouth disease
Jing Zhang
Chinese Centers for Disease Control and Prevention, Beijing, P. R. CHINA

HHMD in Western Pacific area in 2008
- Hong Kong: 163 (EV71: 98)
- Macao: 395 (EV71: 56)
- Taiwan: 373 severe cases with 14 deaths
- Malaysia: 1, 943 cases
- Mongolia: 3, 214 cases
- Singapore: 29, 686 cases with 1 death
- Vietnam (20 southern provinces): 5, 865 cases with 23 deaths.

HFMD outbreaks in mainland China after 2000
- In 2000: Zhaoyuan county (Shandong)
- In 2003: Taian city (Shandong)
- In 2007: Linyi city (Shandong)
- In 2008: Fuyang city (Anhui)
- In 2009: Shangqiu city (Henan), Heze city (Shandong).

Risk factors for HFMD disease
- Children under 5 years are more susceptible
- Poor personal hygiene
- Contact with HFMD patients
- Contact with asymptomatic carriers (children and adults)
- Children in settings gathering with a long time and close contact (kindergartens, schools)
- Contamination of toys, furnitures and premises.

Transmission dynamics
- Asymptomatic case: source of infection
  - Prevalence and infectivity of the asymptomatics
  - Children to adults or adults to children
- Infectious after onset of the symptoms and may last more than 2 weeks after recovery
- Infection spreading quickly among children
  - Picture of transmission in childcare centers, schools and households
- Less cross protection among different serotypes of enteroviruses.

Estimation of disease burden of HFMD in mainland China
- About 84% outpatients in reported cases
- About 16% hospitalizations
- About 2% PICU needed in hospitalization cases
- About 2, 6 deaths per 10, 000 reported cases
- Heavy economic burden: 0, 5 – 1 billion RMB / year.
HFMD should be considered as an important public health issue
EV71 infection is an important fatal cause for young children and is a new challenge for China
The understanding on nosogenetic, host factors, and other risk factors of the EV71 infection is limited
EV71 vaccine development is one of the key measures for the control and reducing of this infection.

**Nosocomial Respiratory Viral Infections**
**Allison McGeer**
Mt. Sinai Hospital, University of Toronto, Canada

* Nosocomial influenza
  o Incidence
    ▪ 3 / 1000 admissions (California, 87)
    ▪ 8 / 1000 admissions (Virginia, 88 – 94)
    ▪ 6 / 1000 admissions (Houston, 88)
  o Case fatality rate
    ▪ 7 % (14 / 213) – (Multiple sources)
  o Cost
    ▪ 4,050 USD / case (S. Dakota, 93)
    ▪ 3,622 USD / case (US, 2000)

**Why worry about hospital-acquired RVIs?**
  o Incidence may be higher in the hospital than in the community
  o Disease is more severe in hospitalized patients
    ▪ RSV: 4, 4 % noso CFR (Langley, Ped 1997)
    ▪ Ad7h: 16 % pediatric noso CFR (Larranaga, JCV 2007)
    ▪ Influenza: 15 % noso CFR (unpublished information)
  o Outbreaks occur
  o Occupational disease occurs in staff.

**What are hospital influenza reservoirs?**
  o Vanhems (ECCMID 2009)
    ▪ 6 Pt – Pt, 7 Pt – HCW, 6 HCW – HCW
  o Cheng (JHI 2009)
    ▪ 1 Pt – Pt, 1 Pt – HCW, 2 HCW – HCW
  o McGeer (unpublished)
    ▪ 3 HCW – HCW, 1 HCW – 1 Pt

**Challenges**
  o Most adult patients with fever and respiratory symptoms don’t have a communicable disease
Some patients without fever, do have a communicable disease
Staff work with acute respiratory illnesses
How are respiratory viruses transmitted?
Potential interventions
- Increase winter humidity
- Increase space between patients
- Improve adherence to hand hygiene
- Use additional precautions for some patients
- Require symptomatic staff and patients to wear masks
- Wear chirurgical masks when in contact with other staff
- Wear N95 respirators when in contact with other staff.

Clinical

Bocavirus
Olli Ruuskanen
Turku University, Turku, Finland

Highlights:
- Difficult to culture
- No animal models
- Prevalence 5 – 10%
- Occurs usually during winter months
- All children infected before 5 years of age, rare in adults
- High copy number in NPA
- Should be included to multiplex PCR assays
- Often with other respiratory viruses, interaction?
- Repeated infections occur?
- Common cold, acute wheezing, pneumonia, otitis media, diarrhea
- Not much asymptomatic shedding, no long-term persistence

Enhancing identification of known and emerging respiratory viruses
(satellite symposium sponsored by ibis biosciences)

PLEX-ID: A new technology for identification of influenza and other respiratory viruses
Charlotte Gaydos
Division of Infectious Disease, Medecine, John Hopkins University, Baltimore, Maryland, USA

Challenges and requirements for surveillance of respiratory viruses
- Epidemics happen quickly: ≈ 4 weeks for pdm H1N1
- Surge capacity is an issue
Challenge to have a surveillance tool that gives a rapid diagnosis, as well as genotype, that can provide public health guidance for «just in time» diagnostics.

**Current types of tests for influenza and other respiratory viruses**
- **Rapid tests**
  - Insensitive, non-specific
- **DFA**
  - Rapid, less sensitive, specific
- **Shell vial culture**
  - Sensitive
- **Roller tube culture**
  - Sensitive, slow
- **Real time qPCR / RT-PCR**
  - Sensitive, slow
- **Pyrosequencing**
  - Expensive
- **Flow through microsphere array**
  - Rapid, expensive

**PLEX-ID as a new surveillance tool**
- PLEX-ID technology combines broad amplification with PCR, and electrospray ionization mass spectrometry
- Designed to provide broad identification, detailed genotyping and characterization, and recognition of known and emerging strains
- Designed to track a potential pandemic in real-time.

**Remark**: data mentioned below were obtained on the T 5000 (early version of the PLEX-ID)

**Respiratory virus surveillance I assay**
- Influenza virus validation at Ibis
  - > 650 blinded samples
    - Nasal aspirates, nasal swabs, nasal washes, throat swabs, bronchial washes, tracheal aspirates
    - Samples from 1999 – 2006, across USA
    - Correctly identified all influenza A types
      - 149 H3N2
      - 34 H1N1
    - 67 influenza B
    - Sensitivity: 96.8 %
    - Specificity: 97.5 %
    - PPV: 96.0 %
    - NPV: 98.0 %

**Respiratory virus surveillance II assay**
Provides a single test platform for surveillance-level detection and identification coverage for 6 families of respiratory viruses
- Adenovirus: 2 primer pairs (hexon gene)
- Coronavirus: 2 primer pairs (RdRp and HSP14 in orf 1ab)
- RSV, hMPV: 2 primer pairs (RNA polymerase large subunit L gene)
- Influenza virus: 4 primer pairs (PB1, PB2, NP, NS2), distinguishes A, B and C
- Respirovirus: 2 primer pairs (respirovirus L gene), PIV1 and PIV3
- Rubulavirus: 2 primer pairs (rubulavirus L gene), PIV2 and PIV4

Influenza surveillance II assay
- Identification, characterization, and differentiation of influenza A, B and C viruses directly from human, avian, animal or environment samples
  - Human, avian, equine, swine, etc...
  - Pdm H1N1
  - Seasonal H3N2 / H1N1
  - Newly emerging variants
- Core segments of influenza covered: PB1, PB2, Nuc, M1, PA, NS1
  - Designed to monitor shift and drift
- Rapid analysis
  - Sample to first result in 6 hours
- Throughput of 250 samples / day for surveillance activities
- The system was able to detect newly assorted and shifted or drifted strains (including avian strains such as H5 and other H and N types).

Clinical (continued)

Clinical aspects of Pandemic H1N1 and Human Infection with Highly Pathogenic H5N1

Tim Uyeki
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Clinical spectrum: 2009 H1N1
- Generally uncomplicated illness predominates (mild-to-moderate self-limited illness)
  - Acute upper respiratory tract illness
    - Febrile and afebrile (rarely conjunctivitis)
  - Other symptoms
    - Myalgias
    - Gastrointestinal symptoms (nausea, vomiting, diarrhea)
- Complicated to fatal disease
  - Severe lower respiratory tract disease
    - 16,713 deaths reported to WHO (under-estimated)
Affected populations: Complicated disease
  o 2009 H1N1 virus
    • Children, young adults worldwide
    • Severe disease in persons with underlying high-risk conditions (young adults)
  o H5N1 virus
    • Children, young adults in H5N1-affected countries
    • Severe disease in previously healthy persons

2009 pandemic H1N1: Testing of upper respiratory specimens
  o Rapid influenza diagnostic tests
    • Sensitivity: 40 – 60% (ranging from 10 to 70%) vs rRT-PCR
    • Sensitivity to detect 2009 H1N1 virus lower than detection of seasonal influenza viruses (false negatives)
    • Cannot distinguish between seasonal and or pandemic H1N1 virus infection
    • Positive and negative results need interpretation
  o Immunofluorescence
    • Sensitivity: 47 – 93%
    • Cannot distinguish between seasonal and or pandemic H1N1 virus infection
    • Positive and negative results need interpretation

2009 H1N1 testing and specimen source
  o Upper respiratory tract
    • Viral replication generally detectable for 5 – 6 days in uncomplicated disease, longer in some patients
    • Slower clearance of viral RNA in severe disease
    • Maybe prolonged viral detection with corticosteroid use, immunosupressed
    • 10 – 19% negative rRT-PCR compared to BAL specimens
  o Lower respiratory tract
    • Higher, prolonged viral replication in severe disease
    • Endotracheal aspirate, BAL specimens have high yield
  o Collect multiple specimens from multiple sites for rRT-PCR if 2009 H1N1 virus infection is clinically suspected

H5N1 testing on respiratory specimens
  o Antigen detection
    • Rapid influenza diagnostic tests, immunofluorescence
      • Sensitivity low: limited clinical data
      • Should not be used to detect H5N1 virus infection
  o RT-PCR
    • WHO proficiency testing
- Need updated H5 primers; match clades, subclades to locally circulating virus strains.

**H5N1 testing and specimen source**
- Upper respiratory tract (limited data)
  - H5N1 viral RNA detectable in upper respiratory tract specimens, but yield is generally higher from throat swabs
- Lower respiratory tract
  - High yield in endotracheal specimens
- Collect multiple specimens from multiple sites for rRT-PCR if H5N1 virus exposure and infection is clinically suspected.

**H5N1 surveillance issues**
- Focus is on hospital-based surveillance for pneumonia in persons with sick/dead poultry exposure
- Clinically mild H5N1 pediatric cases identified (Hong Kong, Indonesia, Turkey, Cambodia, Bangladesh)
  - Investigations of contacts of severe cases
  - Expanded surveillance (Egypt)
  - Routine surveillance
  - Sero-surveys (Cambodia)

**What is the true H5N1 case fatality?**
- 488 confirmed H5N1 cases, 286 deaths
- Case fatality: 59% since 2003/ range: 30 – 83%
- 20 cases in Egypt, Vietnam and Indonesia (2010).

**Risk factors for H5N1 virus infection**
- Visiting a live poultry market (Hong Kong 1997)
- Direct or close contact with sick/dead poultry (Thailand, Vietnam, China)
- Having sick/dead poultry in home (Vietnam)
- Preparing poultry for previously sick/dead poultry for consumption (Vietnam)
- Independent risk factors: direct contact or indirect contact with sick/dead poultry, visiting a live poultry market (China)
- Some cases without identified risk factors
- Limited human-to-human transmission documented

**Risk factors for complications 2009 H1N1**
- Age: young, elderly
- Underlying conditions
  - Chronic pulmonary disease
  - Chronic cardiac, renal, hepatic disease
  - Immunosuppression
  - Metabolic disease (diabetes)
  - Hemoglobinopathy
- Pregnancy
- Obesity
  - 25 – 30 % without known risk factors

**2009 H1N1 complications**
- Predominant complication: viral pneumonia with rapid progression (4 – 6 days from onset)
  - Acute lung injury: respiratory failure
    - Diffuse alveolar damage, ARDS, refractory hypoxemia
- Multi-organ failure (renal failure)
- Shock requiring vasopressors
- Exacerbation of chronic disease (COPD, asthma)
- Bacterial pneumonia (at admission or VAP)
- Myocarditis
- Encephalopathy, encephalitis
- Hemophagocytosis

**H5N1 signs and symptoms**
- Early stage
  - Fever, cough, occasionally diarrhea with fever
- Disease progression
  - Fever, non productive cough, dyspnea, shortness of breath
- Presenting signs at admission (4 – 6 days after onset)
  - Fever, cough, shortness of breath, tachypnea, dyspnea, chest pain, diarrhea, leucopenia, lymphopenia, thrombocytopenia
  - Others: malaise, sore throat, abdominal pain, vomiting
  - Atypical presentations: fever and diarrhea

**H5N1 complications**
- Most common: pneumonia
  - Progressing to respiratory failure
- Acute respiratory distress syndrome (ARDS)
- Multi-organ failure
  - Cardiac and renal dysfunction
- Gastrointestinal involvement
- Sepsis-like syndrome, shock, hemophagocytosis

**Role of bacteria co-infection**
- 2009 H1N1
  - Implicated in fatal cases
    - 26 % in US autopsy case series (n = 100)
    - Streptococcus pneumoniae, MRSA, MSSA, GAS
- H5N1
  - Very rarely detected (limited data)
  - Few autopsies, limited bacteriological testing
Impact upon pregnant women

- **2009 H1N1**
  - Severe illness, fatal outcomes reported in pregnant women worldwide (includes < 2 weeks postpartum)
    - Very high mortality with pregnancy and HIV
  - Impact highest in 3rd trimester and second trimester
    - Emergency Cesarean deliveries in ICU

- **H5N1**
  - 4 of 6 pregnant women with H5N1 died (Anhui province, China)
    - 2 who had spontaneous miscarriages survived

Extrapulmonary 2009 H1N1 virus infection?

- Viremia not conclusively demonstrated, but suggested in severe illness
  - Sporadic cases of viral RNA detection in blood in critical ill patients (virus not isolated)
  - Case report of suspected vertical (transplacental) transmission
  - Transmission to newborn infant in Thailand

- Autopsy analyses do not detect extrapulmonary infection to date

Extrapulmonary H5N1 virus infection

- Neurological involvement: 2004, in Vietnam
  - 4-year old male fatal case of encephalitis (seizures, coma) with diarrhea
    - H5N1 isolated from CSF, serum, throat and rectal swabs
- Viremia in critically ill patients (fatal cases)
  - H5N1 viral RNA and virus detected in blood, serum, plasma.
- H5N1 viremia can occur with dissemination in critically ill patients

H5N1 pathogenesis

- Infection, high viral pulmonary replication
  - Local acute lung injury, apoptosis of alveolar epithelial cells: induces proinflammatory cytokines
- Host inflammatory response
  - High H5N1 virus levels are associated with high levels of proinflammatory cytokines: fatal outcomes
    - Cytokines dysregulation
    - Increased plasma IL-10, IL-6, IFN-
  - Early antiviral treatment needed to suppress viral replication, prevent cytokine dysregulation
- Other factors
  - Viremia, viral dissemination, hemophagocytosis
  - Prolonged viral shedding (up to 16 days)

2009 H1N1 clinical management

- Early antiviral treatment with neuraminidase inhibitors (Oseltamivir, Zanamivir)
Consider higher dosing and longer duration for severe disease

- Oxygen therapy – ensure adequate oxygenation
- Advanced respiratory support – mechanical ventilation – follow guidelines for sepsis-associated ARDS
- Antibiotic treatment following evidence-based guidance for community-acquired pneumonia
- Corticosteroids: no routine use
  - Low dose for septic shock requiring vasopressors and have adrenal insufficiency
- No aspirin or aspirin-containing products for < 18 years old persons

**H5N1 clinical management**

- Infection control
  - Isolate the patient
  - Implement infection control precautions
  - PPE for HCWs and family members
- Supportive care (ICU)
  - Pulmonary
    - Supplemental oxygen, invasive mechanical ventilation for respiratory failure, lung protective strategies
  - Good ICU management of complications
- Treatment
  - Antiviral medications (Oseltamivir)
  - Corticosteroid treatment not recommended

**Conclusions**

- 2009 H1N1 and H5N1 viral infections can cause similar critical syndromes
- Most severe disease with 2009 H1N1 occurs in persons with underlying illness
- Most patients with 2009 H1N1 virus infection do not become critically ill
- Despite extensive exposure to H5N1 virus, human disease appears rare
- Host factors and host response are important in influencing disease severity.

**Vaccinology / Prevention**

**Key Immunological Observations from the Clinical Development of Sanofi Pandemic Influenza A (H1N1) Vaccines**

Yanee Hutagalung
Sanofi Pasteur, Singapore

- Clinical studies were rapidly initiated in more than 6,000 subjects from 6 months of age to establish the safety and immunogenicity of Sanofi Pasteur’s candidate inactivated split-virion vaccines
Key immunological finding from the studies

- A single administration of either AF03-adjuvanted or unadjuvanted influenza A (H1N1) vaccine stimulated high level immunogenicity, largely exceeding the criteria for vaccine registration
- Robust antibody responses elicited by the pdm H1N1 vaccine in adults and older children could be explained, at least in part, by preexisting immune priming conferred by exposure to previous seasonal strains
- However, irrespective of prior immunity to influenza, the candidate pdm H1N1 vaccine appeared more immunogenic than previous seasonal strains and much more immunogenic than unadjuvanted candidate pdm vaccines based on the influenza A (H5N1) strains
- A second vaccine administration helped further increase antibody titers
  - Particularly true in children below 9 years of age
  - Also observed in elderly subjects
  - These observations triggered recommendations for 1 or 2 vaccine injections for vaccination of the different age populations
- Interestingly, the levels of antibodies required for protection against pdm influenza remain largely unknown, and therefore the protective benefit of antibody responses to the levels attained after 2 injections remains to be determined
- The 3.8 g hemagglutinin (HA) per dose adjuvanted vaccine displayed similar immunogenicity compared to the 15 g HA per dose unadjuvanted vaccine in adult and elderly subjects as well as older children
  - More immunogenic than the unadjuvanted vaccine in children below 9 years of age

Conclusions

- The results confirm the excellent immunogenicity of the AF03-adjuvanted H1N1 vaccine in all age groups, especially in young children
- H1N1 pdm vaccines manufactured by Sanofi Pasteur were shown to be highly immunogenic
- Ongoing large scale studies will confirm their safety and effectiveness profile.

Poster session

Neuraminidase inhibitor resistance in influenza viruses

The detection of oseltamivir-resistant pandemic (H1N1) 2009 influenza viruses using real-time PCR assay

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Background
Neuraminidase inhibitor resistance in influenza viruses has been very uncommon until the emergence of oseltamivir-resistant seasonal influenza A (H1N1) viruses during the 2007 / 08 northern hemisphere influenza season. These resistant viruses subsequently spread and became the dominant H1N1 strain in many countries including Australia during 2008 / 09. Oseltamivir resistance is due to a point mutation in the N1 gene that results in a tyrosine to histidine substitution at amino acid position 275 (H275Y). The seasonal influenza A (H1N1) virus has now been replaced in much of the world by the pandemic (H1N1) 2009 influenza virus, which was first reported in April 2009 in Mexico and the USA. There are concerns that oseltamivir resistance will develop in pandemic (H1N1) 2009 influenza virus due either to mutation in the N1 gene, or to reassortment with the resistant seasonal H1N1 virus, thereby reducing the effectiveness of oseltamivir for the treatment and prevention of this infection.
Fortunately only a small number of resistant strains of pandemic (H1N1) 2009, all with the H275Y mutation, have been detected, including several in Australia.

This group developed a real-time reverse transcription PCR (rRT-PCR) assay for the rapid detection of the H275Y mutation, suitable for routine diagnosis. They selected 390 clinical respiratory specimens collected from May to September 2009 and known to be positive in the pandemic (H1N1) 2009 rRT-PCR assay. 2 rRT-PCR assays were designed to detect the wild-type strain or the H275Y neuraminidase gene mutant of the pandemic influenza virus. To validate those assays, a conventional RT-PCR assay was also designed for sequence analysis of that mutation site.

The H275Y mutation was detected in 3 hospitalized patients with pandemic (H1N1) 2009 infection. 2 of these patients admitted to the ICU, showed a varying mixture of wild-type and mutant virus in both the rRT-PCR assays and the sequencing analysis chromatograms in samples collected over several days. These findings were independently confirmed by fluorescent-based NA inhibition and pyrosequencing assays. In all 3 patients, the resistant virus disappeared either spontaneously or following treatment with zanamivir.

As expected, some strains of the pandemic (H1N1) 2009 influenza virus have developed resistance to the neuraminidase inhibitor, oseltamivir. Thus far, resistance has not been widespread with 39 sporadic cases reported worldwide as of October 2009 and all infections have resolved. However, in view of the experience with seasonal H1N1 viruses, continued surveillance is required to monitor the emergence and possible spread of future oseltamivir-resistant strains of pandemic (H1N1) 2009 influenza virus.