

pH1N1 – H3N2: A Novel Influenza Virus Reassortment

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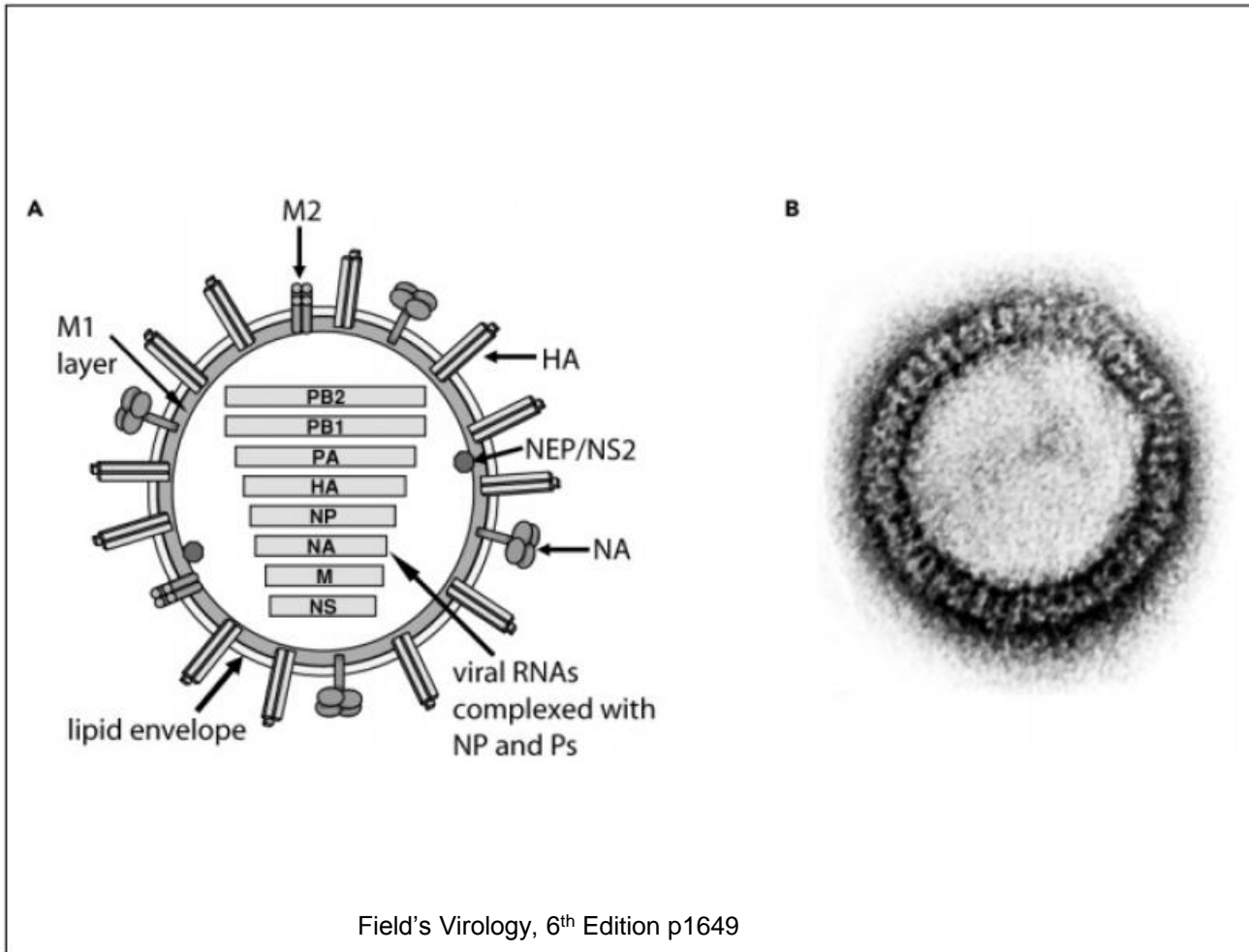
June 16, 2011



pH1N1 – H3N2 Reassortment: Talk Overview

- Explain strain typing of influenza viruses and genetic drift.
- Review molecular changes observed in seasonal H3N2 during 2010/11 influenza season in Ontario
 - Discuss relationship between molecular changes and antigenic drift
- Review genetic shift in influenza viruses
 - Highlight reassortments that have not resulted in pandemics.
- Summarize recent Ontario case of coinfection with pH1N1 and H3N2 followed by reassortment.
 - Discuss implications of this finding.

Influenza Structure



H1N1 hemagglutinin antigenic sites and receptor binding sites

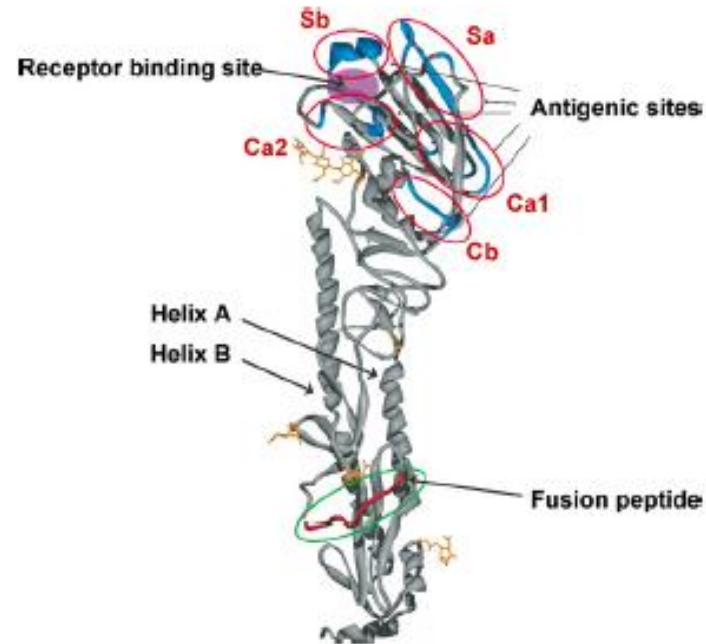


Fig. 1. Ribbon diagram of an uncleaved hemagglutinin monomer from the 1918 influenza A virus (H1N1), the causative agent of the “Spanish flu” pandemic. The head contains the sialic acid receptor-binding site, which is surrounded by the five predicted antigenic sites (Sa, Sb, Ca1, Ca2, and Cb). The stem comprises helices A and B and the fusion peptide, as shown. (Adapted from a figure, kindly provided by James Stevens and Ian Wilson, in [1].)

Fields Virology. Philadelphia: LippincottWilliams &Wilkins; 2007.

Hemagglutination Assay

A serial dilution of virus is mixed with a fixed amount of RBCs

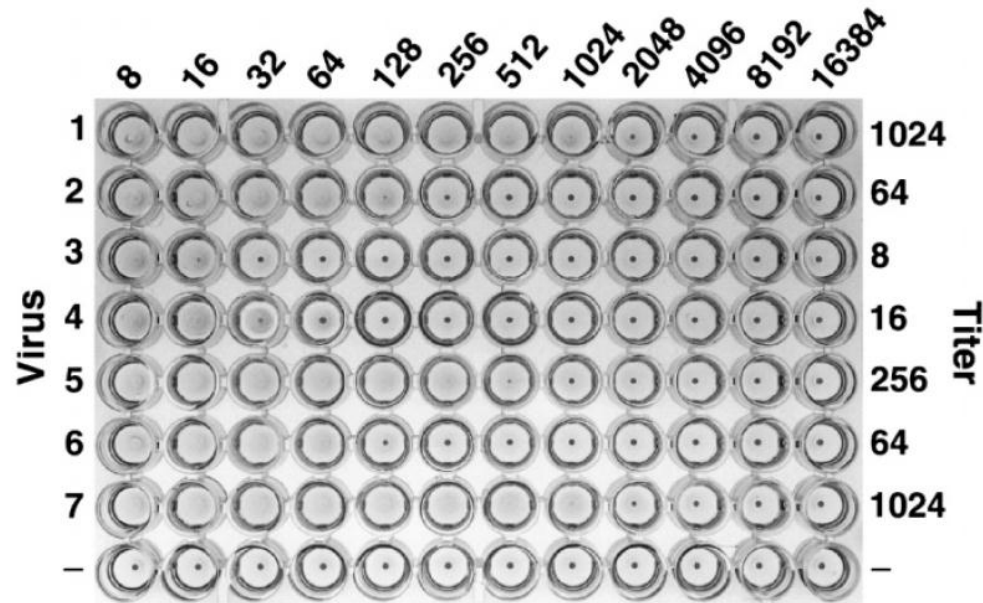
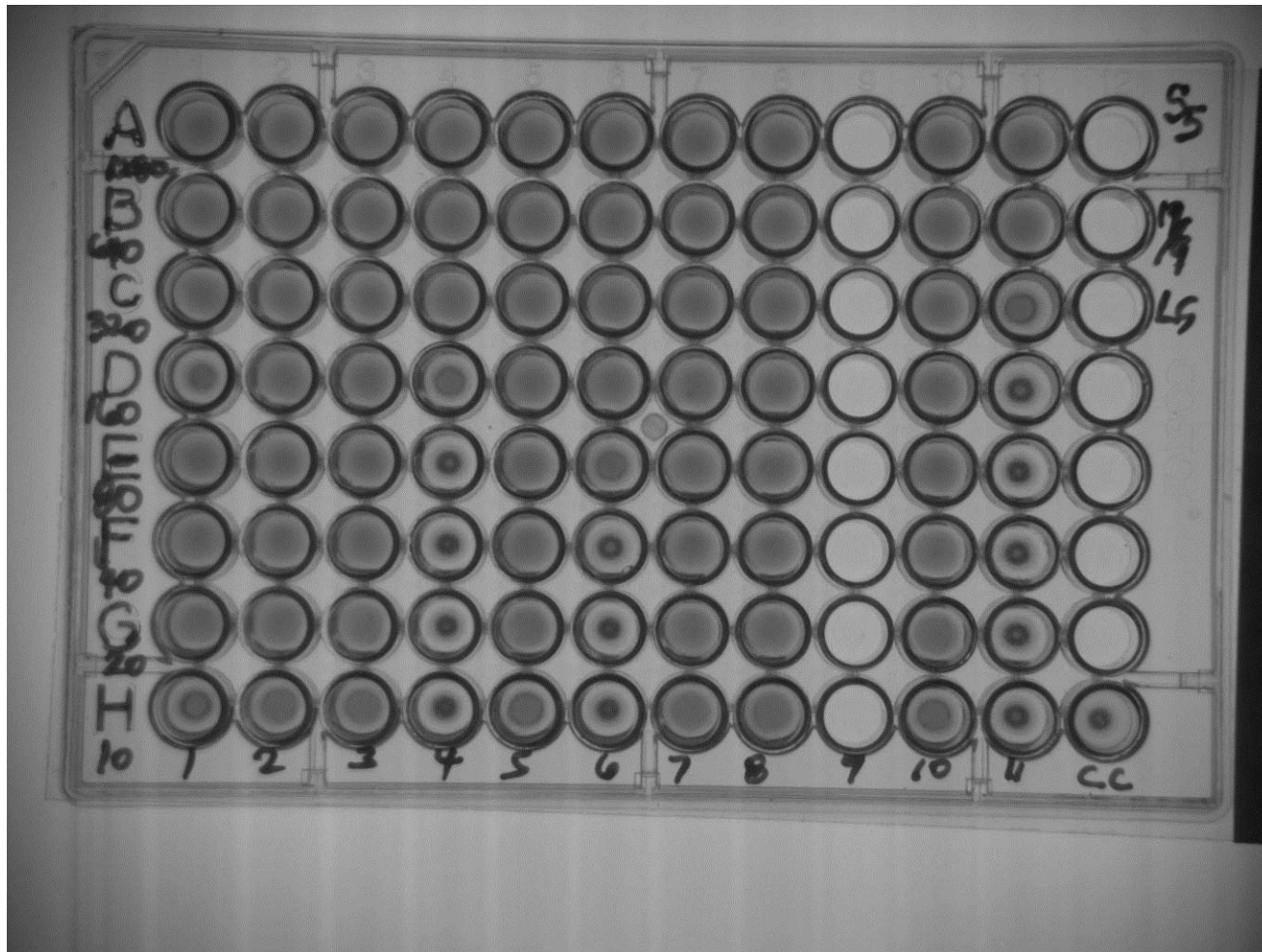


Figure 2.9 Hemagglutination assay (HA): Seven different samples of influenza virus, numbered 1–7 at the left, were serially diluted as indicated at the top, mixed with chicken red blood cells (RBC), and incubated on ice for 1–2 hours. Wells in the bottom row contain no virus. Agglutinated RBC coat wells evenly, in contrast to nonagglutinated cells, which form a distinct button at the bottom of the well. The HA titer, shown at the right, is the last dilution that shows complete hemagglutination activity. (Courtesy of Drs. J. Talon and P. Palese.)

Hemagglutination Inhibition Assay

A fixed amount of virus and RBC is mixed with a serial dilution of antibody



Influenza Antigenic Drift

- Mutations in nucleotides result in amino acid alterations, predominantly at antigenic sites.
- Drift is suspected in an influenza virus when hemagglutination is poorly inhibited in the HAI assay using a panel of antibodies against known circulating influenza viruses.
- Confirmed when the virus is injected into animals (e.g ferrets) and the antibody formed gives a higher HAI titre than any antibody panels to other circulating influenza viruses.

Influenza genetic drift

- H genes undergo coding nucleotide substitutions at higher rate than other influenza genes.
- Influenza viruses have 5 antigenic sites in HA.
 - designated A to E for the H3 strains
 - Ca1 , Ca2, Cb, Sa, and S in H1
- H3 and H1 mutate at a rate to give
 - amino acid changes in HA1 of 0.8% to 1% per year.
 - 5×10^{-3} nucleotide substitutions per site per year.
- Influenza B slightly lower rate mutation in HA1:
 - 0.5% amino acid change per year and 4×10^{-3} nucleotide substitutions per site per year

Influenza genetic drift

- Each new drift variant of epidemiologic importance has generally had a total of four or more amino acid substitutions across two or more of the antigenic sites.
- On average 17 unique a.a mutations in exposed surface portion of H1 and H3 per year occur between epidemic years.
 - Equates to one major epitope, or 20% of each of the 5 antigenic sites mutating per year.
 - Wilson & Cox. Annu, Rev, Immunol. 1990,8:737-71

Sequencing of early H3N2 Perth influenza isolates in Ontario

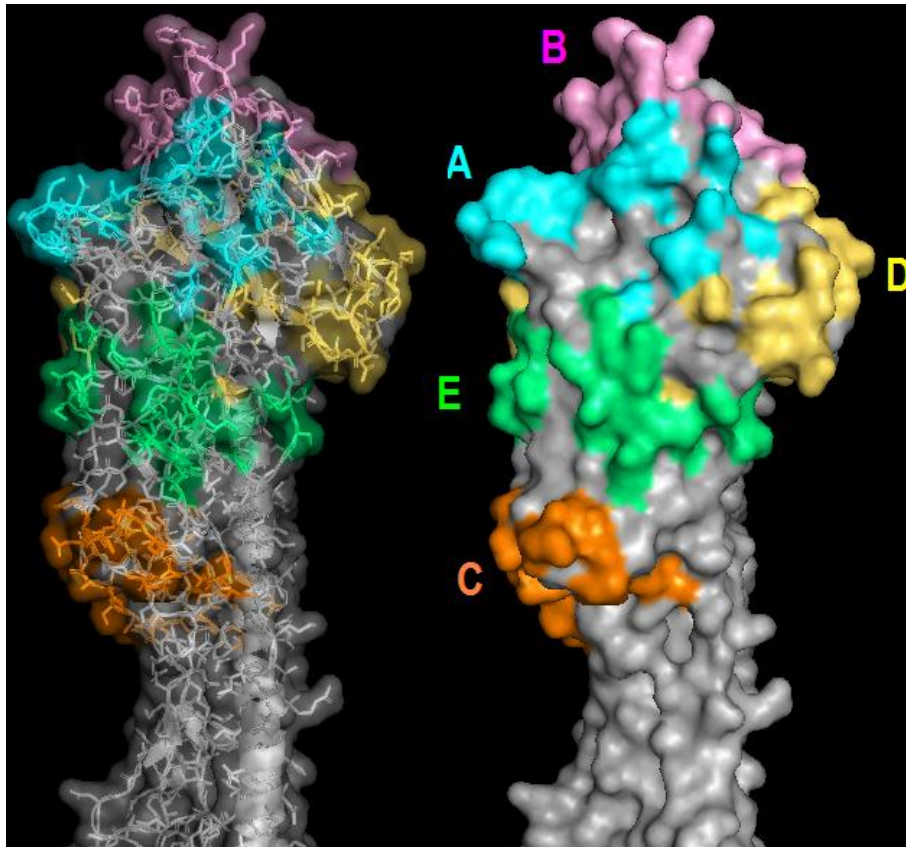
Up to 10 a.a. mutations across the 5 antigenic sites.

| | | | Perth/16/2009 | Site C | | | | | Site A | | | | Site D | | | | Site E | | | | | Site B | | Extra mutations |
|------------|--------|----|---------------|--------|----|-----|-----|-----|--------|-----|-----|-----|--------|-----|-----|-----|--------|-----|----|----|----|--------|-------|---------------------|
| | | | | 50 | 53 | 280 | 312 | 304 | 140 | 144 | 146 | 168 | 208 | 212 | 214 | 230 | 260 | 261 | 75 | 62 | 94 | 199 | 159 | |
| | | | | E | D | E | N | A | I | K | N | M | R | T | S | I | I | R | Q | K | Y | S | F | |
| 10U0010611 | Aug-09 | 1 | Honduras | . | . | . | S | . | . | N | . | . | . | A | I | . | . | . | . | . | . | . | . | D291E, K468T, D489N |
| 10C0432471 | Aug-10 | 2 | Mexico | . | N | A | . | . | . | N | . | . | K | A | I | V | . | . | . | E | H | . | . | |
| 10H0001995 | Sep-14 | 3 | Hamilton | . | N | A | . | . | . | N | . | . | . | A | I | V | . | . | . | E | H | . | . | |
| 10C0526567 | Sep-15 | 4 | -OB | . | N | A | . | . | M | N | . | . | . | A | I | V | . | . | . | E | H | . | . | |
| 10C0537546 | Sep-17 | 5 | -OB | . | N | A | . | . | M | N | . | . | . | A | I | V | . | . | . | E | H | . | . | R220K |
| 10N0091737 | Sep-22 | 6 | -OB | . | N | A | . | . | . | N | . | . | . | A | I | V | . | . | . | E | H | A | . | |
| 10N0091738 | Sep-22 | 7 | -OB | . | N | A | . | . | . | N | . | . | . | A | I | V | . | . | . | E | H | A | . | |
| 10C0575475 | Sep-29 | 8 | -OB | . | N | A | . | . | M | N | . | . | . | A | I | V | . | . | . | E | H | . | . | |
| 10C0575478 | Sep-29 | 9 | -OB | . | N | A | . | . | M | N | . | . | . | A | I | V | . | . | . | E | H | . | . | C524G |
| 10C0575591 | Sep-30 | 10 | HONG KONG | K | . | . | . | T | . | . | . | I | . | . | I | . | M | Q | . | . | . | . | P162S | |
| 10C0575589 | Oct-01 | 11 | Hosp | . | N | A | . | . | M | N | . | . | . | A | I | V | . | . | . | E | H | . | . | |
| 10C0575590 | Oct-01 | 12 | Hosp | . | N | A | . | . | M | N | . | . | . | A | I | V | . | . | . | E | H | . | . | |
| 10C0582096 | Oct-02 | 6 | | . | N | A | . | . | M | N | . | . | . | A | I | V | . | . | . | E | H | . | . | |
| 10C0543493 | Sep-20 | 13 | | . | N | A | . | . | . | N | G | . | . | A | I | V | . | . | . | E | H | . | Y | |
| 10C0582144 | Oct-03 | 14 | | . | N | A | . | . | M | N | . | . | . | A | I | V | . | . | . | E | H | . | . | |
| 10C0582093 | Oct-04 | 15 | | . | N | A | . | . | . | N | . | . | . | A | I | V | . | . | . | E | H | A | . | |
| 10C0627683 | Oct-08 | 16 | | . | . | K | . | . | . | . | . | . | . | . | I | . | M | Q | H | . | . | . | . | P162S, G510E |
| 10P0021546 | Oct-09 | 17 | -OB | G | N | A | . | . | M | N | . | . | . | A | I | V | . | . | . | E | H | . | . | |
| 10P0021548 | Oct-09 | 18 | -OB | G | N | A | . | . | M | N | . | . | . | A | I | V | . | . | . | E | H | . | . | |
| 10C0610464 | Oct-13 | 19 | | . | N | A | . | . | M | N | . | . | . | A | I | V | . | . | . | E | H | . | . | |

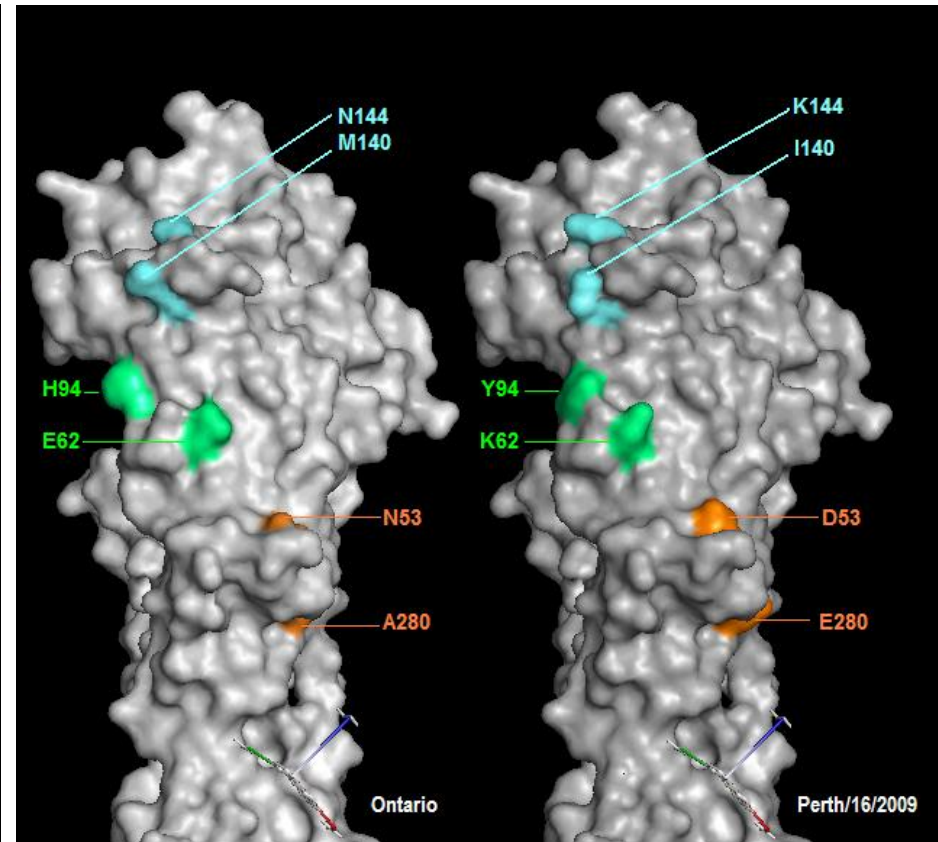
- No reduction in HAI titre to vaccine strain done at NML
- Although represents the “norm” need ongoing vigilant molecular surveillance.

Alireza Eshaghi

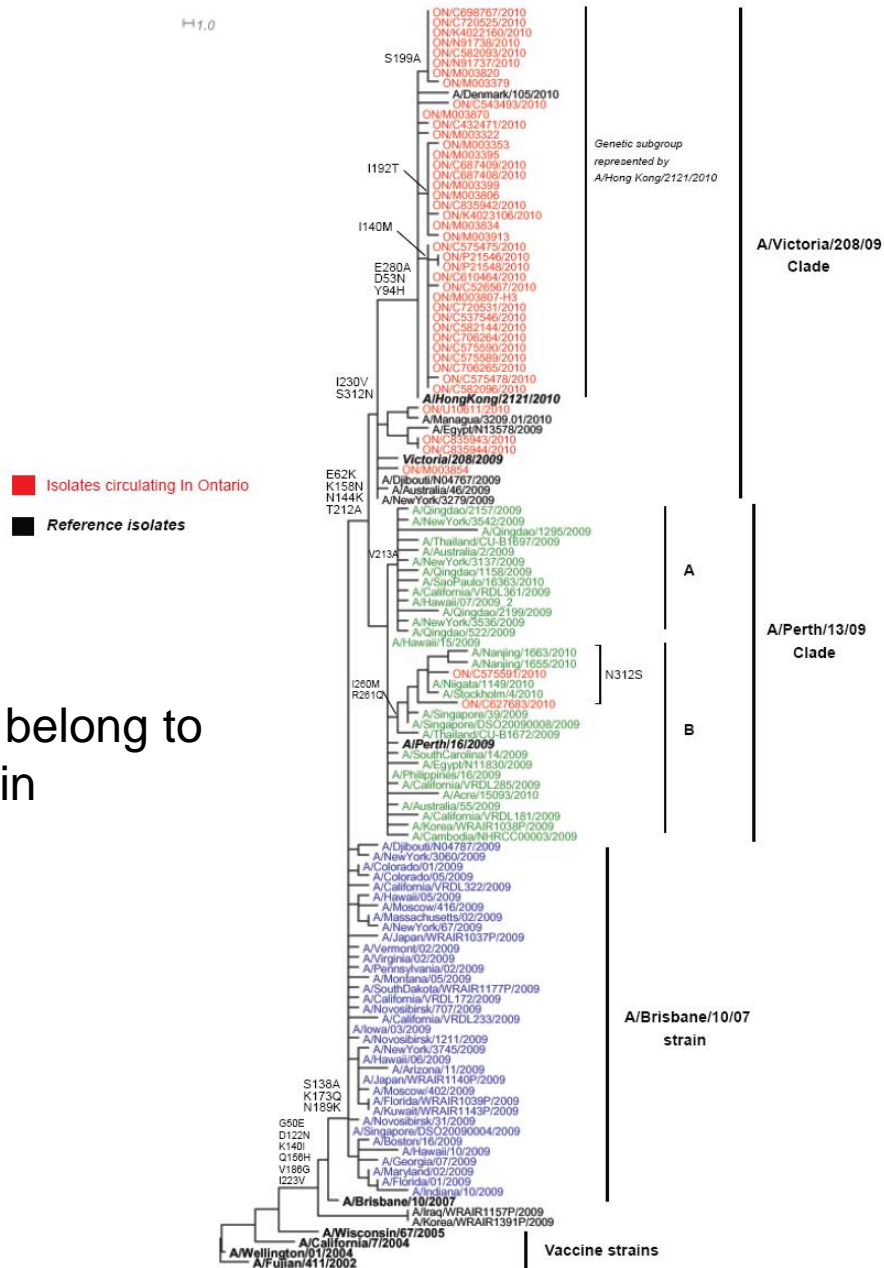
Antigenic sites of influenza H3N2; structural changes due to mutations at antigenic sites



Antigenic sites A-E in HA (H3N2)



Two mutations at each antigenic site A, E and C



H3N2 circulating in Ontario belong to Victoria Clade of Perth Strain

Figure 1: Phylogenetic comparison of influenza A (H3N2) HA genes: 124 complete hemagglutinin gene of influenza H3N2 including 41 isolates from Ontario have been used. The phylogenetic tree was constructed by Maximum Parsimony in MEGA version 5.05 using Close-Neighbor-Interchange (CNI) on Random Tree search method. The bars indicate the proportion of amino acids changes. Sequences from other countries obtained from GISAID and NCBI.

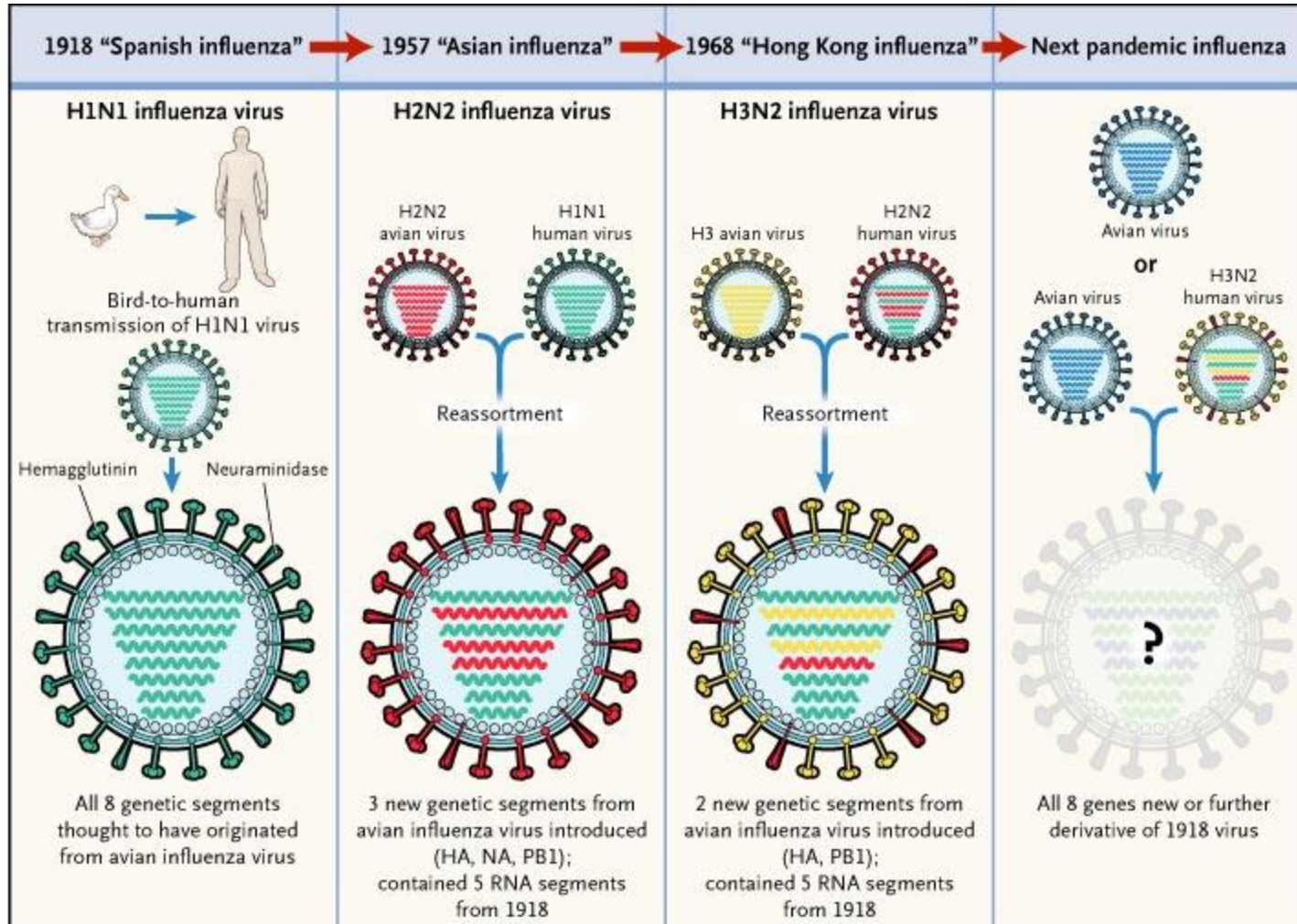
H3N2 Strain Typing NML. September 1, 2010 to May 16, 2011.

- **Influenza A (H3N2):**
Of the 269 H3N2 viruses characterized, 266 (98.9%) were antigenically related to A/Perth/16/2009
 - influenza A/H3N2 component recommended for the 2010-11 Northern Hemisphere influenza vaccine.
- Three viruses (1.1%) tested showed reduced titer with antiserum produced against A/Perth/16/2009.
- **Yan Li, Ph.D.**
Chief, Influenza and Respiratory Viruses Section
National Microbiology Laboratory
Public Health Agency of Canada
- **Should these findings be a cause for concern??**
 - WHO/CDC say no.

Genetic Shift and Reassortment In Influenza

- Antigenic shift occurs when a new HA or NA subtype is introduced into the human population.
- Coinfection of cells with two different influenza A viruses that swap segments = reassortment
- Can theoretically result in 256 different genotypes (2^8)

Antigenic Shift/Reassortment Resulting in Pandemics



Antigenic Shift/Reassortment in Swine

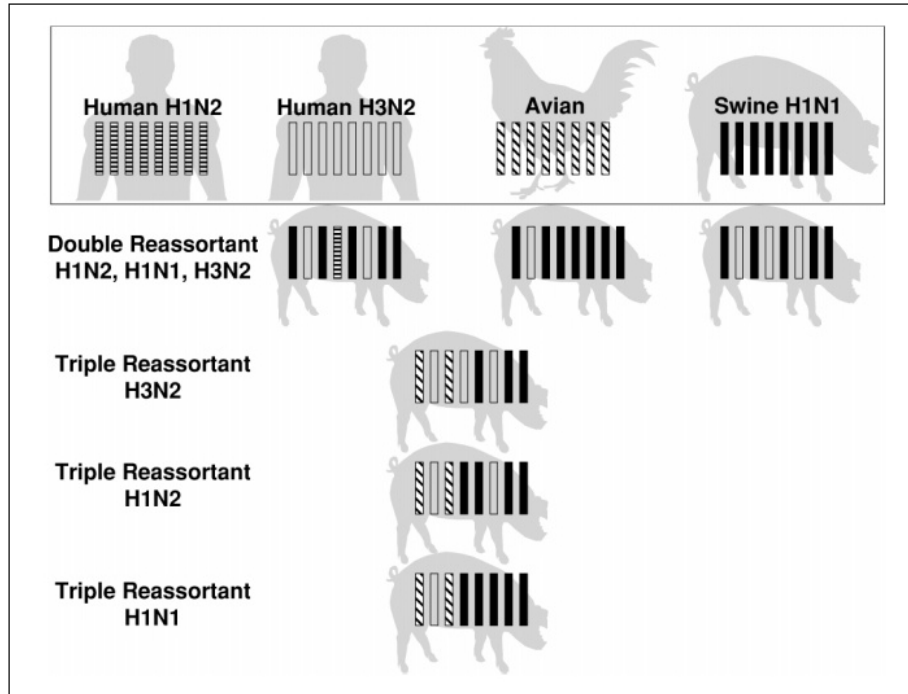
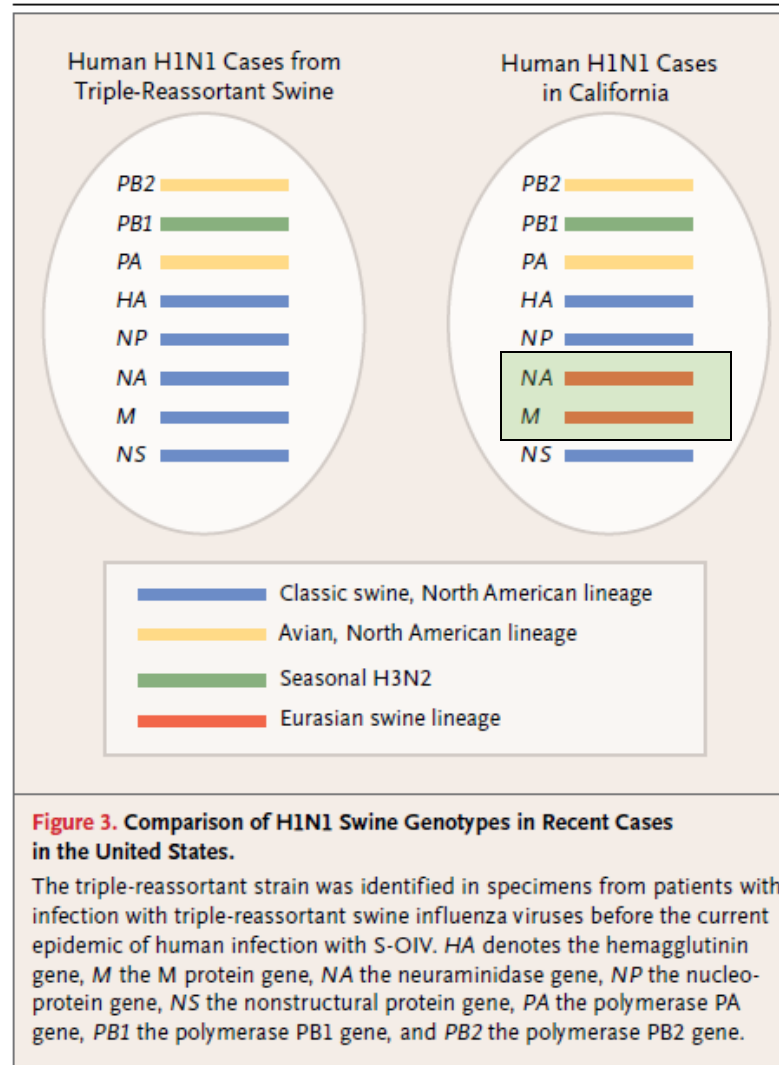


Figure 48.8 Recent reassortment events among North American swine viruses. In 1998, triple reassortant viruses emerged in North American pig populations that contained PB2 and PA genes of avian origin; PB1, HA, and NA genes of human origin; and NP, M, and NS genes that originated from classical H1N1 swine viruses. Subsequent reassortment events resulted in triple reassortant H1N2 and H1N1 viruses. In addition, human/swine reassortants of different genotypes have been isolated from North American pigs since 1998. The eight viral RNA segments are arranged from left to right according to their lengths, starting with the longest segment (PB2).

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Field's Virology, Fifth Edition, by David E. Knipe, Peter M. Howley, Diane E. Griffin, Malcolm A. Martin, etc.

Emergence of a Novel Swine-Origin Influenza A (H1N1) Virus in Humans

Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team*



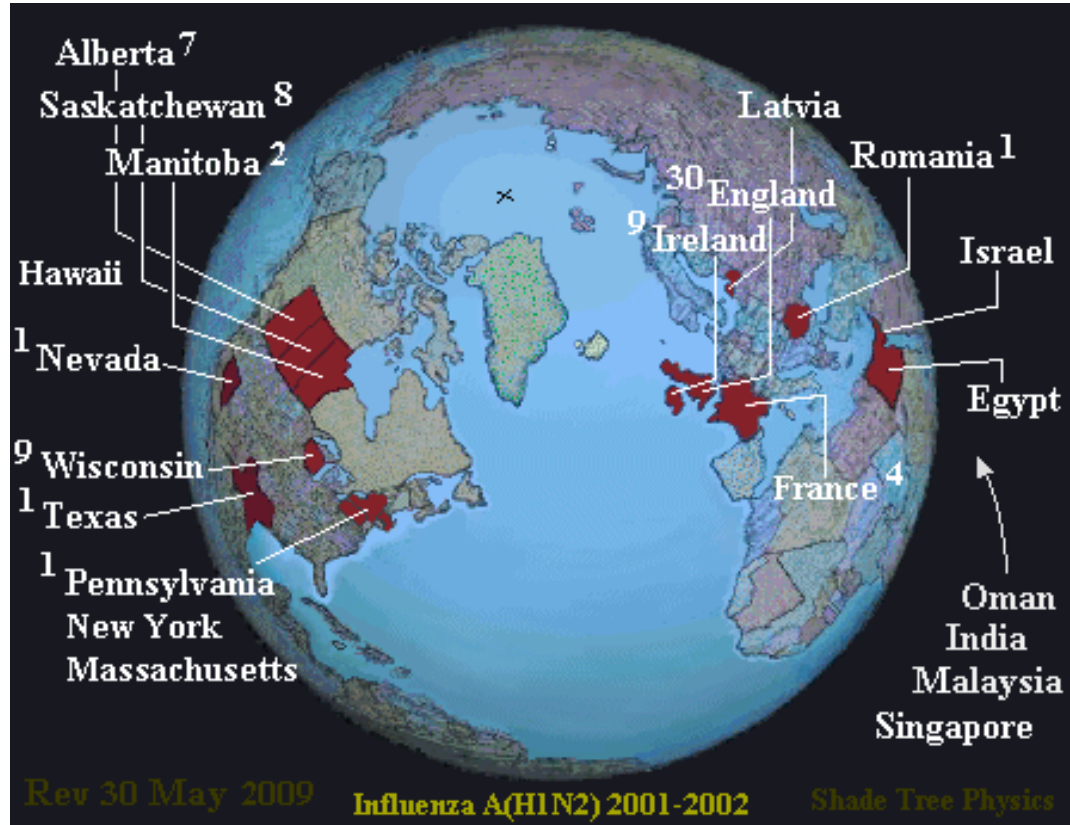
Human Infections with Triple Reassortant Swine H3N2 2010/2011 Influenza Season

- CDC reported 5 cases in the US between Sept and Nov 2011
- 2 cases occurred in September (Pennsylvania and Wisconsin).
- 1 case in October (Pennsylvania),
- 2 cases in November (Minnesota).
- 2 adult, 3 children; 2 hospitalized, all 5 recovered fully.
- The cases in Wisconsin and Pennsylvania had direct contact with swine or lived in areas close to swine farms.
- The two cases from Minnesota occurred in a father (index case) and child.
 - Father had direct swine contact, daughter did not.
 - MMWR - Morb Mortal Wkly Rep. 60(21):705-12, 2011 Jun 3.

H1N2 Reassortants in Influenza Viruses

- Cocirculation of influenza A H3N2 and H1N1 viruses has led to sporadic reports of H1N1-H3N2 reassortants in humans.
- 1983- H1N2 reassortant case in China
- During 1988-89, 19 H1N2 viruses identified in 6 cities in China.
- Dec 2001 – H1N2 reassortant identified in Wisconsin in 6 month old child
- Subsequently, 51 H1N2 viruses identified from 890 H1 viruses examined from 41 countries.
 - Canada, Singapore, Egypt, Malaysia, India, Oman, Romania, UK, USA.

Influenza A(H1N2) 2001-2002



<http://www.datasync.com/~rsf1/vel/1918h1n2.htm>

How common is influenza co-infection?

Characterization of an influenza A and influenza B co-infection of a patient in a long-term care facility with co-circulating influenza A and influenza B

International Journal of Infectious Diseases (2009) 13, e127–e128

AliReza Eshaghi
Joanne Blair
Laura Burton
Kam Wing Choi
Cedric De Lima
Carla Duncan
Cyril Guyard
Rachel Higgins
Ernesto Lombos
Donald E. Low
Tony Mazzulli
Steven J. Drews*

*Central Public Health Laboratory,
Toronto, Ontario, Canada*

2001: Reassortant influenza B viruses possessing B/Vic lineage HA and I B/Yagamata NA identified in USA and Hong Kong.

Shaw, M.W., Xu, X., Li, Y., Normand, S., Ueki, R.T., Kunimoto, G.Y., Hall, H., Klimov, A., Cox, N.J., Subbarao, K., 2002. Reappearance and global spread of variants of influenza B/Victoria/2/87 lineage viruses in the 2000–2001 and 2001–2002 seasons. *Virology* 303 (1), 1–8.

Ontario's Reassortant H3N2-pH1N1: Case History

- January 24, 2011: A 16-month-old infant admitted to a Greater Toronto Area Hospital on with respiratory and gastrointestinal symptoms.
 - Normal CXR.
 - Admitted for intravenous rehydration
- After 15 hours, was discharged home and subsequently recovered uneventfully.
- Two nasopharyngeal swabs were collected on the day of admission and sent to Public Health Ontario Laboratories for influenza testing by real-time PCR.

Reassortant H3N2-pH1N1: Initial Laboratory Testing

- Influenza A was detected in both specimens by real-time PCR
- Influenza Subtyping
 - Both positive for hemagglutinin (H3) gene in a moderately high copy number (cycle thresholds 29 and 31).
 - They were also noted to be positive for the pH1N1 neuraminidase (NA) gene at a very low level (CT values 38 and 39)
 - Suspecting contamination, primary samples were reextracted and retested; identical results were obtained.

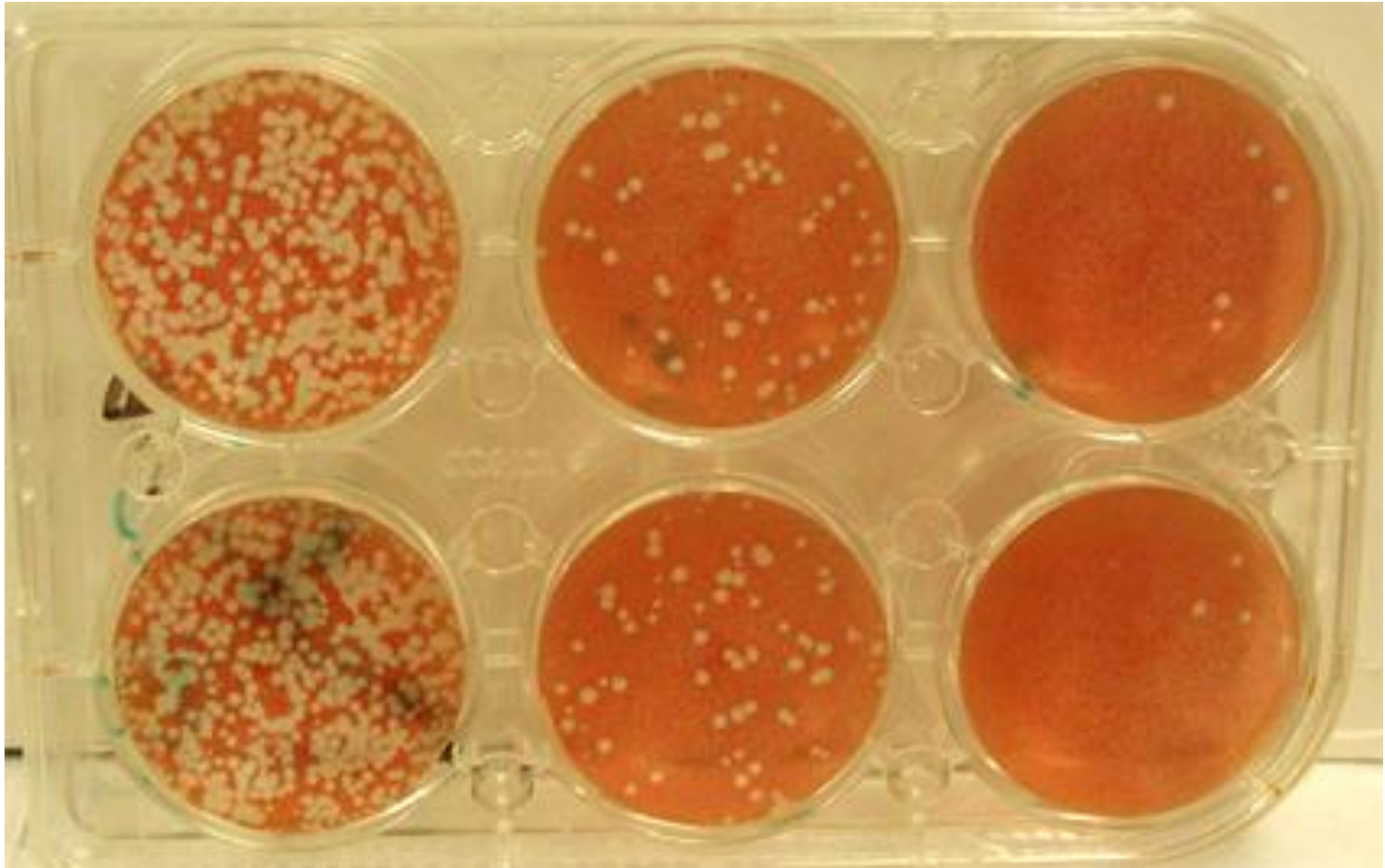
Reassortant H3N2-pH1N1: Sequencing on Primary Specimens

- Further sequencing confirmed presence of the following genes in the primary samples:
 - A. Pandemic H1N1 2009 genes: Matrix, NS, H1, N1
 - (H1 sequencing was conducted on one primary sample only)
 - B. Seasonal H3N2 genes: N2
 - (H3 sequencing was not conducted on either primary sample)

Sequencing of viral culture material (rhesus monkey kidney cells).

- Whole genome sequence analysis of culture isolate:
 - H3 and N2 of seasonal H3N2
 - PB2, PB1, PA, NS, NP and M of pandemic H1N1.
- Gene sequences obtained in culture and primary specimen were identical.
- The H3 and N2 gene sequences most closely matched the currently circulating A/Perth/16/2009 strain.
- The matrix and NS genes amplified from primary sample were identical to currently circulating pH1N1, most closely matching A/California/07/2009 strain.

Plaque Assays



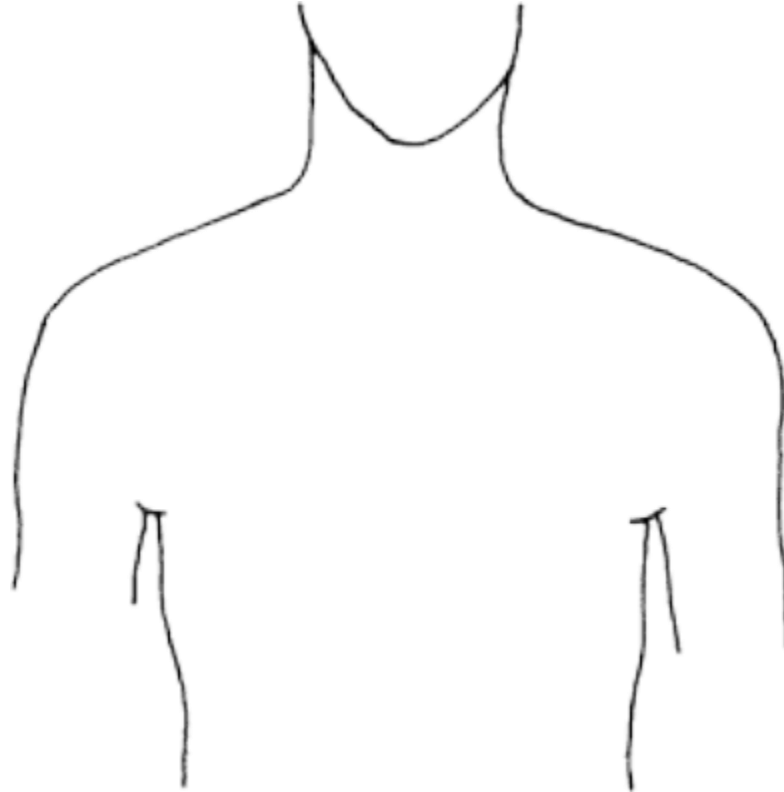
H3N2-pH1N1 Reassortant In Ontario: Further work at Canada's National Microbiology (NML)

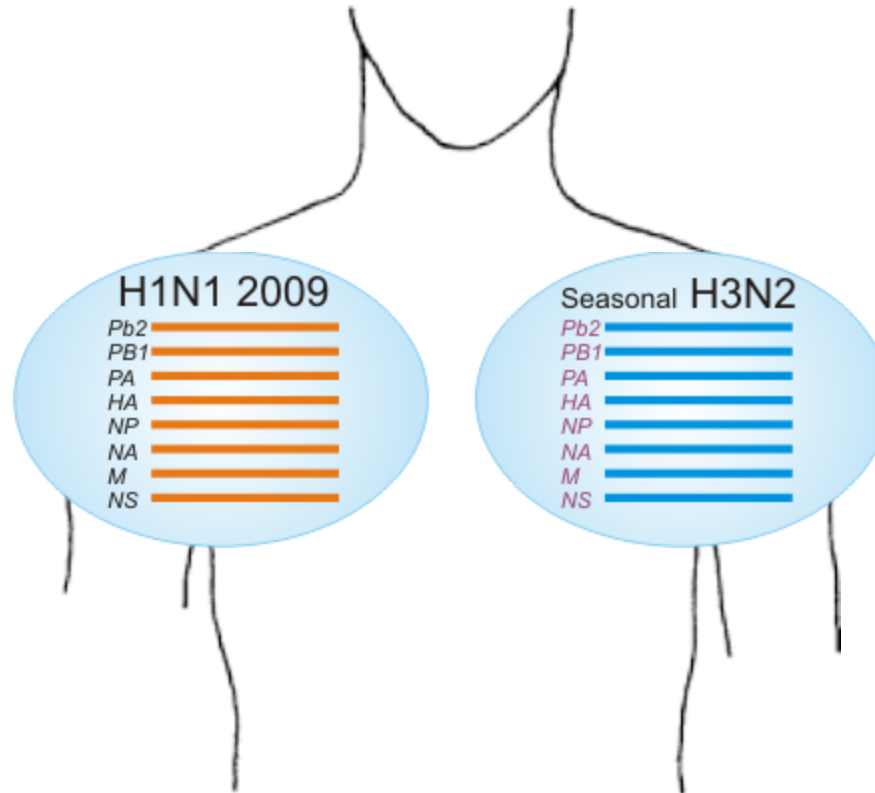
- NML performed plaque forming assays on primary sample and culture material using Madin-Darby canine kidney cell lines.
- Sequencing of individual plaque material in both primary sample and culture reconfirmed a reassortant virus
 - No evidence of the HA and NA genes of pH1N1 within plaques.
 - All sequences matched those from PHL.
- In addition, gene sequences obtained in both plaque assays matched each other, and were also identical to those obtained at PHO.
- Strain type by hemagglutination inhibition assay: A/Perth/16/09 (H3N2) .

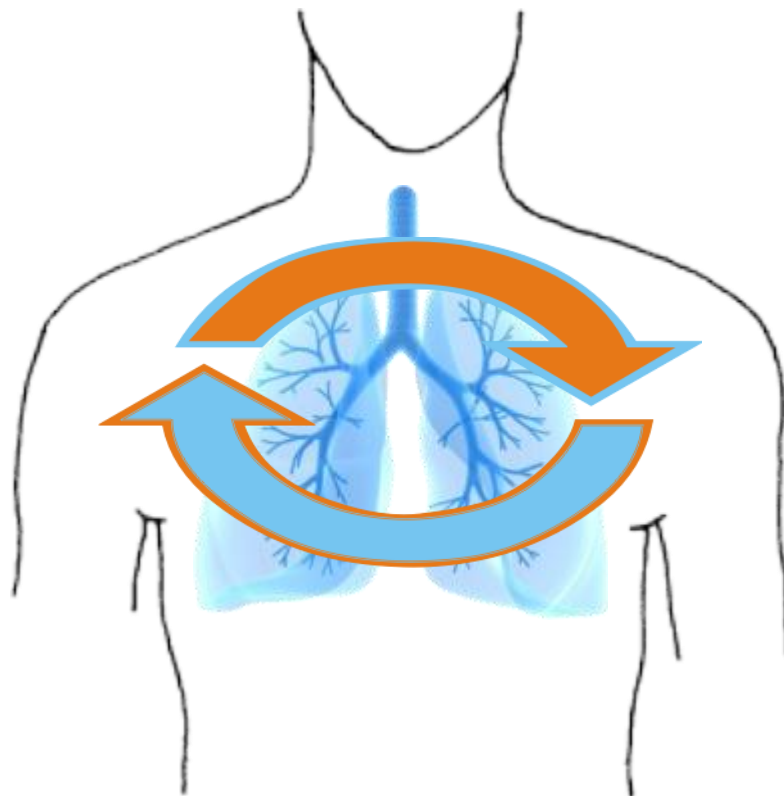
H3N2-pH1N1 Reassortant: Summary of Findings

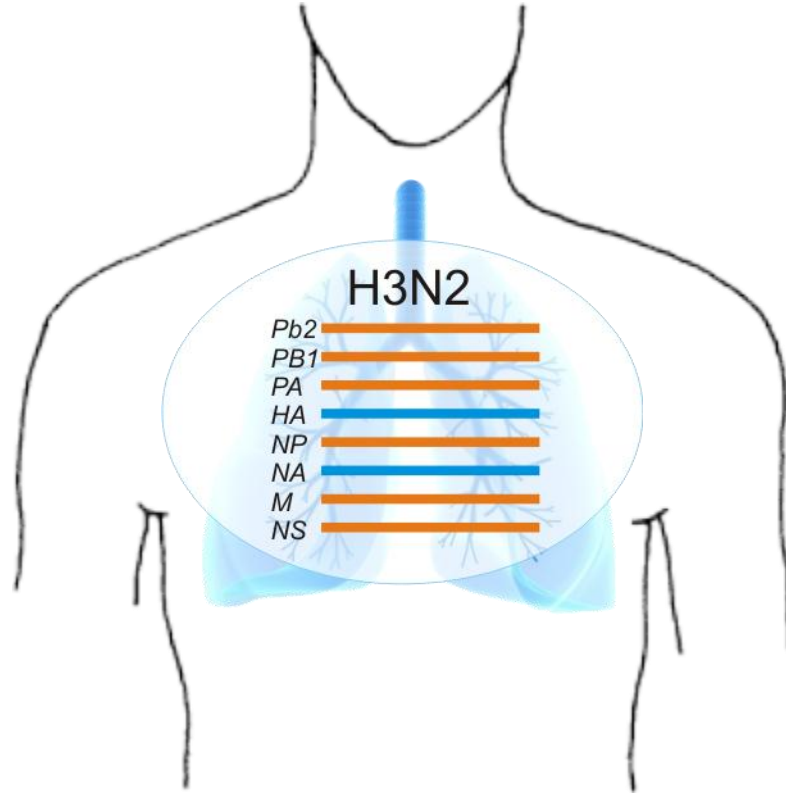
- Case represents coinfection with H3N2 and pH1N1, followed by reassortment in the patient (*in vivo* reassortment).
- A low level of pandemic H1N1 2009 NA gene was present in the primary sample.
 - indicates reassortment occurred in the child and lab detected a remnant of NA left behind.
- The subsequent reassortant virus consisted of HA and NA of H3N2 together with the remaining 6 genes (PB2, PB1, PA, NS, NP and M) of pH1N1.

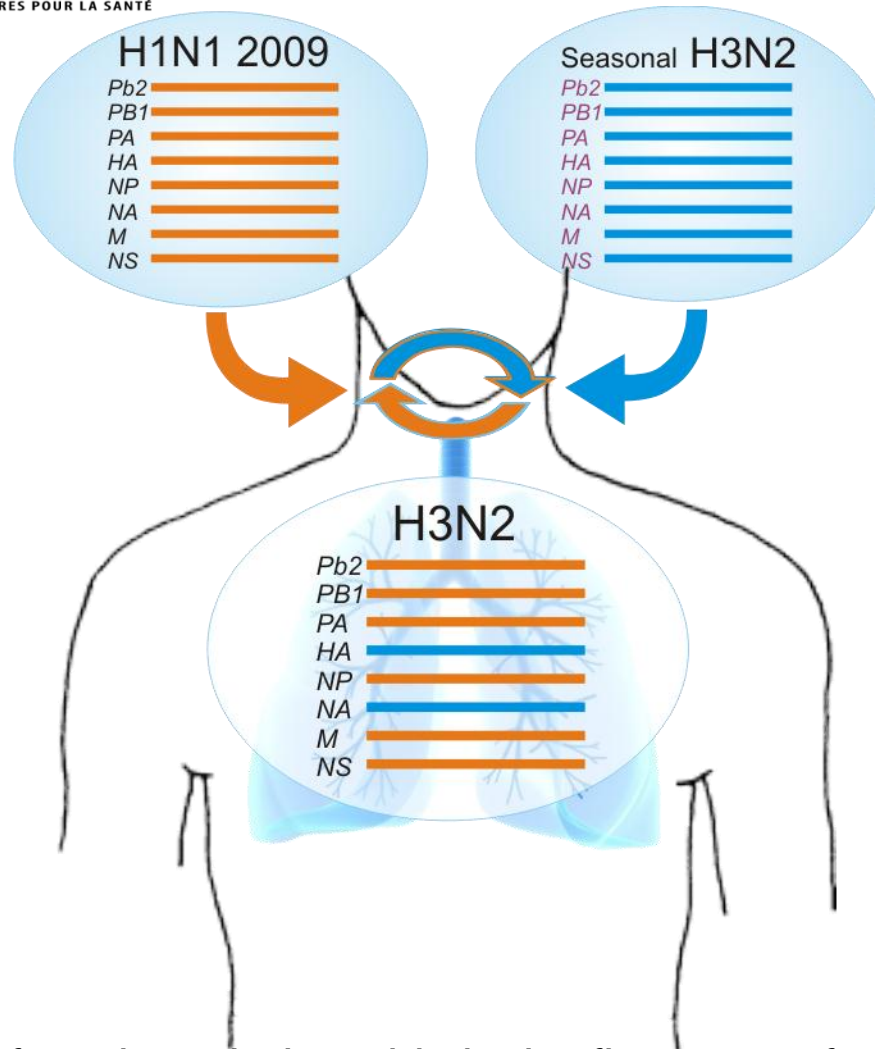
And this is what it looked like!











To the best of our knowledge, this is the first case of reassortment involving pH1N1 and seasonal H3N2.

Could this reassortant transmit?

- There was no documented transmission in this child
 - Reassortment can affect viral fitness.
 - It may increase, decrease or have no influence on transmissibility.
- Recent study: reverse genetics was used to generate a laboratory reassortant of this type.
 - Infection of 6 ferrets with this reassortant resulted in higher levels of virus and more severe respiratory damage when compared to wild-type pH1N1.
 - Schrauwen et al. Emerg Infect Dis. 2011 Feb;17(2):200-8.

Would the current influenza vaccine protect against this reassortant?

- The current vaccine should be active against this reassortant
 - made up of two viruses that are currently circulating.
- In particular, it contains the H3 and N2 of the current seasonal H3N2 subtype of influenza.
- Further assessment will be needed to confirm this.

Would current testing detect this subtype if it is circulating?

- Routine influenza subtyping would not differentiate the reassortant from a regular seasonal H3N2 virus.
- However, a proportion of isolates across Ontario, Canada and internationally are fully gene-sequenced
 - this work would detect any significant circulation of a new reassortant such as this one.

Enhanced surveillance and investigation is needed to better understand:

- How common the virus is (prevalence)
- How well the virus transmits from person-to-person (transmissibility)
- Cross-protection from previously circulating influenza viruses.
- Vaccine efficacy/effectiveness of currently used vaccines against the new reassortant

Are all Reassortants of Equal Significance?

- Reassortants involving viral subtypes not previously circulating in humans would likely be of much greater public health significance
 - human population would have no or only some cross-protective immunity to the new subtype formed.

Summary

- We have observed a high frequency of amino acid mutations at antigenic sites in seasonal H3N2 in Ontario in 2010 2011 influenza season.
 - Appears to have not caused antigenic drift according to HAI assays.
 - Impact on clinical influenza still not clear.
- We have documented the first case of coinfection followed by reassortment between pH1N1 and H3N2.
 - Is not expected to cause a serious public health concern as it is made up of influenza viruses currently circulating in humans.
 - Further laboratory study of this virus will better characterize its fitness, transmissibility and pathogenicity .
- Ongoing molecular and surveillance and strain typing of influenza viruses is critical for prompt detection and characterization of novel influenza strains.

Thanks To....

- **All Public Health Laboratory Staff**
 - Specimen Triage/DASH
 - Virus Detection:
 - Molecular Diagnostics:
 - Research Staff: Reza Eshaghi, Aimin Li, Rachel Higgins.
 - Samir Patel, Dr Low, medical/clinical microbiologists, management team.
- **Surveillance and Epidemiology**
 - Natasha Crowcroft, Anne-Luise Winter, Adriana Peci, Romy Olsha
- **NML/PHAC**
 - Yan Li, Nathalie Bastien (Influenza and Resp Viruses Section, NML)
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- **Public Health Units**
- **Ministry**