

2 March 2010

This supplement has been prepared to present scientific and technical news items that may be of more interest to technical personnel at RDT&E activities and the labs, or the medics rather than the broader readership of the basic CB Daily. Due to the nature of the material, the articles, if available online, are usually only available through subscription services thus making specific links generally unavailable. Thus, usually only the bibliographic citation is available for use by an activity's technical library.

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Chem-Bio News – Pandemic Influenza Edition #101

1. INITIAL RESPONSE OF HEALTH CARE INSTITUTIONS TO EMERGENCE OF H1N1

INFLUENZA: EXPERIENCES, OBSTACLES, AND PERCEIVED FUTURE NEEDS: "Future efforts to optimize the response to H1N1 should include curtailing personal stockpiling of antivirals and vaccine development with consideration of mandatory vaccination of health care workers."

2. DEVELOPMENT OF A REAL-TIME RT-PCR ASSAY FOR A NOVEL INFLUENZA A (H1N1)

VIRUS: "This sensitive and specific real-time RT-PCR assay will contribute to the early diagnosis and control of the emerging H1N1 influenza pandemic."

3. MODELING GENE SEQUENCES OVER TIME IN 2009 H1N1 INFLUENZA A VIRUS

POPULATIONS: "Antigenic regions relevant for vaccine development can differ from previous vaccine strains and vary among patients."

4. LUNG PATHOLOGY IN FATAL NOVEL HUMAN INFLUENZA A (H1N1) INFECTION: "Autopsies have shown that the main pathological changes associated with S-OIV infection are localized to the lungs, where three distinct histological patterns can be identified."

5. DESIGN AND CLINICAL APPLICATION OF A MOLECULAR METHOD FOR DETECTION AND TYPING OF THE INFLUENZA A/H1N1PDM VIRUS:

"The novel method is suitable for the diagnosis of A/H1N1pdm, and is also suitable, at least in the screening phase, for laboratories not equipped with the real-time PCR technology."

6. NUCLEAR IMPORT AND ASSEMBLY OF INFLUENZA A VIRUS RNA POLYMERASE STUDIED IN LIVE CELLS BY FLUORESCENCE CROSS-CORRELATION SPECTROSCOPY:

"Our study sheds light on the interplay between the nuclear import of the subunits and the assembly of the influenza virus polymerase and provides a methodological framework to analyze the effects of different host range mutations in the future."

7. RAPID QUANTIFICATION OF SINGLE-NUCLEOTIDE MUTATIONS IN MIXED INFLUENZA A VIRAL POPULATIONS USING ALLELE-SPECIFIC MIXTURE ANALYSIS:

"Results from the current study demonstrate that FluASMA is a highly sensitive and quantitative SNP analysis method, even for minor mutant components (<1%)."

8. PNEUMOCOCCAL SURFACE PROTEIN A CONTRIBUTES TO SECONDARY STREPTOCOCCUS PNEUMONIAE INFECTION AFTER INFLUENZA VIRUS INFECTION:

"Our findings indicate that PspA contributes to secondary *S. pneumoniae* infection after influenza virus infection and that PspA immunization mitigates early secondary pneumococcal lung infections."

9. EFFICACY OF THE NEW NEURAMINIDASE INHIBITOR CS-8958 AGAINST H5N1 INFLUENZA VIRUSES:

"We found that R-125489 bound to NA more tightly than did any other NA inhibitor tested. Our results indicate that CS-8958 is highly effective for the treatment and prophylaxis of infection with H5N1 influenza viruses, including oseltamivir-resistant mutants."

10. STUDY SAYS HUMIDITY IS KEY FACTOR IN US FLU OUTBREAKS:

"Winter influenza outbreaks in the United States typically follow periods of unusually low absolute humidity (AH), and this pattern suggests it may be possible to develop short-term forecasts of flu epidemics, according to a study published this week."

11. HONG KONG REPORTS SWINE-PANDEMIC FLU REASSORTANT: "A laboratory at Hong Kong University (HKU) detected a reassortant made up of a swine influenza virus and the pandemic H1N1

virus in a sample obtained from a slaughterhouse pig as part of a surveillance program, officials announced today."

CB Daily Report

Chem-Bio News

INITIAL RESPONSE OF HEALTH CARE INSTITUTIONS TO EMERGENCE OF H1N1 INFLUENZA: EXPERIENCES, OBSTACLES, AND PERCEIVED FUTURE NEEDS

Preventive Medicine Week

February 21, 2010

"To assess attitudes and responses of health care epidemiology professionals to the H1N1 influenza crisis, we conducted a cross-sectional survey of members of the Society for Healthcare Epidemiology of America. We assessed beliefs regarding (1) importance of H1N1, (2) institutional preparedness, (3) time spent on the H1N1 crisis, and (4) the institution's response to H1N1. Of 323 respondents, 195 (60.4%) reported that their hospitals were well prepared for a pandemic. Furthermore, the majority reported that senior administrators provided adequate political support and resources (85.1% and 80.2%, respectively) to respond to H1N1. However, 163 (50.9%) respondents reported that other important infection prevention activities were neglected during the H1N1 crisis. Shortages of antiviral medication were reported by 99 (30.7%) respondents. Furthermore, 126 (39.0%) reported that personal stockpiling of antiviral medications occurred at their institution, and 166 (51.4%) reported that institutional actions were initiated to prevent personal stockpiling. Also, 294 (91.0%) respondents believed that H1N1 influenza would reappear later this year. Vaccine development, health care worker education, and revisions of pandemic influenza plans were identified as the most important future initiatives. Finally, 251 (77.7%) respondents felt that health care workers should be mandated to receive influenza vaccine. Although generally institutions are well prepared for the H1N1 crisis, substantial revisions of pandemic preparedness plans appear to be necessary."

"Future efforts to optimize the response to H1N1 should include curtailing personal stockpiling of antivirals and vaccine development with consideration of mandatory vaccination of health care workers."

The full article can be found at: (E. Lautenbach, et. al., "Initial response of health care institutions to emergence of H1N1 influenza: experiences, obstacles, and perceived future needs". *Clinical Infectious Diseases*, 2010;50(4):523-7). Link not available.

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DEVELOPMENT OF A REAL-TIME RT-PCR ASSAY FOR A NOVEL INFLUENZA A (H1N1) VIRUS

World Disease Weekly

February 23, 2010

"In this study, a real-time reverse transcriptase PCR (RT-PCR) assay based on the hemagglutinin gene was developed that discriminates the novel H1N1 from swine influenza virus, seasonal H1N1/H3N2 virus and the highly pathogenic H5N1 avian influenza virus."

"The sensitivity of this assay was 0.2 50% tissue culture infective dose of virus and 200 copies of in vitro-transcribed target RNA. Three hundred and forty-eight clinical specimens from suspected H1N1 patients were tested using this assay, and forty-two (12.07%) were found to be positive. Tests using the real-time PCR assay recommended by WHO and virus isolation gave identical results."

"This sensitive and specific real-time RT-PCR assay will contribute to the early diagnosis and control of the emerging H1N1 influenza pandemic."

The full article can be found at: (T. Jiang, et. al., "Development of a real-time RT-PCR assay for a novel influenza A (H1N1) virus". Journal of Virological Methods, 2010;163(2):470-3). Link not available.

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MODELING GENE SEQUENCES OVER TIME IN 2009 H1N1 INFLUENZA A VIRUS POPULATIONS

Health Risk Factor Week

February 16, 2010

"In order to gain insight into the mode of evolution of these new H1N1 strains, we performed a Bayesian coalescent Markov chain Monte Carlo (MCMC) analysis of full-length neuraminidase (NA) gene sequences of 62 H1N1 IAV strains (isolated from March 30(th) to by July 28(th), 2009)."

"The results of these studies revealed that the expansion population growth model was the best to fit the sequence data. A mean of evolutionary change of 7.84×10^{-3} nucleotide substitutions per site per year (s/s/y) was obtained for the NA gene. A significant contribution of first codon position to this mean rate was observed. Maximum clade credibility trees revealed a rapid diversification of NA genes in different genetic lineages, all of them containing Oseltamivir-resistant viruses of very recent emergence. Mapping of naturally occurring amino acid substitutions in the NA protein from 2009 H1N1 IAV circulating in 62 different patients revealed that substitutions are distributed all around the surface of the molecule, leaving the hydrophobic core and the catalytic site essentially untouched. High evolutionary rates and fast population growth have contributed to the initial transmission dynamics of 2009 H1N1 IAV. Naturally occurring substitutions are preferentially located at the protein surface and do not interfere with the NA active site."

"Antigenic regions relevant for vaccine development can differ from previous vaccine strains and vary among patients."

The full article can be found at: (N. Goni, et. al., "Modeling gene sequences over time in 2009 H1N1 Influenza A Virus populations". Virology Journal, 2009;6():215). Link not available.

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LUNG PATHOLOGY IN FATAL NOVEL HUMAN INFLUENZA A (H1N1) INFECTION

Pharma Law Weekly

February 16, 2010

"The autopsy findings of 21 Brazilian patients with confirmed S-OIV infection are presented."

"These patients died in the winter of the southern hemisphere 2009 pandemic, with acute respiratory failure. Lung tissue was submitted to virologic and bacteriologic analysis with real-time reverse transcriptase polymerase chain reaction and electron microscopy. Expression of toll-like receptor (TLR)-3, IFN-gamma, tumor necrosis factor-alpha, CD8(+) T cells and granzyme B+ cells in the lungs was investigated by immunohistochemistry. Patients were aged from 1 to 68 years (72% between 30 and 59 yr) and 12 were male. Sixteen patients had preexisting medical conditions. Diff use alveolar damage was present in 20 individuals. in six patients, diffuse alveolar damage was associated with necrotizing bronchiolitis and in five with extensive hemorrhage. There was also a cytopathic effect in the bronchial and alveolar epithelial cells, as well as necrosis, epithelial hyperplasia, and squamous metaplasia of the large airways. There was marked expression of TLR-3 and IFN-gamma and a large number of CD8(+) T cell sand granzyme B+ cells within the lung tissue. Changes in other organs were mainly secondary to multiple organ failure. Autopsies have shown that the main pathological changes associated with S-OIV infection are localized to the lungs, where three distinct histological patterns can be identified. We also show evidence of ongoing pulmonary aberrant immune response."

"Our results reinforce the usefulness of autopsy in increasing the understanding of the novel human influenza A (H1N1) infection."

The full article can be found at: (T. Mauad, et. al., "Lung Pathology in Fatal Novel Human Influenza A (H1N1) Infection". American Journal of Respiratory and Critical Care Medicine, 2010;181(1):72-79). Link not available.

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DESIGN AND CLINICAL APPLICATION OF A MOLECULAR METHOD FOR DETECTION AND TYPING OF THE INFLUENZA A/H1N1PDM VIRUS

World Disease Weekly
February 23, 2010

"In March/April 2009, Mexico experienced an outbreak of respiratory illness, due to a new influenza of swine origin virus, which spread rapidly via human-to-human transmission, and became pandemic (A/H1N1pdm). Because of its unique genome composition, which includes gene segments of swine, avian and human origin, and to the considerable differences to the human influenza A viruses that have circulated so far, the currently used molecular methods proved inadequate."

"Based on published sequences, a primer set targeting the nucleoprotein gene was designed, which provided enhanced sensitivity for the new strain and proved suitable for sequence-based strain identification. The novel nucleoprotein reverse-transcription-PCR showed higher sensitivity for A/H1N1pdm than a commercial test for influenza A, and was comparable to the real-time-based method developed by the Centers for Disease Control and Prevention. It was used to screen 177 clinical samples referred to the laboratory for suspected A/H1N1pdm infection, detecting 17 (9.6%) infections that were confirmed by sequence analysis (100% sensitivity as compared to the real-time kit)."

"The novel method is suitable for the diagnosis of A/H1N1pdm, and is also suitable, at least in the screening phase, for laboratories not equipped with the real-time PCR technology."

The full article can be found at: (E. Lalle, et. al., "Design and clinical application of a molecular method for detection and typing of the influenza A/H1N1pdm virus". Journal of Virological Methods, 2010;163(2):486-8). Link not available.

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NUCLEAR IMPORT AND ASSEMBLY OF INFLUENZA A VIRUS RNA POLYMERASE STUDIED IN LIVE CELLS BY FLUORESCENCE CROSS-CORRELATION SPECTROSCOPY

Medical Imaging Week
February 27, 2010

"Intracellular transport and assembly of the subunits of the heterotrimeric RNA-dependent RNA polymerase constitute a key component of the replication cycle of influenza virus. Recent results suggest that efficient polymerase assembly is a limiting factor in the viability of reassortant viruses."

"The mechanism of nuclear import and assembly of the three polymerase subunits, PB1, PB2, and PA, is still controversial, yet it is clearly of great significance in understanding the emergence of new strains with pandemic potential. In this study, we systematically investigated the interactions between the polymerase subunits and their localization in living cells by fluorescence cross-correlation spectroscopy (FCCS) and quantitative confocal microscopy. We could show that PB1 and PA form a dimer in the cytoplasm, which is imported into the nucleus separately from PB2. Once in the nucleus, the PB1/PA dimer associates with PB2 to form the trimeric polymerase. Photon-counting histogram analysis revealed that trimeric polymerase complexes can form higher-order oligomers in the nucleus. We furthermore demonstrate that impairing the nuclear import of PB2 by mutating its nuclear localization signal leads to abnormal formation of the trimeric polymerase in the cytoplasm. Taken together, our results demonstrate which of the previously discussed influenza virus polymerase transport models operates in live cells."

"Our study sheds light on the interplay between the nuclear import of the subunits and the assembly of the influenza virus polymerase and provides a methodological framework to analyze the effects of different host range mutations in the future."

The full article can be found at: (S. Huet, et. al., "Nuclear import and assembly of influenza A virus RNA polymerase studied in live cells by fluorescence cross-correlation spectroscopy". Journal of Virology, 2010;84(3):1254-64). Link not available.

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RAPID QUANTIFICATION OF SINGLE-NUCLEOTIDE MUTATIONS IN MIXED INFLUENZA A VIRAL POPULATIONS USING ALLELE-SPECIFIC MIXTURE ANALYSIS

Health Risk Factor Week

February 23, 2010

"Monitoring antiviral resistance in influenza is critical to public health epidemiology and pandemic preparedness activities. Effective monitoring requires methods to detect low-level resistance and to monitor the change in resistance as a function of time and drug treatment."

"Resistance-conferring single-nucleotide mutations in influenza virus are ideal targets for such methods. In the present study, five sets of paired TaqMan allele-specific PCR (ASPCR) assays were developed and validated for quantitative single-nucleotide polymorphism (SNP) analysis. This novel method using Delta Ct is termed allele-specific mixture analysis (ASMA) or FluASMA. The FluASMA assays target L26F, V27A, A30T, and S31N mutations in the A/Albany/1/98 (H3N2) M2 gene and H275Y mutation in the A/New Caledonia/20/99 (H1N1) NA gene and have a limit of quantification of 0.25-0.50% mutant. The error for % mutant estimation was less than 10% in all FluASMA assays, with intra-run Delta Ct coefficient of variance (CoV) at $\leq 2\%$ and inter-run Delta Ct CoV at $\leq 5\%$."

"Results from the current study demonstrate that FluASMA is a highly sensitive and quantitative SNP analysis method, even for minor mutant components ($< 1\%$)."

The full article can be found at: (C.M. Liu, et. al., "Rapid quantification of single-nucleotide mutations in mixed influenza A viral populations using allele-specific mixture analysis". Journal of Virological Methods, 2010;163(1):109-15). Link not available.

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PNEUMOCOCCAL SURFACE PROTEIN A CONTRIBUTES TO SECONDARY STREPTOCOCCUS PNEUMONIAE INFECTION AFTER INFLUENZA VIRUS INFECTION

Pharma Investments, Ventures & Law Weekly

February 21, 2010

"We compared the growth of Streptococcus pneumoniae mutants with a disruption in the gene for either pneumococcal surface protein A (PspA-), neuraminidase A (NanA-), or hyaluronidase (Hyl-) to that of the parental strain D39 by means of a competitive growth model in mice with and those without prior influenza virus infection. The numbers of total bacteria recovered from mice with prior influenza virus infection were significantly greater than those recovered from mice without prior influenza virus infection."

"Although the Hyl- and NanA- mutants did not display attenuation in mice with or without prior influenza virus infection, the PspA- mutant exhibited attenuation both in mice with and in mice without prior influenza virus infection. This defect was severe in influenza virus-infected mice, for which growth of the PspA- mutant was 1800-fold lower than that of the parental strain D39. Furthermore, PspA immunization significantly reduced secondary bacterial lung burdens and concentrations of specific markers of lung damage in mice receiving serotypes 2, 3, and 4 pneumococci."

"Our findings indicate that PspA contributes to secondary S. pneumoniae infection after influenza virus

infection and that PspA immunization mitigates early secondary pneumococcal lung infections."

The full article can be found at: (Q.O. King, et. al., "Pneumococcal surface protein A contributes to secondary Streptococcus pneumoniae infection after influenza virus infection". Journal of Infectious Diseases, 2009;200(4):537-45). Link not available.

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EFFICACY OF THE NEW NEURAMINIDASE INHIBITOR CS-8958 AGAINST H5N1 INFLUENZA VIRUSES

By Maki Kiso, Shuku Kubo, Makoto Ozawa, Quynh Mai Le, Chairul A. Nidom, Makoto Yamashita, Yoshihiro Kawaoka
PLoS Pathogens
February 26, 2010

"Currently, two neuraminidase (NA) inhibitors, oseltamivir and zanamivir, which must be administered twice daily for 5 days for maximum therapeutic effect, are licensed for the treatment of influenza. However, oseltamivir-resistant mutants of seasonal H1N1 and highly pathogenic H5N1 avian influenza A viruses have emerged. Therefore, alternative antiviral agents are needed. Recently, a new neuraminidase inhibitor, R-125489, and its prodrug, CS-8958, have been developed. CS-8958 functions as a long-acting NA inhibitor in vivo (mice) and is efficacious against seasonal influenza strains following a single intranasal dose. Here, we tested the efficacy of this compound against H5N1 influenza viruses, which have spread across several continents and caused epidemics with high morbidity and mortality. We demonstrated that R-125489 interferes with the NA activity of H5N1 viruses, including oseltamivir-resistant and different clade strains. A single dose of CS-8958 (1,500 µg/kg) given to mice 2 h post-infection with H5N1 influenza viruses produced a higher survival rate than did continuous five-day administration of oseltamivir (50 mg/kg twice daily). Virus titers in lungs and brain were substantially lower in infected mice treated with a single dose of CS-8958 than in those treated with the five-day course of oseltamivir. CS-8958 was also highly efficacious against highly pathogenic H5N1 influenza virus and oseltamivir-resistant variants. A single dose of CS-8958 given seven days prior to virus infection also protected mice against H5N1 virus lethal infection. To evaluate the improved efficacy of CS-8958 over oseltamivir, the binding stability of R-125489 to various subtypes of influenza virus was assessed and compared with that of other NA inhibitors. We found that R-125489 bound to NA more tightly than did any other NA inhibitor tested. Our results indicate that CS-8958 is highly effective for the treatment and prophylaxis of infection with H5N1 influenza viruses, including oseltamivir-resistant mutants."

The full article can be found at: <http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1000786;jsessionid=E5D53485768A4FED9DA0925B23ED267B>

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STUDY SAYS HUMIDITY IS KEY FACTOR IN US FLU OUTBREAKS

By Robert Roos
CIDRAP News (Center for Infectious Disease Research & Policy – University of Minnesota)
February 25, 2010

"Winter influenza outbreaks in the United States typically follow periods of unusually low absolute humidity (AH), and this pattern suggests it may be possible to develop short-term forecasts of flu epidemics, according to a study published this week.

A five-member research team led by Jeffrey Shaman of Oregon State University found the association by comparing humidity records with the timing of flu epidemics nationwide over 30 years. They reported their findings in PLoS Biology.

The team also developed a mathematical model of flu transmission in which transmissibility was governed largely by absolute humidity. Outbreak patterns generated by the model matched up well

with actual death rates from pneumonia and flu in five states, they report.

"The results indicate that AH affects both the seasonality of influenza incidence and the timing of individual wintertime influenza outbreaks," the report says. "The association of anomalously low AH conditions with the onset of wintertime influenza outbreaks suggests that skillful, short-term probabilistic forecasts of epidemic influenza could be developed."

Why seasonal flu outbreaks occur in the winter in temperate regions—a pattern that does not hold for pandemic flu—has long been a mystery. The new study builds on recent experimental evidence that aerosolized flu viruses survive much better at low humidity and flu in lab animals spreads much more easily at low humidity."

The full article can be found at:

<http://www.cidrap.umn.edu/cidrap/content/influenza/general/news/feb2510humid.html>

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HONG KONG REPORTS SWINE-PANDEMIC FLU REASSORTANT

By Lisa Schnirring

CIDRAP News - Center for Infectious Disease Research & Policy (University of Minnesota)

February 26, 2010

"A laboratory at Hong Kong University (HKU) detected a reassortant made up of a swine influenza virus and the pandemic H1N1 virus in a sample obtained from a slaughterhouse pig as part of a surveillance program, officials announced today.

It is the first reported reassortant between the two types of viruses. The virus was detected in a pig that was imported from the Chinese mainland, which has been notified about the finding, Hong Kong's agriculture department said in a statement. It was detected during HKU's regular influenza surveillance.

The agriculture department said in statement that the finding doesn't pose a public health risk or present any food-safety issues.

Dr Malik Peiris, a microbiology professor who heads HKU's surveillance program, said in the statement that the finding isn't unexpected, likely occurs worldwide, and was only detected in Hong Kong because of intensive surveillance. He said further tests are under way to further characterize the virus.

A spokesman for Hong Kong's Centre for Health Protection, however, said that preliminary findings suggest the reassortant is sensitive to oseltamivir (Tamiflu)."

The full article can be found at:

<http://www.cidrap.umn.edu/cidrap/content/influenza/swineflu/news/feb2610swine.html>

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