National Collaborating Centre for Infectious Diseases
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Purple Paper

Pandemic pH1N1 Weekly Literature Synthesis
(Week of November 22-28, 2009)

Lineage of pH1N1 Viruses

From where did the 2009 'swine-origin' influenza A virus (H1N1) emerge?
Gibbs AJ et al. *Virol J. Published online November 24, 2009.*

The complete genetic sequences of the pH1N1 virus were deciphered at the end of April 2009, soon after the first isolation of the virus. By comparing the genetic sequences of pH1N1 to sequences of other known influenza A viruses, several scientist groups concluded pH1N1 is a reassortant of North American and Eurasian swine influenza A viruses. One theory for the emergence of pH1N1 is that movement of live pigs between North America and Eurasia might have facilitated the mixing of diverse influenza A viruses leading to the generation of the novel pH1N1 strain. It was suggested that the pH1N1 virus might have been circulating unnoticed for a long time due to a lack of influenza surveillance in pigs and probably emerged into the human population on a single occasion around January 2009.

In a similar genetic analysis based on different parameters, the authors of this study attempted to trace the origin of the pH1N1 virus. They also proposed that the pH1N1 might have been an escape virus due to a laboratory error – possibly during vaccine research and/or production processes. Evidence presented in the paper supporting this claim included specific genetic features of pH1N1 and its "temporal-familial" relationship to other known swine influenza A viruses.

NCCID Comments:
Results of this study, not yet published at the time, were made known to the WHO in May 2009. Given the potential implications of the findings, WHO enlisted scientists from its five Collaborating Centres for Influenza (of which CDC is a part), World Organization for Animal Health (OIE), Food and Agriculture Organization of the United Nations (FAO), and other human and animal influenza virologists to review the evidence and determine the validity of the hypothesis. At a press conference on May 14, 2009 [1], Dr. Keiji Fukuda, Assistant Director-General ad Interim for Health Security and Environment at WHO, announced that the scientists had concluded that "the hypothesis does not really stand up to scrutiny. In fact, the evidence suggests that [pH1N1] is a naturally-occurring virus and not a laboratory-derived virus." As Dr. Fukuda had pointed out, scientists of the review panel found that much of data presented were actually within the expected "behaviour" of swine influenza A viruses. The importance of enhancing global surveillance of influenza in pigs remains.

Differentiation of two distinct clusters among currently circulating influenza A(H1N1)v viruses, March-September 2009.
Fereidouni SR et al. *Euro Surveill 2009; 14(46):pii=19409.* Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19409

Influenza A virus has 8 gene segments encoding 11 viral proteins. In this study, the authors compared more than 300 complete gene sequences of pH1N1 available as of September 10, 2009, to determine if sub-groups of the circulating pH1N1 virus exist.

Results show that there were two closely related but distinct clusters of the circulating pH1N1 virus. The two clusters, arbitrarily named cluster 1 and 2, could be differentiated by nine single genetic-base changes – 3 found in the genes for the surface proteins hemagglutinin (HA) and neuraminidase (NA), and 6 in the genes for 4 internal proteins. Of the 9 genetic changes, 4 caused an amino acid change. All genetic changes did not appear to be located in regions of the virus' genetic makeup responsible for any known phenotypic difference or biological functions.

It is estimated that cluster 1 occurred two weeks before cluster 2. Most pH1N1 viruses from Mexico, Texas and California belonged to cluster 1, whereas most pH1N1 viruses from New York belonged to cluster 2. The reason for or significance of this dichotomy is unclear.

Cluster 1 could be further divided into 3 sub-clusters. While sub-cluster 1.1 contained a unique combination of genetic changes that was distinguishable from cluster 2, sub-clusters 1.2 and 1.3 contained a variable number of genetic changes specific for cluster 2. A great majority of Canadian pH1N1 isolates that were available for analysis belonged to cluster 1.1.

Oseltamivir-Resistant Seasonal Influenza

Oseltamivir-resistant influenza A(H1N1) viruses detected in Europe during season 2007-8 had epidemiologic and clinical characteristics similar to co-circulating susceptible A(H1N1) viruses.
Ciancio BC et al. *Euro Surveill 2009; 14(46):pii=19412* Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19412

To identify possible risk factors associated with infection with an oseltamivir-resistant seasonal H1N1 virus and to determine whether such infection is associated with a more severe disease outcome, investigators from the European Centre for Disease Prevention and Control used **case-control and prospective cohort study designs**, respectively, to analyze pooled data from **5 European countries**. Of 6 eligible countries selected based on preset selection criteria, **Germany, Luxembourg, the Netherlands, Norway and the UK** agreed to participate. Influenza surveillance data were collected from both sentinel- and non-sentinel based settings during the 2007-8 season from week 40(2007) to week 20(2008). Cases were defined as individuals with laboratory-confirmed seasonal H1N1 infection whose isolates showed oseltamivir resistance, and controls were individuals with laboratory-confirmed seasonal H1N1 infection whose isolates were susceptible to oseltamivir. Oseltamivir resistance or susceptibility was confirmed by laboratory assays.

The estimated level of oseltamivir resistance during the study period varied among participating countries: Germany (13.1%), Luxembourg (26.0%), the Netherlands (26.9%), Norway (64.7%) and the UK (11.0%). After adjusting for age, reporting country and source of the sample (sentinel vs. non-sentinel setting), none of the examined risk factors (age, sex seasonal influenza vaccination, any chronic medical conditions, diabetes, immuno-suppression, cardiovascular disease and respiratory disease) was significantly associated with an increased risk of infection with an oseltamivir-resistant virus. There was no difference in symptoms between oseltamivir-resistant and oseltamivir-sensitive influenza-infected patients at the time of sampling. Furthermore, infection with a resistant virus did not increase the individual's risk of more severe disease.

Some limitations of this study were incomplete data sets, and recall bias in the information collected from clinicians and patients. Information bias could also occur if information gathered for one study group was more accurate than that for the other study group.

NCCID Comments:
Oseltamivir-resistance is ubiquitous among seasonal H1N1 strains isolated in many countries. Despite the widespread use of oseltamivir during the current pandemic, oseltamivir-resistant pH1N1 viruses have thus far only occurred sporadically. According to a report in WHO's *Weekly Epidemiological Record*, a total of 39 oseltamivir-resistant cases had been described as of October 22, 2009 since the beginning of the pandemic [2]. Of the 16 cases identified in the Americas, 2 cases occurred in Canada – one was associated with the prophylactic use of oseltamivir and the other with treatment with oseltamivir. An interesting parallel between oseltamivir-resistant pH1N1 cases and their seasonal counterparts is that almost all patients with the resistant pH1N1 had typical ILI and recovered without complication. The number of oseltamivir-resistant cases has continued to climb since the publication of the WHO report. At the time of this writing, the total number of oseltamivir-resistant cases has increased to 75

worldwide. At this juncture, case studies of oseltamivir-resistant pH1N1 cases suggest that transmission of these viruses does not take place beyond the immediate setting in which they are discovered. However, it is foreseeable that continual evolution of oseltamivir-resistant pH1N1 viruses may enable enhanced transmission.

Clinical effectiveness of oseltamivir and zanamivir for treatment of influenza A virus subtype H1N1 with the H274Y mutation: a Japanese, multicenter study of the 2007-2008 and 2008-2009 influenza seasons.
Kawai N *et al. Clin Infect Dis. Published online November 13, 2009.*

In **Japan**, the incidence of oseltamivir-resistant seasonal H1N1 infection increased from 3% during the 2007/08 season to 97% during the 2008/09 season. On February 14, 2009, the Infectious Disease Surveillance Center of Japan further concluded that 99.5% of seasonal H1N1 isolates in Japan were resistant to oseltamivir. To examine the clinical effectiveness of oseltamivir in patients infected with the oseltamivir-resistant seasonal H1N1 virus, investigators of this study compared the illness outcome among patients treated with either oseltamivir or zanamivir during the 2007/08 and 2008/09 seasons using a **case-control study** design. Data were gathered and analyzed for laboratory-confirmed influenza cases with influenza-like illness who attended clinics belonging to the Influenza Study Group of the Japan Physicians Association.

The main groups for comparison were:
- 2007/08 seasonal H1N1 infection (both oseltamivir and zanamivir treatment groups)
- 2008/09 seasonal H1N1 infection (both treatment groups)
- 2008/09 seasonal H3N2 infection (both treatment groups).

The illness outcome measures were:
- the duration of fever, temperature ≥ 37.5^{o}C *after symptom onset*
- the duration of fever, temperature ≥ 37.5^{o}C *after the first dose of anti-virals*.

Laboratory assays confirmed that all seasonal H1N1 isolates during the 2008/09 season, but none during the 2007/08 season, were resistant to oseltamivir.

Results showed patients who were treated with oseltamivir for the 2008/09 seasonal H1N1 influenza had significantly longer fever episodes *after symptom onset* and *after the start of therapy* than patients who received oseltamivir for the 2007/08 seasonal H1N1 influenza and patients who received the same treatment for the 2008/09 seasonal H3N2 influenza. When comparing oseltamivir and zanamivir treatments among patients who were infected with the 2008/09 seasonal H1N1 influenza, patients receiving oseltamivir had significantly longer fever episodes than their counterparts receiving zanamivir both *after symptom onset* and *after the start of therapy*.

NCCID Comments:
There are several major limitations in this study. The number of study subjects was small. No information was available regarding underlying medical conditions that subjects might have or the severity/type of symptoms presented at the time of the clinic visit. Time/date of onset of symptoms and resolution of fever episodes were self-reported by the patients or their family members; as a result, bias might have inadvertently introduced in the data set. Some patients were using antipyretics during their course of anti-viral treatment, thus possibly skewing the measured outcome. The most important limitation was perhaps the lack of an untreated control group in this study. In the discussion section of the article, the authors argued that although oseltamivir was less effective than zanamivir in treating patients with oseltamivir-resistant seasonal H1N1 influenza, it was still more effective compared to no treatment. This claim was based on the observations that:

1. 2008/09 seasonal H1N1 influenza patients who were treated with oseltamivir experienced shorter fever episodes than 2003/04 and 2004/04 seasonal influenza patients who received no anti-viral therapy.
2. The plasma concentration of oseltamivir detected in treated patients with the 2008/09 seasonal H1N1 influenza exceeded the concentration required to inhibit the activity of oseltamivir-resistant neuraminidase by 50% in a laboratory assay.

The basis of these arguments is questionable. First, the use of untreated controls from previous seasons is problematic, because inherent differences in the patients and influenza viruses from different seasons would confound the result. Second, there is no direct correlation between an effective dose of oseltamivir in a laboratory assay and in the human body. Therefore, with no direct comparison between untreated and oseltamivir-treated groups in the same influenza season, the clinical effectiveness of oseltamivir in patients with resistant influenza infections is at present inconclusive.

Oseltamivir Side Effects

In vitro pharmacological selectivity profile of oseltamivir prodrug (Tamiflu®) and active metabolite.
Lindemann L *et al. Eur J Pharmacol. Published online November 13, 2009.*

Neuropsychiatric adverse effects have been reported in some influenza patients receiving oseltamivir (Tamiflu®) treatment. To address this issue, a group of scientists from F. Hoffmann-La Roche Ltd., maker of Tamiflu®, investigated the interaction of oseltamivir with different potential human receptor targets using various laboratory experimental systems. A prodrug is the original formulated medicine that is inactive until it is metabolized by the body and converted into an active metabolite that exerts the desired effect of the medicine. In this study, both the oseltamivir phosphate prodrug and its active metabolite, oseltamivir carboxylate, were tested against a panel of receptor targets. The test panel included in vitro synthesized human neuraminidases (NA), NA isolated from non-human primate and rodent brain tissue, seasonal influenza A H3N2 NA, and other molecular targets responsible for cardiovascular function, endocrine and metabolic function, cellular house-keeping function, and mood, cognition and behaviour. The influenza NA served as a positive control for these experiments. The rodent NA, which is known to be substantially different from human NA, served as the negative control.

Results showed oseltamivir is highly specific for influenza NA as little interaction was observed between oseltamivir and human NA and other human receptor targets. These findings suggest that the neuropsychiatric adverse events in some influenza patients treated with oseltamivir may be a disease- rather than a drug-related phenomenon.

pH1N1 in Pregnant and Immunocompromised Patients

H1N1 novel influenza A in pregnant and immunocompromised patients.
Lapinsky SE et al. *Crit Care Med. Published online November 23, 2009.*

Pregnant women are at a higher risk of viral pneumonia, respiratory failure and mortality due to complications as a result of influenza infection than the general population, especially during pandemics. During the 1918 influenza pandemic, the rate of mortality among pregnant women was estimated to be 27%. In the 1957 epidemic, the maternal mortality rate was 50%. The current pH1N1 pandemic is also associated with an increased rate of morbidity and mortality among pregnant women. In one study, the rate of admission of pregnant women was shown to be 4x higher than in the general population with pH1N1 infection. Although hospitalization of pregnant women due to seasonal influenza complications does not appear to cause significant adverse perinatal outcomes, pH1N1 has been associated with a high incidence of perinatal morbidity and mortality. Among 6 pregnant women in Winnipeg requiring mechanical ventilation, 2 maternal deaths occurred, resulting in 3 fetal losses and one fetus with severe hypoxic encephalopathy.

Oseltamivir is extensively used in pregnant women for the treatment of pH1N1 with good outcomes. Special considerations should be made regarding intubation and mechanical ventilation of pregnant patients who require oxygenation.

Immunocompromised people – for example, patients who are undergoing hematopoietic stem cell transplantation or solid organ transplantation, people infected with HIV, and patients with malignant disease undergoing chemotherapy and receiving corticosteroid treatment for inflammatory conditions – may experience more severe seasonal influenza-associated

complications. They are also at an increased risk of prolonged viral shedding and subsequent development of oseltamivir resistance. Among HIV patients, receiving highly active anti-retroviral therapy appears to be associated with improved influenza outcomes.

Given the paucity of data concerning the effect of pH1N1 on immunocompromised patients, similar precautions to seasonal influenza management should be taken. Vaccination continues to be the mainstay of influenza prevention among immuno-compromised patients. Although oseltamivir should be administered within 48 hours of symptom onset to achieve optimal effectiveness, treatment with oseltamivir in immunocompromised patients after the usual cut-off of 48 hours may still be beneficial due to prolonged viral shedding in these patients.

Mitigation Strategy Simulation Modelling

Assessment of intervention strategies against a novel influenza epidemic using an individual-based model.
Morimoto T and Ishikawa H. *Environ Health Prev Med. Published online November 26, 2009.*

To assess the effectiveness of anti-viral prophylaxis, school closure and restraint (self-imposed isolation) on mitigating the impact of an influenza pandemic, a group of Japanese scientists developed an individual-based simulation model of an H1N1 outbreak in a structured population based on demographic data of Sapporo City, Hokkaido, Japan. The scientists populated the virtual city by assigning each resident his/her individualized information (e.g. age, household, residence district, occupation, places of school and work, mode of transportation, social activity group, and casual contact group) drawn from the National Census of Japan, School Basic Survey and Employment Status Survey. Influenza infection dynamics used in this model were derived from observational data from previous pandemics and surveillance data for avian influenza A (H5N1). Each mitigation strategy was considered and analyzed independently and in combination.

In this study, two forms of anti-viral prophylaxis were explored, in addition to school closure and restraints: (1) broadly targeted anti-viral prophylaxis by prescribing anti-virals to symptomatic patients and their contacts; and, (2) school-age targeted anti-viral prophylaxis by prescribing anti-virals to symptomatic school-age children and their household contacts. In general, each examined mitigation strategy could reduce the impact of an influenza outbreak to varying degrees compared to no mitigation. However, by combining different interventions, a synergistic effect could be seen. The most effective mitigation combination was broadly targeted anti-viral prophylaxis, school closure and restraint. This combination could avert the highest number of patients and death, and shorten the duration of an outbreak.

NCCID Comments:
This model has several limitations. First, vaccination strategies were not considered. In other modelling studies that examined vaccination as an integral part of a mitigation plan or as a stand-alone intervention, vaccination has consistently been shown to be more effective than other interventions. Second, the main mitigation strategy presented here was the use of anti-virals. It would be worthwhile to consider how anti-viral resistance may influence the results of this model. Third, the cost of these mitigation strategies, especially school closures and restraint was not considered. Although, in theory, anti-viral chemoprophylaxis, school closures, and broad societal restraint may produce impressive reductions in attack rates and peaks in mathematical models, the costs will need to be factored into future modeling if decision-makers wish to be able to make fully evidence-informed decisions concerning recommending such drastic interventions.

References

[1] WHO 14 May 2009 Press Briefing. Transcript of virtual press conference with Gregory Hartl, WHO Spokesperson for Epidemic and Pandemic Diseases, and Dr Keiji Fukuda, Assistant Director-General ad Interim for Health Security and Environment, World Health Organization. Available online: http://www.who.int/mediacentre/influenzaAH1N1_prbriefing_20090514.pdf

[2] Oseltamivir-resistant pandemic (H1N1) 2009 influenza virus, October 2009. *WHO Weekly Epidemiological Record 2009; 84:453-368.*

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