National Collaborating Centre for Infectious Diseases
Centre de collaboration nationale des maladies infectieuses

Purple Paper

Pandemic pH1N1 Weekly Literature Synthesis
(Week of November 29-December 5, 2009)

Epidemiology of pH1N1

Estimated epidemiologic parameters and morbidity associated with pandemic H1N1 influenza.
Tuite AR et al. *CMJ. Published online December 3, 2009.*

The investigators of this study used laboratory-confirmed pH1N1 cases from **Ontario** between April 13-June 20, 2009 during the first wave of the pandemic to estimate the epidemiological parameters of pH1N1. This cut-off was chosen as routine individual reporting of pH1N1 cases was stopped after this date. A total of 3,152 laboratory confirmed pH1N1 cases was reported during this period, with the mean age of patients being 21.9 years. The investigators concluded that:

1. The median time from exposure to symptom onset was 4 days.
2. The median duration of symptoms was 7 days. The median duration was significantly shorter among patients aged < 18 years (7 days) than older patients (8 days).
3. A total of 140 hospital admissions and 10 deaths were reported during the study period. The estimated risk of hospital admission per case was 4.5%. The estimated case-fatality rate was 0.3%. The estimated case-fatality rate among asymptomatic cases was 0.2%. The risk of hospitalization was highest among infants aged < 1 year and among those aged ≥ 65 years; adolescents had a decreased risk. Although adults > 50 years of age comprised only 7% of cases, 7 of 10 deaths belong to this age group.
4. The mean basic reproduction number (R_0; the average number of secondary cases generated by a typical case in a susceptible population) was 1.31.
5. The mean latent period (the time from infection to when the individual is infectious to others) was 2.62 days.
6. The mean duration of infectiousness (the time period during which an asymptomatic or symptomatic case is able to infect other susceptible individuals) was 3.38 days.
7. The serial interval (the average time from onset of infectiousness in a case to the onset of infectiousness in a person infected by the case; i.e., the sum of the latent period and half the duration of infectiousness) was estimated to be 4-5 days.
8. In the absence of intervention, the overall attack rate (the proportion of individuals who are infected among those who are exposed) was estimated to be 20-50%.

NCCID Comments:
This study's estimate for the basic reproduction number for pH1N1 in Ontario (1.31) is in general agreement with estimates from Mexico (1.2-1.6) [1,2] and the US (1.7-1.8) [3]. Compared to seasonal influenza, whose reproduction number had been estimated to be 1.3 (with year-to-year variability of 0.9-2.1) [4], these estimates indicate that a person with pH1N1 can infect a similar number of people. The serial interval for pH1N1 estimated to be 4-5 days is also comparable to the estimate for seasonal influenza (3.6 days) [5]. In spite of this, the higher attack rate of pH1N1 than seasonal influenza in younger age groups may translate into a greater total number of hospitalizations and deaths associated with pH1N1. For comparison, the serial interval for pH1N1 in the US was estimated to be 2.2-2.3 days [3].

If you have any comments or questions about the *Purple Paper*, please contact us at nccid@icid.com.

pH1N1 Vaccine

Safety of influenza A (H1N1) 2009 monovalent vaccines – United States, October 1-November 24, 2009.
CDC. *MMWR 2009 Dec 4; 58(Early Release):1-6.*

In the **USA**, 2 monovalent vaccines are licensed for the pandemic influenza A/H1N1: (1) a live, attenuated vaccine for intranasal administration; and (2) an inactivated, split virus or subunit vaccine for intramuscular injection. None of the USA vaccines is adjuvanted. To assess the safety profile of pH1N1 vaccines, CDC reviewed reports submitted through the US Vaccine Adverse Event Reporting System (VAERS) and electronic data in Vaccine Safety Datalink (VSD).

Health-care providers and manufacturers are required to report adverse events in vaccinees to VAERS. The general public can also report adverse events voluntarily through the same mechanism. VAERS is an early alert system that flags potential new, rare or unusual patterns of adverse event; however, it cannot be used to infer causality. VAERS reports were coded as fatal or non-fatal serious adverse events, or as non-serious adverse events. Serious adverse events are defined as those resulting in death, life-threatening illness, hospitalization, prolongation of hospitalization, persistent or significant disability, or congenital anomaly.

Between July 1-November 25, 2009, there were 3,783 reports of adverse events after receipt of pH1N1 vaccine, of which 204 were serious. During the same period, VAERS received 4,672 reports of adverse events after receipt of seasonal influenza vaccines, of which 283 were serious. These are equivalent to 82 adverse events per 1 million pH1N1 vaccine doses distributed and 47 per 1 million seasonal influenza vaccine doses distributed. The serious adverse event reporting rates were 4.4 and 2.9 serious adverse events per 1 million doses distributed for pH1N1 and seasonal influenza vaccines, respectively. The percentage of serious adverse events among all reported adverse events after receipt of pH1N1 and seasonal influenza vaccines were 5.4% and 6.1%, respectively.

Thirteen deaths were reported to VAERS following receipt of pH1N1 vaccine. In 9 deaths, underlying co-morbid conditions were present. One death was the result of a car crash. The remaining 3 deaths were under final review. Of 12 reported possible cases of Guillain-Barré syndrome (GBS) – 4 were confirmed, 4 did not meet preset criteria and 4 are still pending. 19 possible cases of anaphylaxis were also received through VAERS – 13 cases met preset criteria, 5 had an anaphylaxis diagnosis on medical record review, and 1 has not been confirmed. 3 of the GBS cases and 15 of the anaphylaxis cases were coded as serious adverse events. The remaining 173 non-fatal serious adverse events after vaccination with pH1N1 vaccines are currently under review. Overall, the reported proportion and type of serious adverse events appear similar for both pH1N1 and seasonal influenza vaccines.

VSD is a collaborative effort between CDC and eight managed-care organizations to monitor vaccine safety by collecting information on vaccinations and health-care encounters from administrative data and electronic medical records. Because of its capacity to follow vaccinated and unvaccinated persons over time, VSD can test hypotheses generated by VAERS reports and delineate associations between adverse/beneficial health events and vaccination. As of November 21, 2009, 428,376 doses of pH1N1 vaccines were administrated under VSD surveillance. Between October 1-November 21, 2009, no cases of GBS and 1 case of anaphylaxis were reported. There was no increase in rates of other monitored adverse events.

Viral Load in Patients with pH1N1

Viral load in patients infected with pandemic H1N1 2009 influenza A virus.
To KKW *et al. J Med Virol 2010; 82:1-7. Published online November 30, 2009.*

In this study, the investigators profiled the pattern of viral shedding at different body sites in hospitalized pH1N1 patients in **Hong Kong**. They examined the viral load in various clinical samples, including respiratory secretions, serum, urine and stool. Respiratory samples from pH1N1 patients were compared with archived respiratory samples from patients with seasonal influenza in 2007-2009.

Serial sampling of respiratory specimens were also conducted among pH1N1 patients to follow the trend of viral shedding as influenza illness progressed. The viral load of all clinical samples was determined by quantitative laboratory assays.

Twenty-two pH1N1 patients and 44 randomly selected seasonal influenza patients were included in this study. Except for coughing, vomiting, diarrhea and duration of symptom before diagnosis, review of their medical charts did not indicate statistically significant differences between the two groups regarding demographics, underlying diseases, presenting symptoms and other clinical features. Whereas 21 of 22 pH1N1 patients received oseltamivir treatment, no seasonal influenza patients received any anti-viral therapy.

Examination of respiratory samples from pH1N1 patients showed that the median duration of viral shedding after symptom onset was 4 days, although some patients had viral shedding for up to 7 days after onset of symptoms. Viral load was at its highest on the day of symptom onset and declined gradually thereafter. A similar trend was observed in respiratory samples collected from seasonal influenza patients. The initial viral load on the day of symptom onset in seasonal influenza cases was higher than that in pH1N1 cases; however, this difference did not appear to be statistically significant. Pandemic H1N1 virus was detected in stool and in urine from 4 of 9 and 1 of 14 patients, respectively. No pH1N1 virus was detected in any serum samples. Prolonged viral shedding in the respiratory tract and higher viral load in stool were associated with young age.

NCCID Comments:
There are several major limitations in this study. The number of study subjects was small. Furthermore, the fact that almost all pH1N1 patients received oseltamivir treatment might have influenced the viral load in various clinic samples. Lastly, as the authors note, during the containment phase of Hong Kong's pandemic control strategy, even pH1N1 patients with mild symptoms were hospitalized and had respiratory specimens collected for virological diagnosis. This is contrary to seasonal influenza period when only severe influenza cases are hospitalized. Therefore, the seasonal influenza patients in this study likely had underlying medical conditions that exacerbated their influenza illness, causing more severe symptoms and possibly a higher initial viral load at diagnosis.

Prevention and Treatment of Influenza in Infants

Trivalent inactivated influenza virus vaccine given to two-month-old children.
Walter EB et al. *Pediatr Infect Dis J 2009; 28:1099-1104.*

In this **open-label, proof-of-concept trial** sponsored by sanofi pasteur, the authors examined the safety and immunogenicity of a trivalent inactivated influenza vaccine (Fluzone®) in children aged 6-12 weeks compared to children aged 6 months. The children were vaccinated in late spring and summer with the 2004/05 influenza vaccine – the same vaccine for the season just completed. The 2004/05 influenza vaccine was composed of influenza A/New Caledonia/20/99 (H1N1), A/Wyoming/03/2003 (H3N2) and B/Jiangsu/10/2003 strains. There are two doses in the series, administered one month apart. During the course of the study, all participants were allowed to receive other childhood vaccinations as scheduled. This study was primarily an observational study and was not adequately powered to reliably detect differences between the two study groups.

Only healthy children were enrolled. Children who had allergies to eggs or chicken proteins; prior influenza vaccines or documented influenza infection; any underlying medical conditions or immunological/developmental disorders; exposure to or infected with HIV, hepatitis B or C; received blood or blood products in the preceding 2 months; or prior history of Guillain-Barré syndrome were excluded from the study.

To assess the safety of the seasonal influenza vaccine in participants, parents were instructed to record the daily maximum severity of injection site reactions (tenderness, redness, and swelling), the maximum daily temperature, and systemic reactions (vomiting, abnormal crying, drowsiness, loss of appetite, and irritability) for 7 days following each injection. Parents were also contacted 6

months after the final dose of the vaccine to elicit any serious adverse events. To assess the immunogenicity of the seasonal influenza vaccine, laboratory assays were performed to determine antibody titers against influenza in sera collected before vaccination and 1 month after the second dose of the vaccine.

A total of 394 children were initially enrolled in the study. After loss to follow-up, a total of 293 (74%) children were available for immunogenicity analysis (149 in the 2-month age group and 144 in the 6-month age group). In general, the seasonal influenza vaccine was well-tolerated among children in the 2-month age group. Reactions or adverse events were mild and comparable between the two groups.

Mean pre-vaccination antibody titers were higher in the 2-month age group than the 6-month age group, probably due to the presence of maternal antibodies in the younger age group. Conversely, mean post-vaccination antibody titers were higher in the 6-month age group than the 2-month age group. The proportion of children who achieved a protective antibody titer against the vaccine influenza A/H1N1, A/H3N2 and B strains was consistently higher in the 6-month group than the 2-month age group. Because the presence of maternal antibodies has previously been associated with development of a diminished antibody response in young children, the authors re-analyzed the data by excluding 2-month old children with baseline seropositivity and maternal receipt of influenza vaccine. Results showed that the proportion of children of this subset who could achieve a protective antibody titer against the vaccine influenza A/H1N1 and A/H3N2 strains was comparable to the 6-month age group. However, the proportion of 2-month children who developed protective response to influenza B remained low.

Safety of oseltamivir compared with the adamantanes in children less than 12 months of age.

Kimberlin DW *et al. Pediatr Infect Dis J. Published online November 25, 2009.*

Oseltamivir is licensed in the US and Canada for the treatment and prophylaxis of seasonal influenza in children aged 1 year and over. Oseltamivir has been shown to shorten the duration of influenza illness, decrease viral shedding and reduce the incidence of acute otitis media in treated children. Despite its potential benefits, concerns over possible neurologic adverse effects preclude the use of oseltamivir in children aged < 1 year. Nonetheless, off-label use of oseltamivir in children aged < 1 year does occur in the US, especially in infants who are seriously ill or may be at high risk of influenza-associated complications. To assess potential neurologic adverse effects of oseltamivir in infants, the National Institute of Allergy and Infectious Diseases (NIAID) Collaborative Antiviral Study Group performed a **retrospective chart review** in 15 participating academic medical centres of infants < 12 months of age who received oseltamivir therapy compared with infants who received adamantanes (amantadine or rimantadine).

Medical charts were identified for 180 infants. Of 180 subjects, 115 (64%) received oseltamivir, 37 (20%) received amantadine, and 28 (16%) received rimantadine. The median dosage of oseltamivir in the subjects was 2.0-2.2 mg/kg/dose for a median duration of 5-6 days. There were no statistically significant differences in the numbers of neurologic abnormalities during therapy in infants treated with oseltamivir compared with those treated with adamantanes. All recorded neurologic adverse effects experienced by infants treated with either oseltamivir or adamantanes included abnormalities consistent with influenza disease (e.g. vomiting, decreased oral intake), related to pre-existing underlying neurologic conditions (e.g. hypoxic ischemic encephalopathy or congenital cytomegalovirus infection), or explainable by a concomitant medication (e.g. lorazepam). There were no statistically significant differences in demographics and other characteristics between subjects who experienced neurologic adverse events and those who did not. In addition, except for the head/eyes/ears/nose/throat (HEENT) body system, there were no statistically significant differences in abnormalities in other body systems observed in infants treated with oseltamivir or adamantanes. Infants treated with oseltamivir were less likely to develop abnormalities in the HEENT body system (e.g. otitis media, conjunctivitis, rhinorrhea etc.) than their

counterparts treated with adamantanes. This observation is consistent with the known benefit of oseltamivir in reducing the incidence of otitis media in children during influenza infection. This paper documents the second study to date that examined possible oseltamivir-associated neurologic side effects in infants and corroborates findings of the initial study in Japan [6].

NCCID Comments:
As the current H1N1 pandemic unfolded during the first wave in spring 2009, it became clear that children under the age of 1 year with pH1N1 experienced higher rates of hospitalization, ICU admissions and deaths compared to other age groups in Canada. Upon Health Canada Interim Order's permit to expand oseltamivir treatment and prophylaxis in children aged < 1 year with pH1N1 infection, PHAC released the *Interim Guidance for emergency use of oseltamivir (Tamiflu®) in children under one year of age in the context of 2009 (H1N1) pandemic* on July 20, 2009. Prescription of oseltamivir to infants is left to clinicians' discretion; and may apply to suspect cases where rapid test is positive, febrile children without another clear cause and a positive contact history, and febrile infants with respiratory compromise. For a copy of the *Interim Guidance* and recommended oseltamivir dosages, visit http://www.phac-aspc.gc.ca/alert-alerte/h1n1/guidance-orientation-07-20-eng.php.

Anti-Viral Resistance in Influenza

Reassortment between amantadine-resistant and -sensitive H1N1 influenza A viruses generated an amantadine-sensitive virus during the 2007-2008 season.
Furuse Y et al. *J Infect Dis 2009; 200:1766-1773.*

Amantadine was brought to the market as a treatment for influenza A in 1966. Since then, resistance to this anti-viral, caused by a mutation in the matrix 2 (M2) surface protein, has increased dramatically in both seasonal influenza A/H3N2 and H1N1 viruses. In **Japan**, transient amantadine resistance in seasonal influenza A/H1N1 viruses had been observed between the 2005/06 and 2008/09 seasons. To investigate how amantadine-resistance was gained and lost in seasonal influenza A/H1N1 viruses, authors of this study:

1. isolated both resistant and sensitive seasonal A/H1N1 strains from clinical samples during consecutive seasons between2005 and 2009 in Sendai,
2. analyzed their sequences, and
3. delineated their "temporal-familial" relationship to other known seasonal influenza isolates of the same period.

Laboratory tests of the clinic samples showed that amantadine resistance first emerged among seasonal A/H1N1 strains in the 2005/06 season. Subsequently, all seasonal A/H1N1 strains were resistant to amantadine in the 2006/07 season. During the 2007/08 season, both amantadine-resistant and -sensitive seasonal A/H1N1 viruses co-circulated, with the majority of isolated viruses being sensitive to amantadine. By the 2008/09 season, all isolated seasonal A/H1N1 strains were sensitive to amantadine.

Further genetic sequence analysis suggested that the co-circulating resistant and sensitive H1N1 strains during the 2005/06 season might have emerged independently via different evolutionary pathways. Amantadine-resistant influenza A/H1N1 strains in the 2006/07 and 2007/08 seasons appeared to be direct descendants of the resistant 2005/06 strain. By contrast, the amantadine-sensitive A/H1N1 strain isolated in the 2007/08 season was a reassortant between resistant and sensitive viruses, in which at least 4 of its 8 gene segments were derived from the initial resistant strain. This 2007/08 amantadine-sensitive influenza A/H1N1 strain later became the predominant circulating H1N1 strain in the 2008/09 season.

In a separate study using similar laboratory methods, the investigators examined the mechanism by which amantadine resistance was developed in the seasonal influenza A/H3N2 strains in the same period [7]. The investigators found that not only did reassortment of the co-circulating resistant and sensitive 2005/06 H3N2 strains generate a novel amantadine-sensitive strain, a new amantadine-resistant H3N2 strain was also produced that dominated the H3N2 seasonal subtype of the following seasons. Together, results from the two studies indicate that the conditions required for sustained amantadine resistance are different for seasonal H1N1 and H3N2 influenza A

viruses. In addition, although anti-viral therapy exerts substantial pressure for selection of resistance in influenza viruses, other environmental and biological factors probably also play an important role.

Oseltamivir Resistance and the H274Y neuraminidase mutation in seasonal, pandemic and highly pathogenic influenza viruses.
Hurt AC et al. *Drugs 2009; 69:2523-2531.*

Two neuraminidase inhibitors became available to the market in 1999: zanamivir (Relenza®, GlaxoSmithKline) and oseltamivir (Tamiflu®, Roche). This **review article** summarizes what is currently known about oseltamivir resistance in seasonal, pandemic and other highly pathogenic influenza A viruses.

Oseltamivir is a drug designed specifically to physically block the enzymatic active site of influenza neuraminidase and prevent the release of new viral particles from infected host cells. Contrary to the adamantanes (M2 inhibitors – amantadine and rimantadine), where resistance to this class of anti-virals is widespread among influenza A viruses, resistance to neuraminidase inhibitors is less common and primarily targets oseltamivir. The most common mutation in the neuraminidase surface protein is R292K (a change from arginine to lysine in amino acid position 292) for oseltamivir-resistant seasonal influenza A/H3N2 viruses, and H274Y (a change from histadine to tyrosine in position 274) for resistant seasonal influenza A/H1N1 viruses. In essence, the latter mutation widens the space in the interface between oseltamivir and N1 neuraminidase. The resulting loss of a tight fit between respective interacting parts renders oseltamivir ineffective against resistant seasonal influenza A/H1N1 viruses.

Despite findings from *in vitro* and animal studies which had demonstrated poor transmission of oseltamivir-resistant influenza A strains, resistant influenza A viruses continue to spread around the globe and reached unprecedented frequencies of > 90% in some countries. For example, in Japan, the incidence of oseltamivir-resistant seasonal H1N1 infection increased from 3% during the 2007/08 season to over 99% at the end of the 2008/09 season. This rapid global spread suggests that oseltamivir-resistant influenza A viruses might have acquired additional mutations that enhance viral fitness, thus enabling them to out-compete sensitive viruses.

Oseltamivir resistance is also observed in other N1 influenza A viruses, including the highly pathogenic avian H5N1 and the current circulating pandemic H1N1 strains. While both viruses possess the H274Y mutation, additional mutations have been found in oseltamivir-resistant A/H5N1 viruses, which may synergistically increase oseltamivir resistance in viruses that already have the H274Y mutation.

NCCID Comments:
So far, evidence does not indicate sustained human-to-human transmission of oseltamivir-resistance pH1N1 viruses [8]. At the time of this writing, 102 oseltamivir-resistant cases had been identified worldwide since the beginning of the pandemic [Source: CDC as of December 11, 2009].

Post-exposure prophylaxis during pandemic outbreaks.
Moghadas S et al. *BMC Medicine. Published online December 2, 2009.*

Emergence of oseltamivir resistance in some pH1N1 patients may jeopardize the use of this anti-viral as a feasible treatment option for pH1N1. The authors of this study developed a **simulation model** to examine the effect of oseltamivir treatment and prophylaxis in reducing morbidity and mortality associated with influenza infection in the presence of transmissible oseltamivir resistance during pandemic periods.

The parameter estimates of this model were largely based on published data for influenza A in humans without pre-existing immunity. Anti-viral effectiveness estimates were derived from studies of seasonal influenza. Parameters that were taken

into consideration included incubation period, duration of clinical disease, time after onset of symptoms when oseltamivir treatment is most effective and ineffective, and transmission fitness of influenza by infectious symptomatic and asymptomatic individuals. The model assumed that oseltamivir treatment will be made available to infected individuals within 2 days after onset of symptoms, and oseltamivir would be ineffective against resistant infection. It also assumed that when an infected individual is treated with oseltamivir, imposed selective pressure could give rise to anti-viral resistant influenza strains. For post-exposure prophylaxis, the model only targeted close contacts of treated index cases.

Findings of this model demonstrated that coverage with oseltamivir prophylaxis must be complementary to a given treatment level in order to minimize the total number of influenza infections. In general, as the proportion of treated influenza cases increases, the coverage of prophylaxis must decrease to minimize the total number of infections and deaths. The most important factor that dictates the incidence of infection and death is the transmission fitness of the resistant strain. Taken together, results suggest that in the presence of transmissible oseltamivir resistance, mitigation strategies involving the use of anti-virals that focus on treatment of ill influenza patients, rather than prophylaxis of suspected cases, would be the most effective method to reduce influenza-associated morbidity and mortality.

This model focuses on selective pressure exerted by the use of oseltamivir as the primary driving force for the development of anti-viral resistance. However, as the authors note, one should keep in mind that the emergence and spread of resistance can also be influenced by other factors. One example is the acquisition of additional mutations by anti-viral-resistant influenza strains that may compensate for the fitness cost associated with development of resistance. This explanation has been suggested for the phenomenon of rapid global spread of oseltamivir-resistant seasonal influenza A/H1N1 viruses.

NCCID Comments:
At present, oseltamivir-resistant pH1N1 cases remain sporadic [8]. According to this simulation model, oseltamivir would continue to be effective in reducing the number of infections and deaths associated with pH1N1. Nonetheless, the use of oseltamivir must be prudent as future enhancement of transmission fitness of the pH1N1 virus appears likely.

Notable Publications

Guidance on novel influenza A/H1N1 in solid organ transplant recipients.
Kumar D et al. *Am J Transplant. Published online December 2, 2009.*

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