

IMMUNITY TO INFLUENZA INFECTION

Unit 7

Paul Thomas

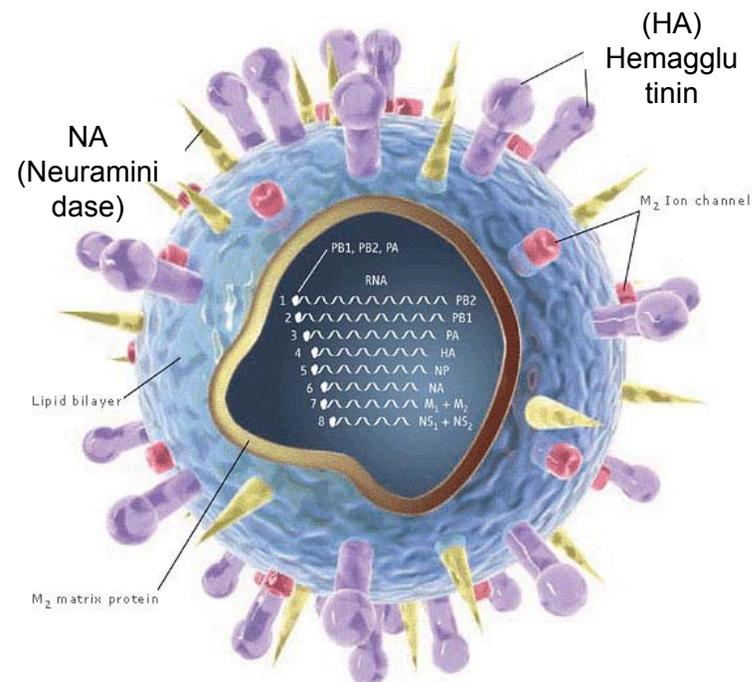
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INFLUENZA A VIRUS

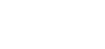
- Negative sense, segmented RNA virus
- *Orthomyxoviridae*
- Eight genes, 11 proteins (three alternate reading frames)
- Two non-structural proteins (NS₁ and PB1-F₂)
- Surface proteins HA and NA determine serotype



Modified from: Kaiser. *Science* 2006, 312:380-382.



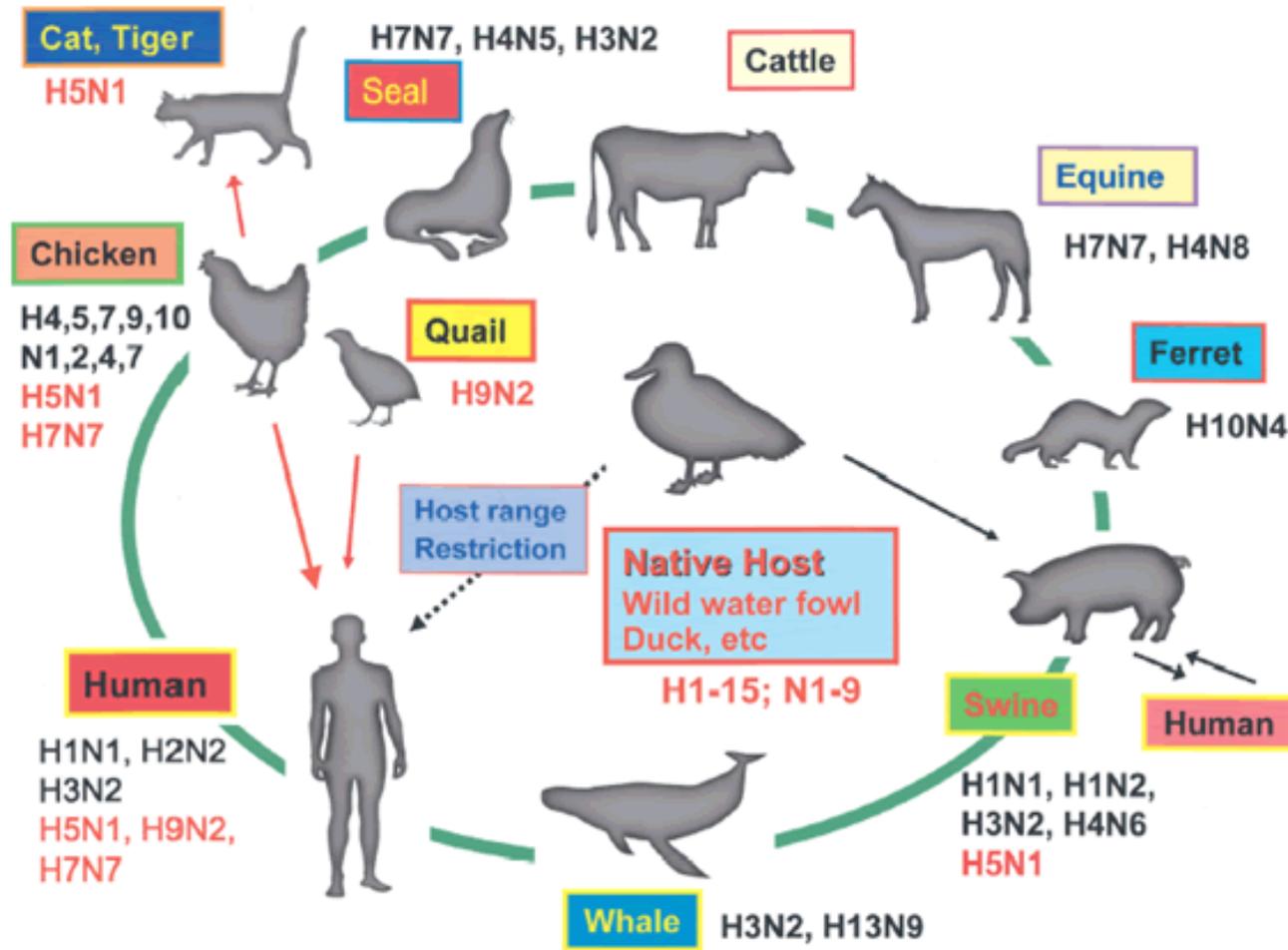
Influenza A HA and NA Subtypes

H1				
H2				
H3				Other Animals
H4				Other Animals
H5				Other Animals
H6				
H7				Other Animals
H8				
H9				
H10				
H11				
H12				
H13				
H14				
H15				
H16				

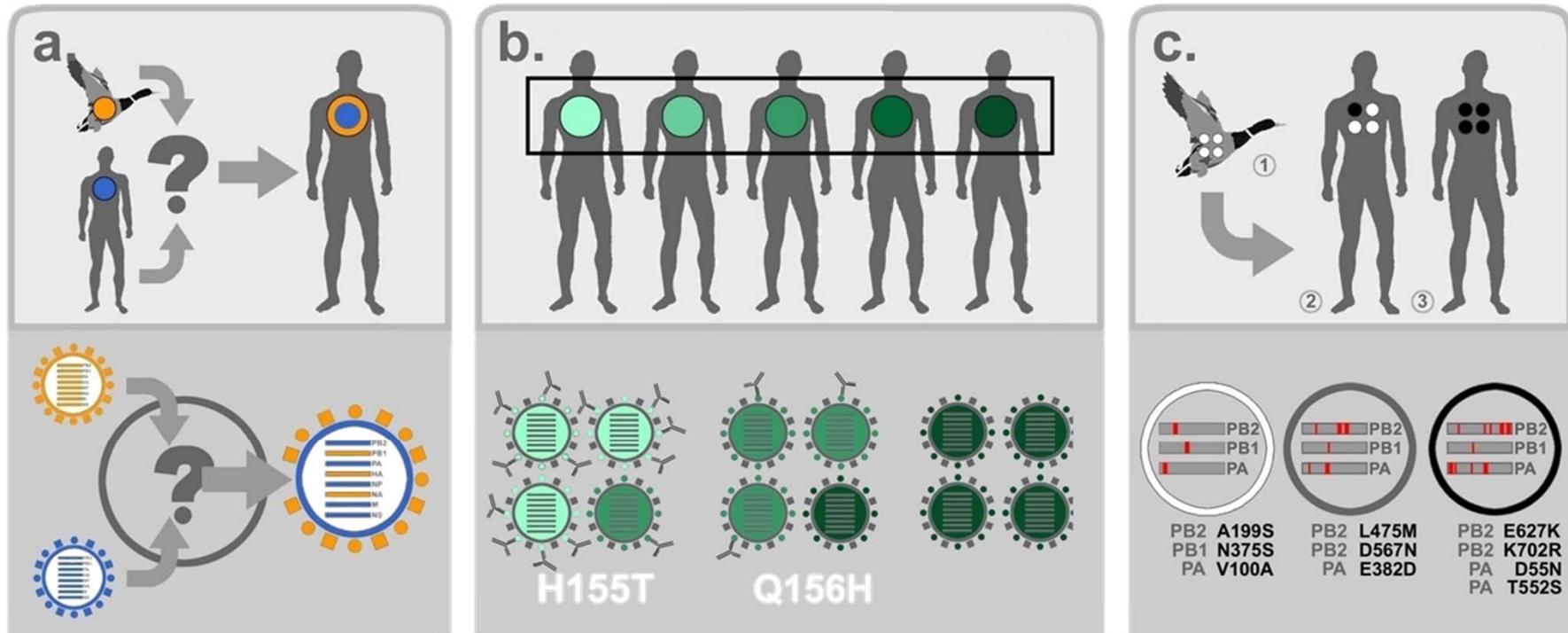
N1				
N2				
N3				
N4				
N5				
N6				
N7				Other Animals
N8				Other Animals
N9				



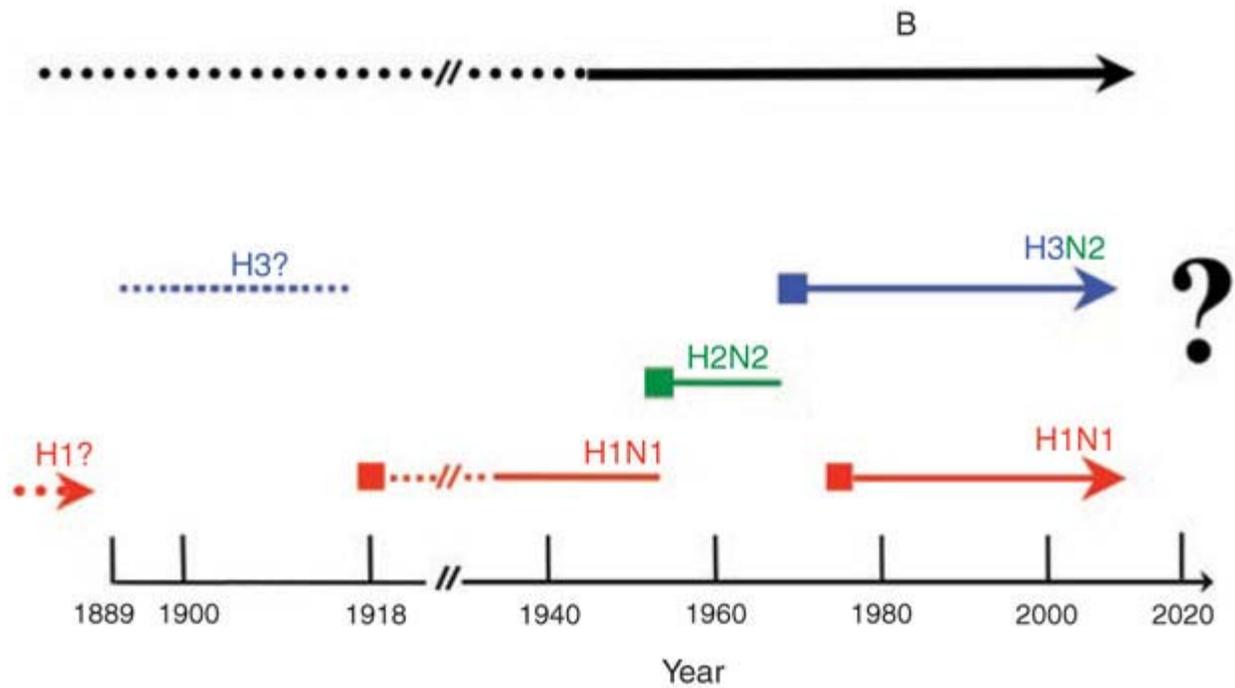
DIVERSE HOST TROPISM ALLOWS RESTRICTION AND RECOMBINATION



INFLUENZA EVOLUTION

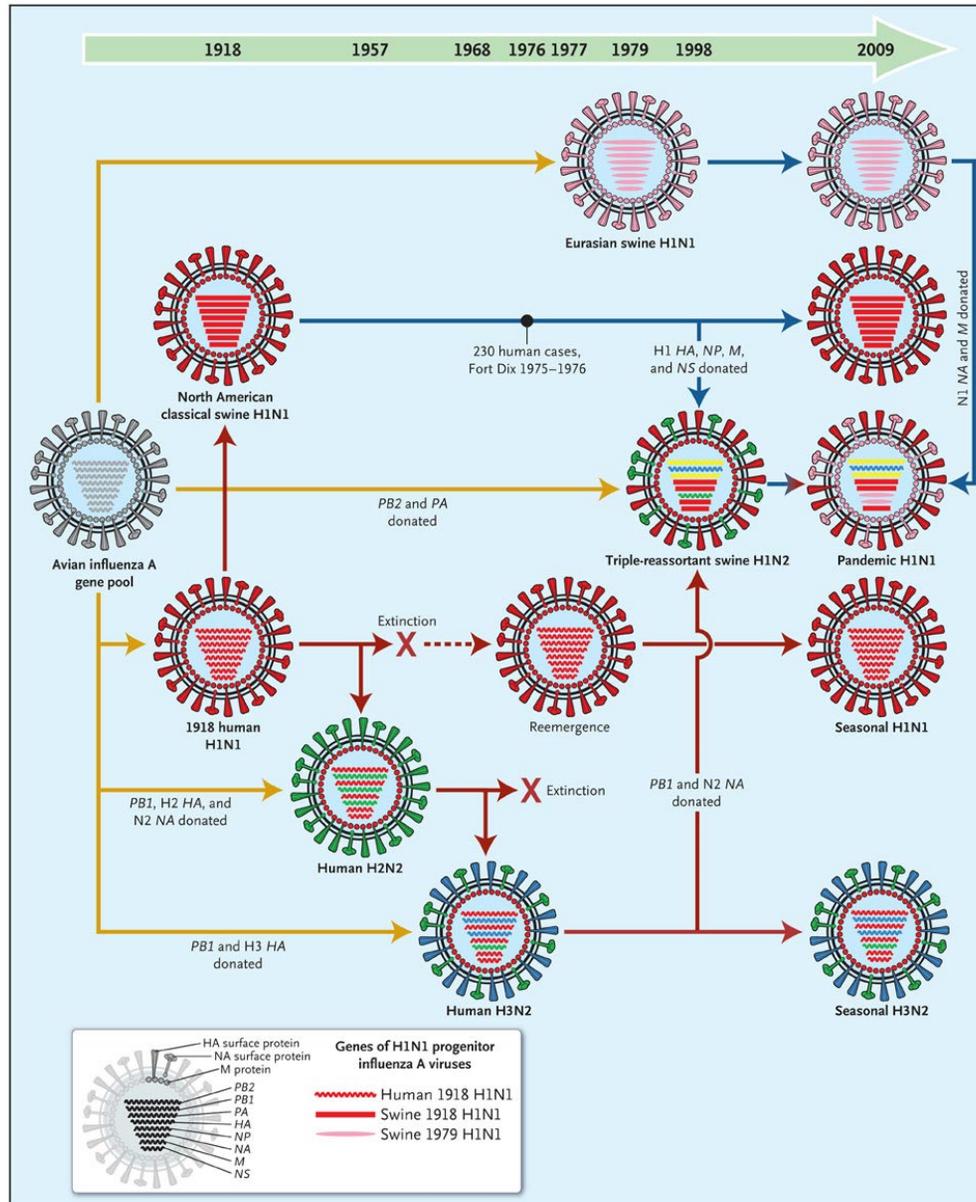


HUMAN INFLUENZA PANDEMICS



EVOLUTION OF HUMAN INFLUENZA FROM 1918

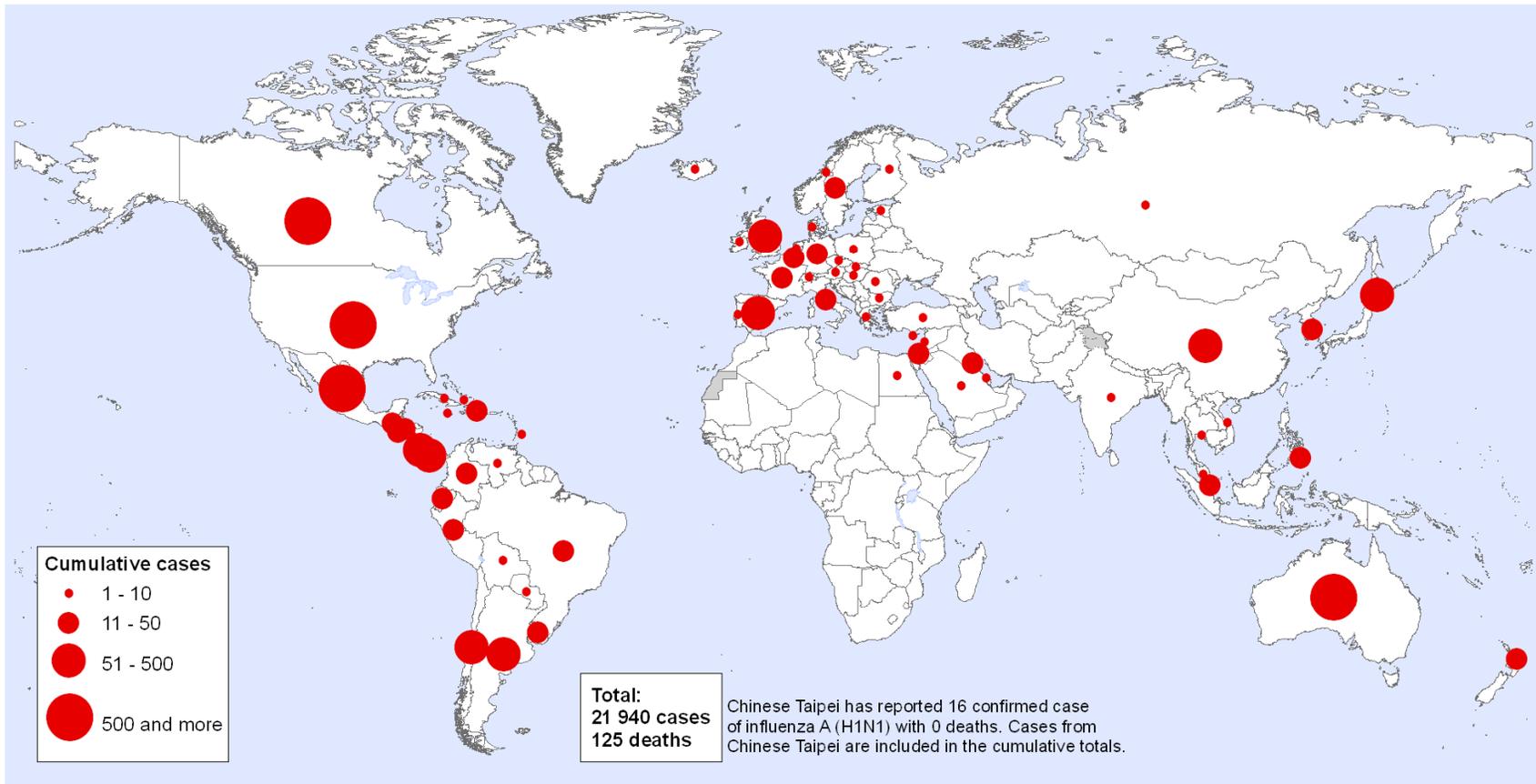
- All current human influenza is majority-derived from the 1918 pandemic
- Distinct reservoirs have allowed evolution to occur with varying pressures, providing diverse sources for new gene introductions into the human pool



SWINE-ORIGIN H₁N₁ INCIDENCE

New Influenza A (H1N1),
Number of laboratory confirmed cases as reported to WHO

Status as of 05 June 2009
06:00 GMT



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Map produced: 05 June 2009 08:10 GMT

Data Source: World Health Organization
Map Production: Public Health Information
and Geographic Information Systems (GIS)
World Health Organization

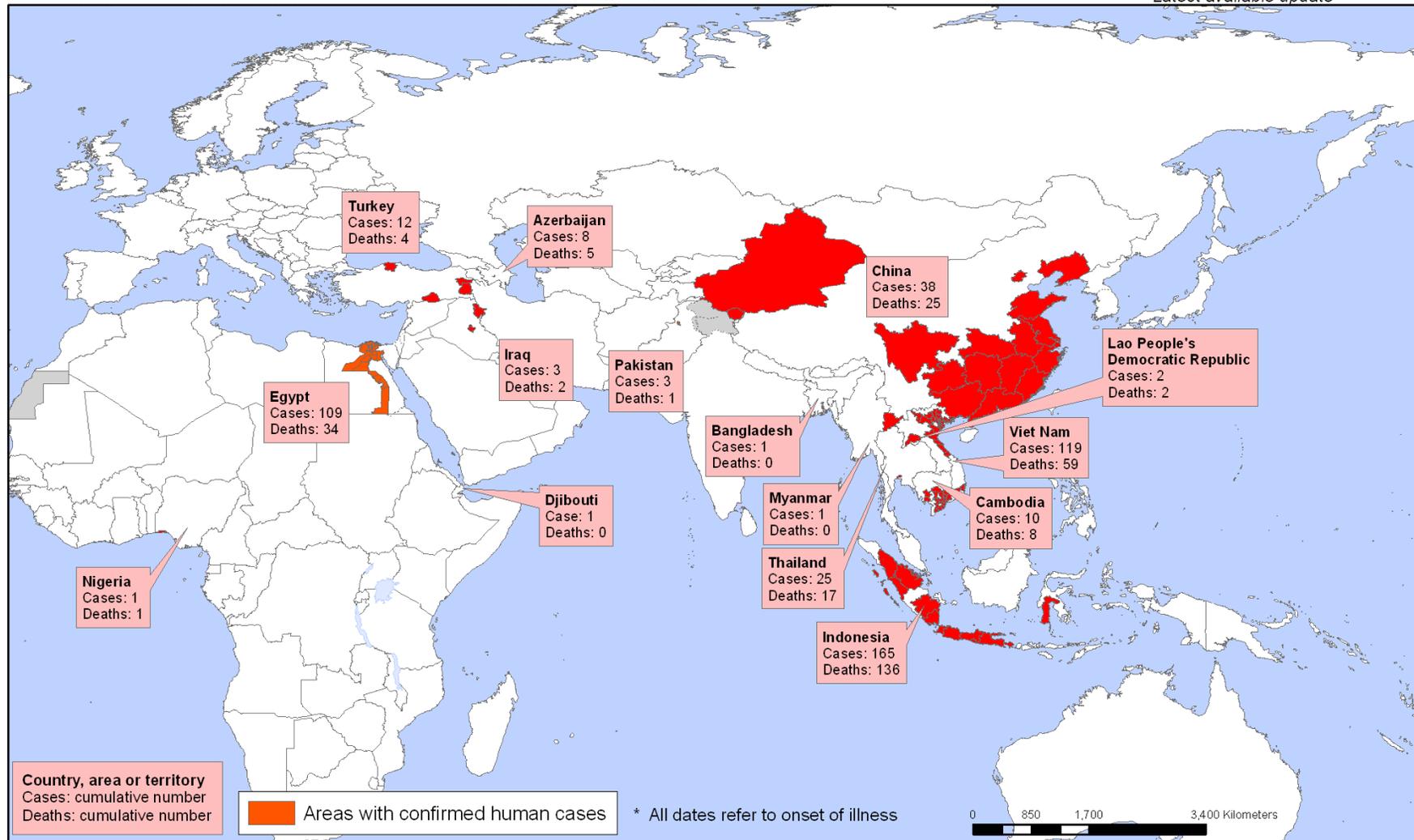


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H5N1 INCIDENCE

Areas with confirmed human cases of H5N1 avian influenza since 2003 *

Status as of 06 May 2010
Latest available update



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. © WHO 2010. All rights reserved.

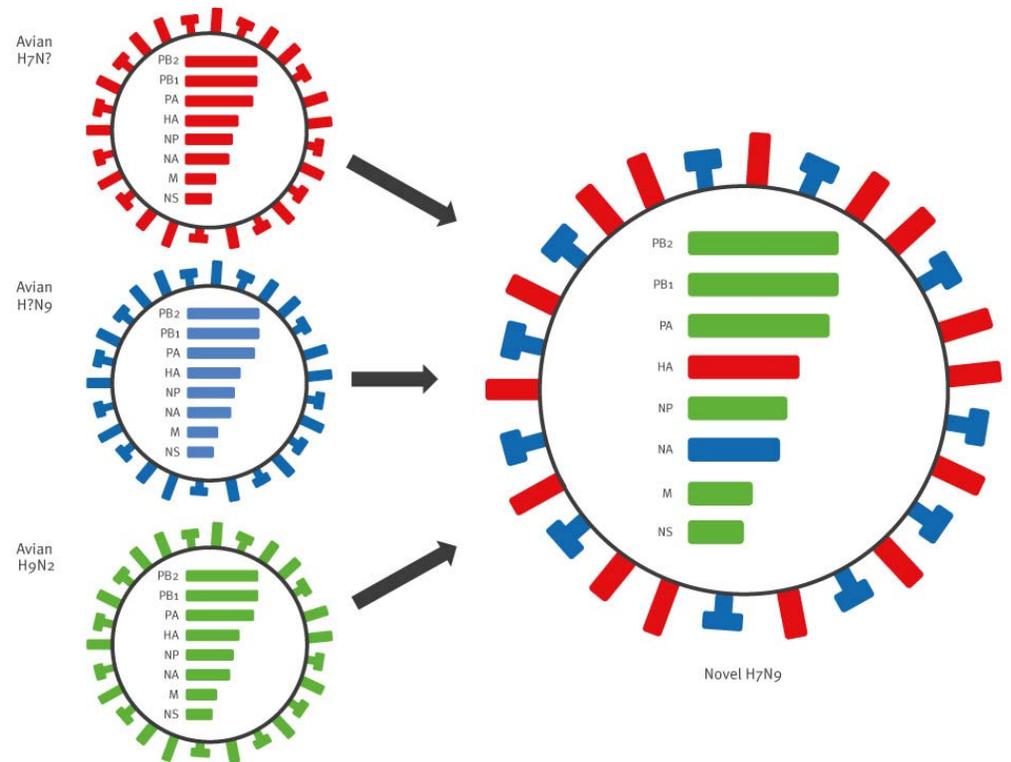
Data Source: WHO
Map Production: Public Health Information and Geographic Information System (GIS)
World Health Organization

H7N9 IS A RECOMBINANT OF H9N2 WITH OTHER AVIAN VIRUSES

- The H9N2 cassette was also the basis of the H5N1 viruses emerging in 1997 and 2002
- However, the theoretical parent H9N2 lineage has never been observed on its own

FIGURE 3

Schematic diagram of novel influenza A(H7N9) virus generation

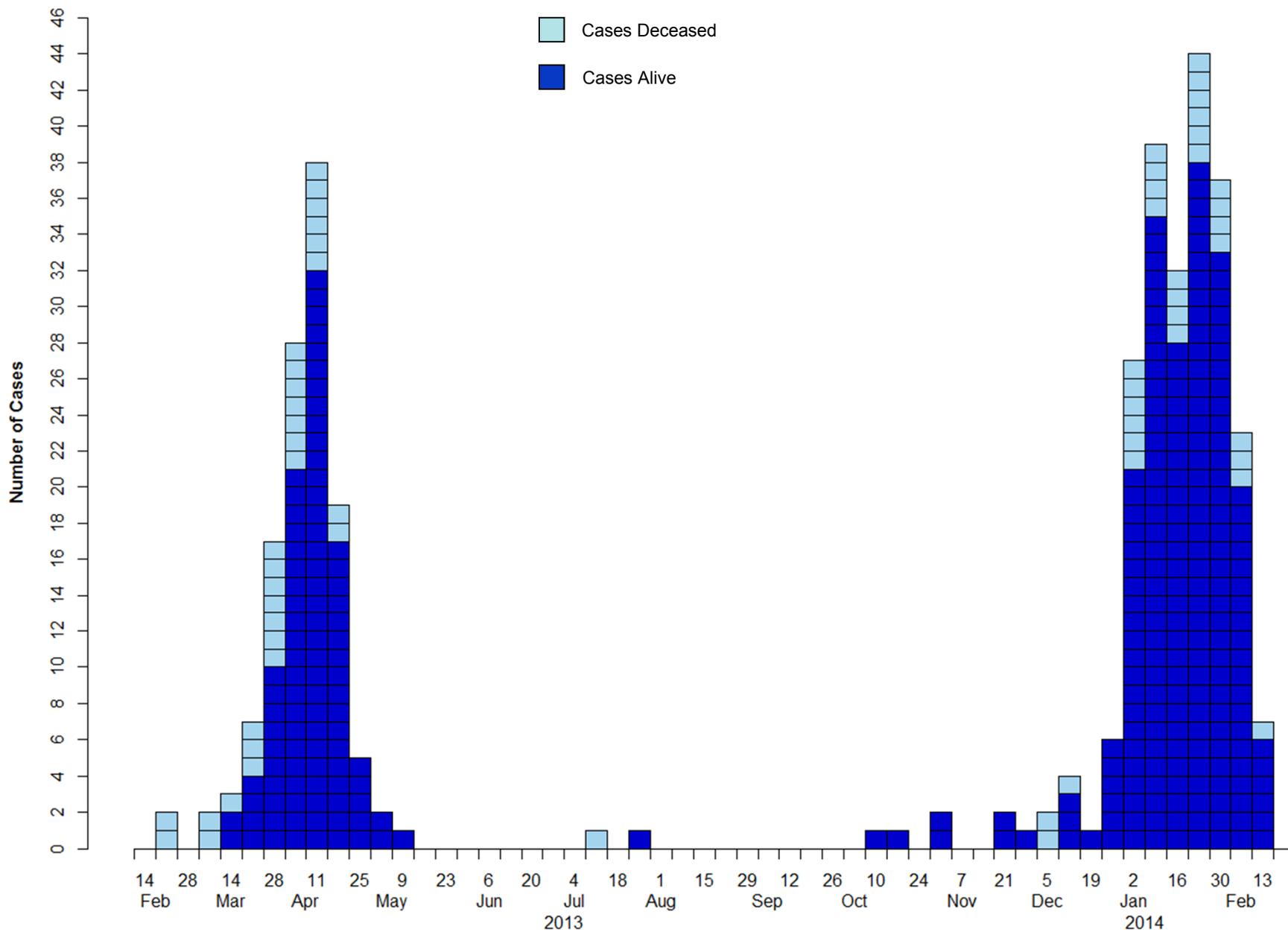


HA: haemagglutinin; NA: neuraminidase.

The novel influenza A(H7N9) viruses are likely to have acquired their HA gene from an avian H7 virus of unknown NA subtype, their NA gene from an avian N9 virus of unknown HA subtype, and their remaining six viral segments from avian H9N2 viruses circulating in poultry.

Cases of H7N9 Influenza in China by Week of Onset (Feb 26, 2014)

373 Total Cases: 114 Deaths
Date of Onset Missing for 18 Cases

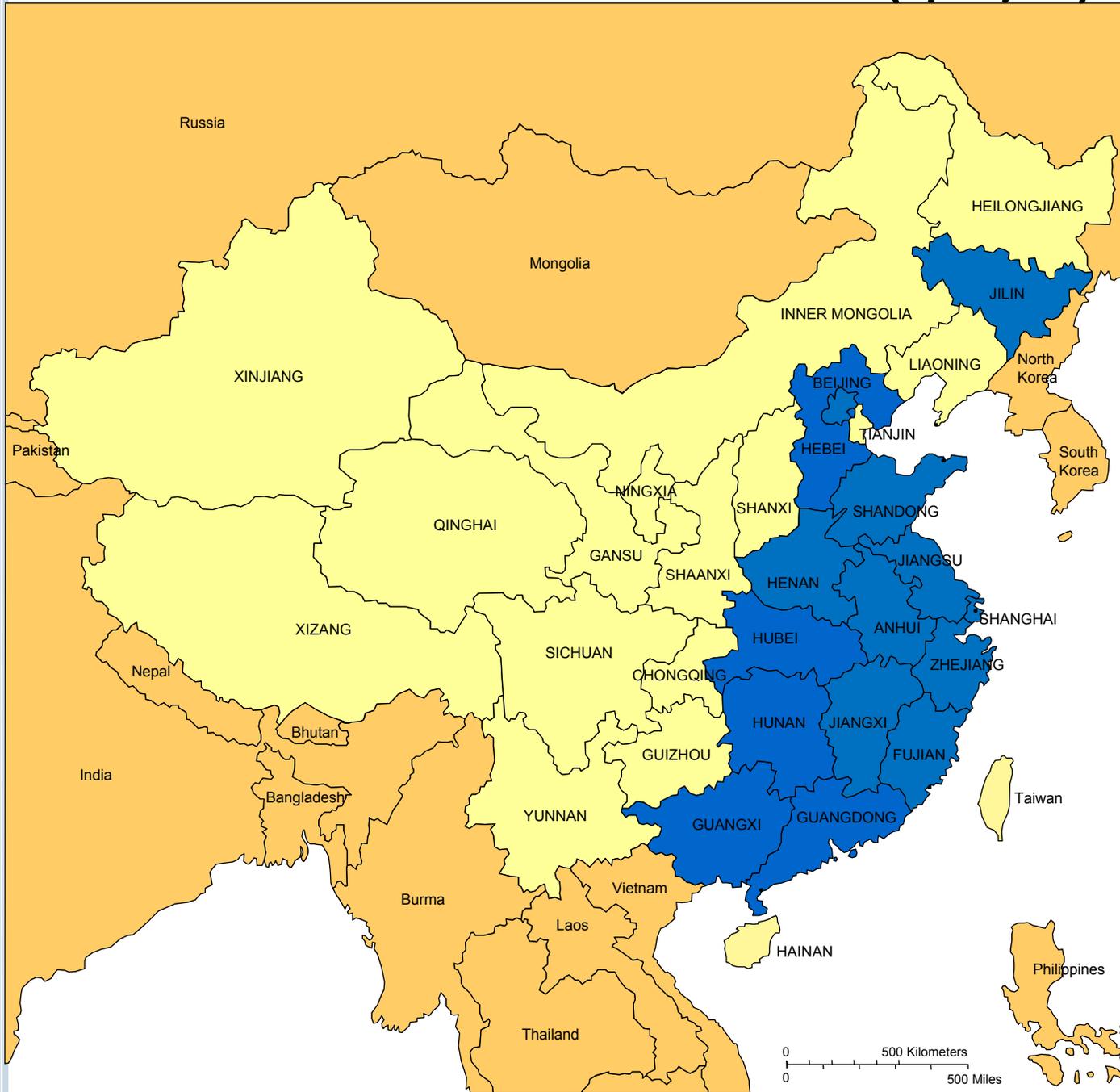


Note: Total cases include an asymptotically infected child in Beijing

Source: Provincial CDC (China), National China CDC, WHO, and news reports

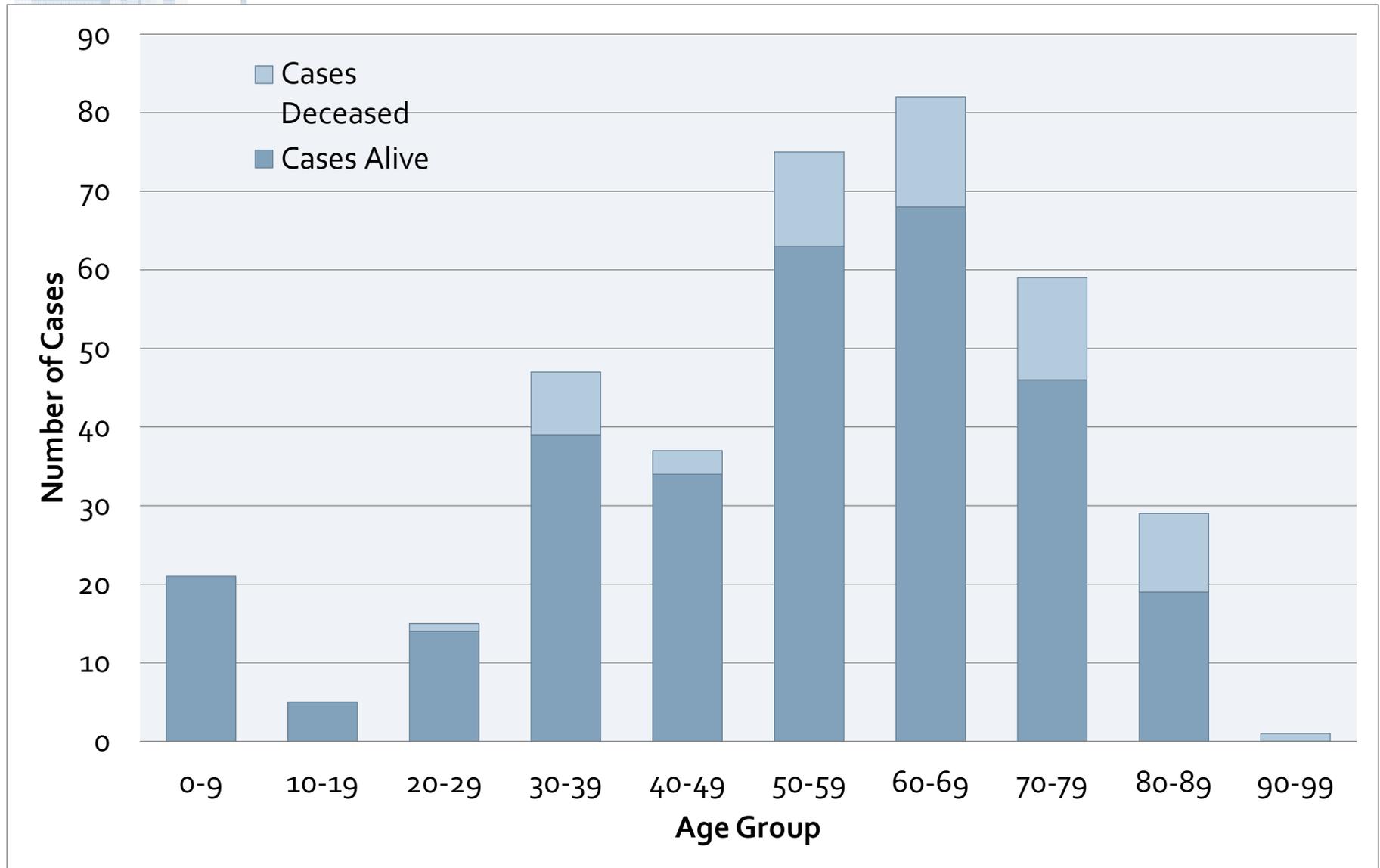
Location of H7N9 Influenza in China (2/26/14)*

*373 total cases/114



Province/ City	Number of Cases
Anhui	9
Beijing	5
Fujian	20
Guangdong	86
Guangxi	3
Hebei	1
Henan	4
Hunan	14
Jiangsu	43
Jiangxi	6
Jilin	1
Shandong	2
Shanghai	42
Zhejiang	137

Cases of H7N9 Influenza in China by Age-Group (2/26/14)*

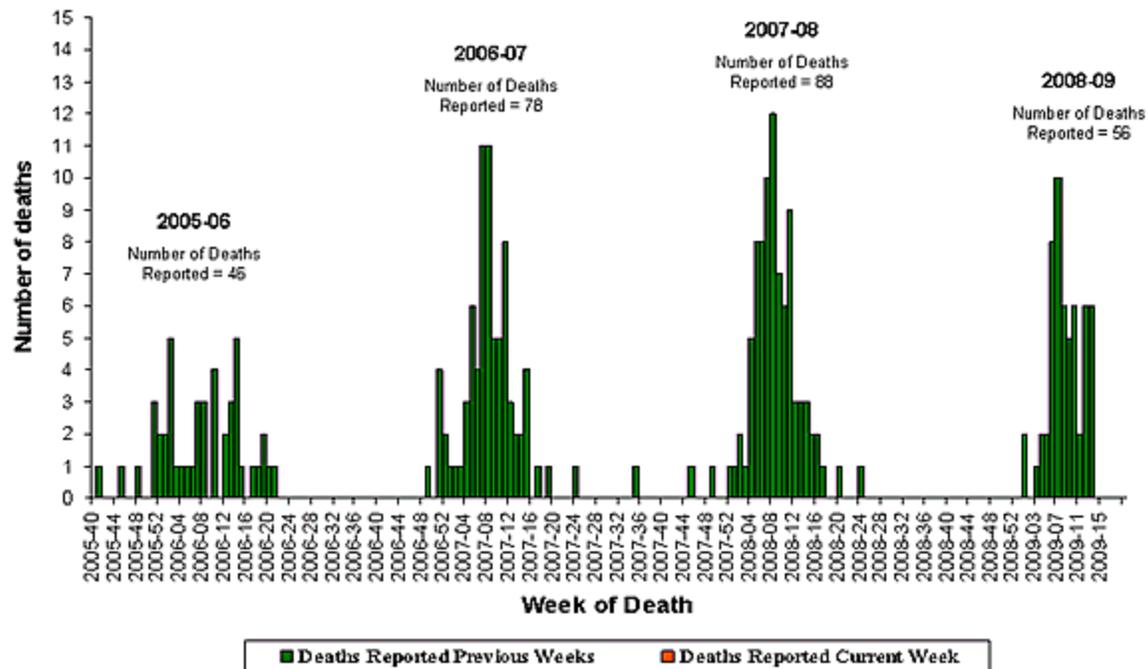


*Total cases = 373

Note: ages of 3 cases are unknown and identity of 52 deaths also unknown

ANNUAL SEASONAL INCIDENCE, U.S.

**Number of Influenza-Associated Pediatric Deaths
by Week of Death:
2005-06 season to present**

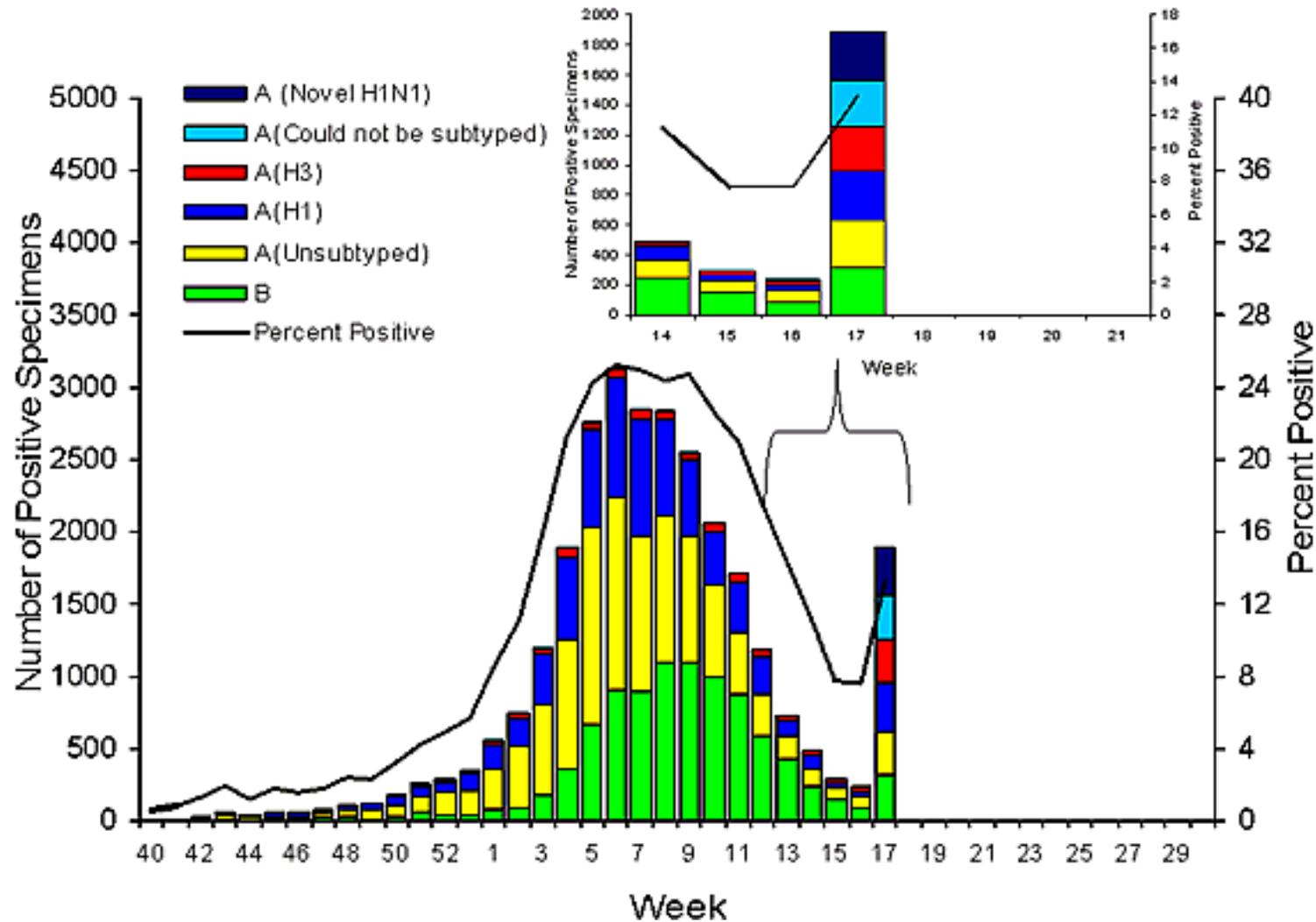


Annual number of deaths within the U.S. attributable to influenza: 41,400 (as of 2004)



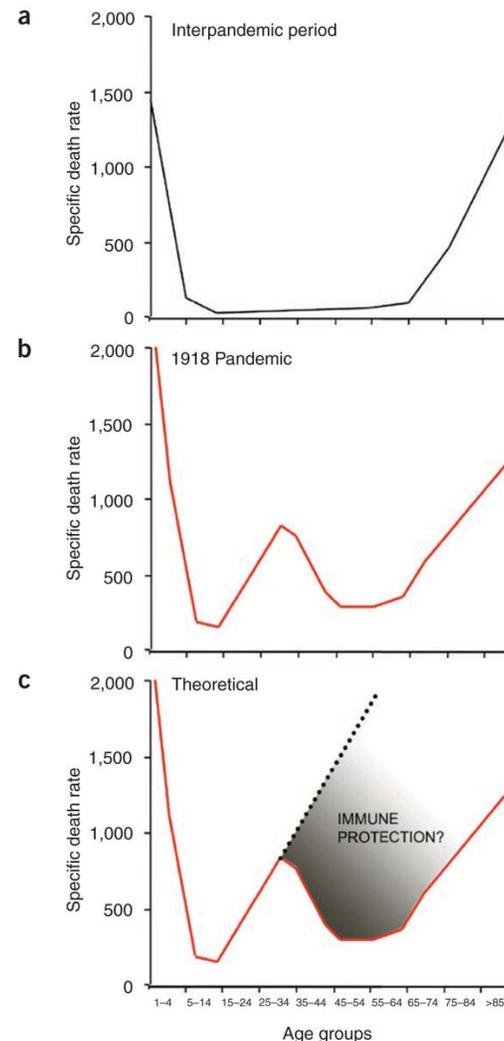
EMERGENCE OF THE 2009 PANDEMIC

Influenza Positive Tests Reported to CDC by U.S. WHO/NREVSS Collaborating Laboratories, National Summary, 2008-09

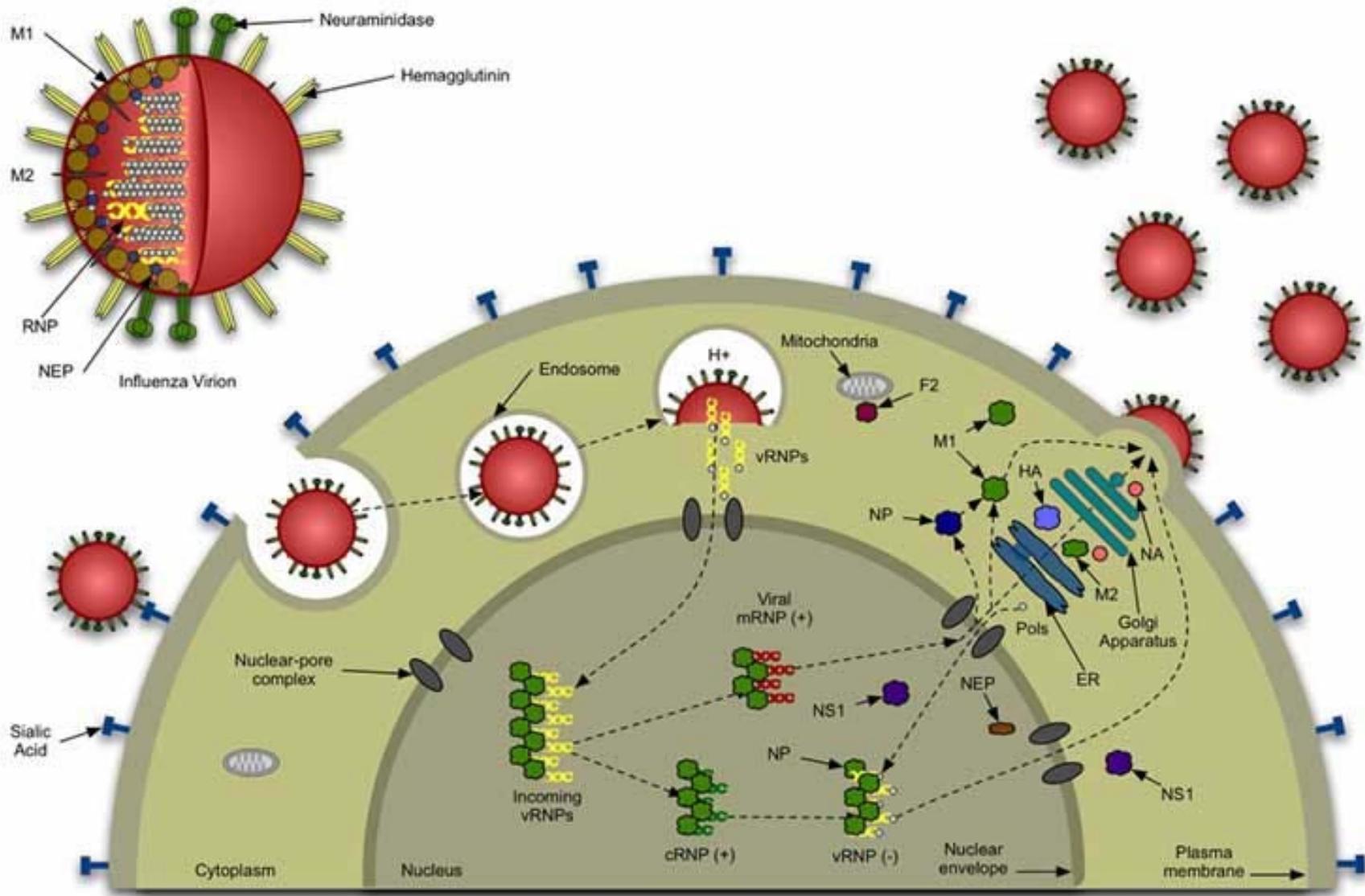


1918 (AND POSSIBLY SWORH1N1) MORTALITY CURVES SUGGEST PREVIOUS EXPOSURE

- The “U” shaped curve of regular influenza infection demonstrates the highest mortality among children (naïve) and the elderly (immunocompromised)
- The 1918 pandemic had a “W” shaped curve, with a spike in deaths among young adults—immunopathology or prior protection for ~40 year olds?

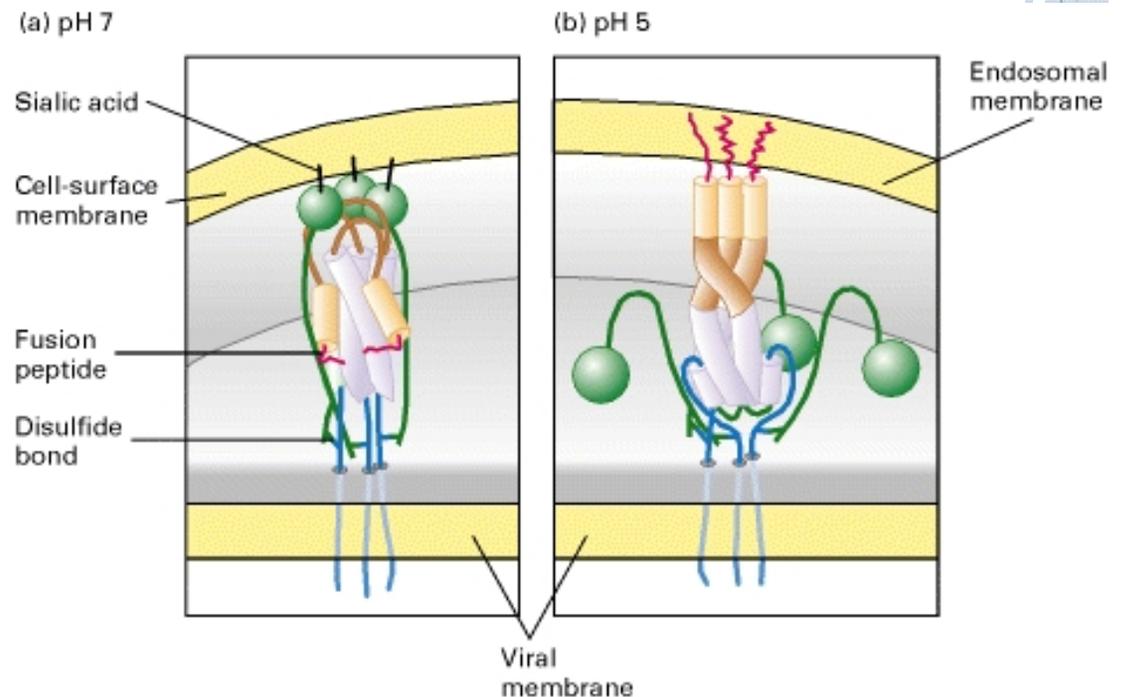


INFLUENZA LIFE CYCLE



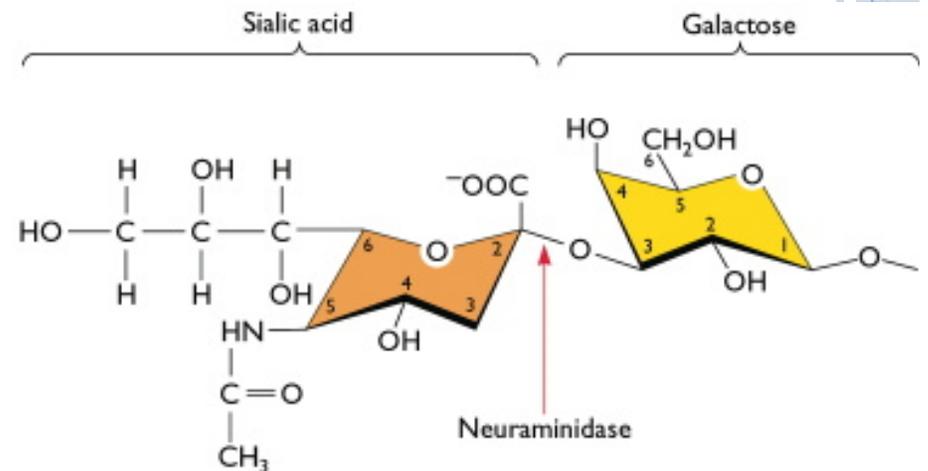
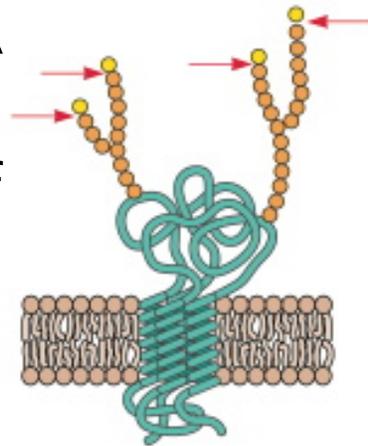
HA IS REQUIRED FOR CELL ENTRY

- HA binding to sialic acid on the surface of cells mediates initial attachment
- Virus is endocytosed, where the endosome is acidified
- This triggers a conformational change in the virus, resulting in membrane fusion
- For HA to be active, it needs to be cleaved by a protease into two pieces—this protease is generally restricted to the respiratory epithelium



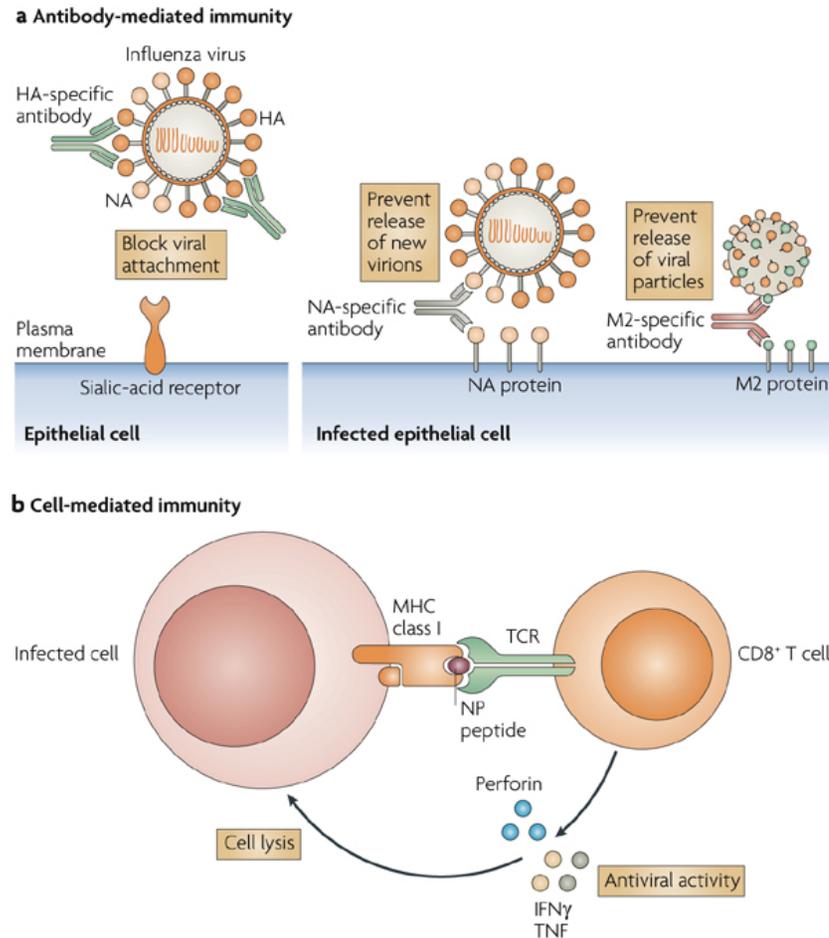
NEURAMINIDASE ACTS TO CLEAVE THE SIALIC ACID RECEPTORS FROM THE CELL SURFACE

- IAV must balance the binding and entry activity of HA with the sialic acid cleavage activity of NA so that virus efficiently enters and buds from the cell surface—thus HA and NA are often “matched” for activity



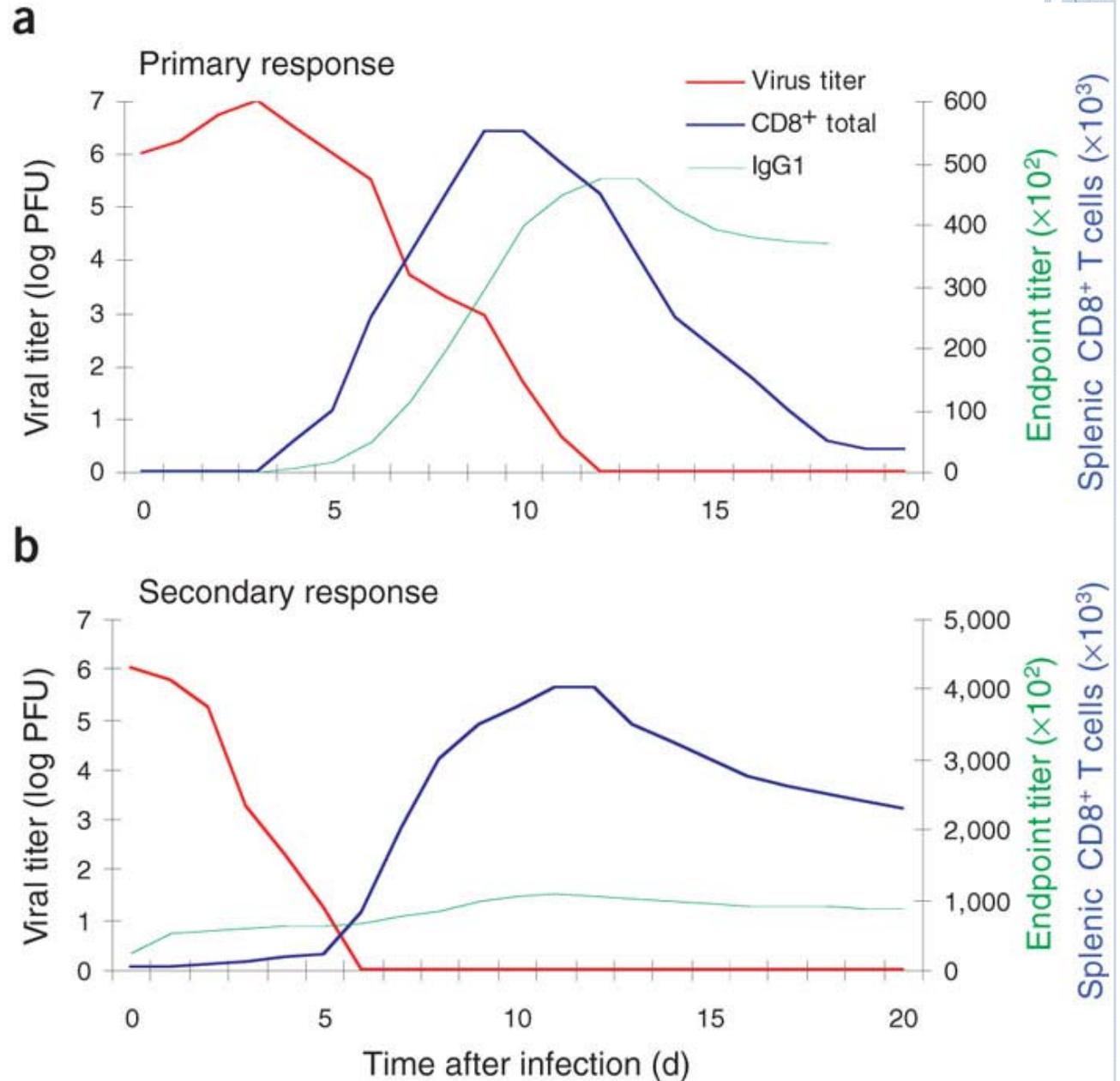
IMMUNE MECHANISMS OF PROTECTION

- Antibody mediated immunity exerts the most pressure on the virus, leading to seasonal antigenic drift and pandemic strains of antigenic shift
- Internal proteins are relatively conserved allowing heterologous cellular protection
- Mutation of dominant CD8 epitopes over time suggests that CTLs provide immunological pressure



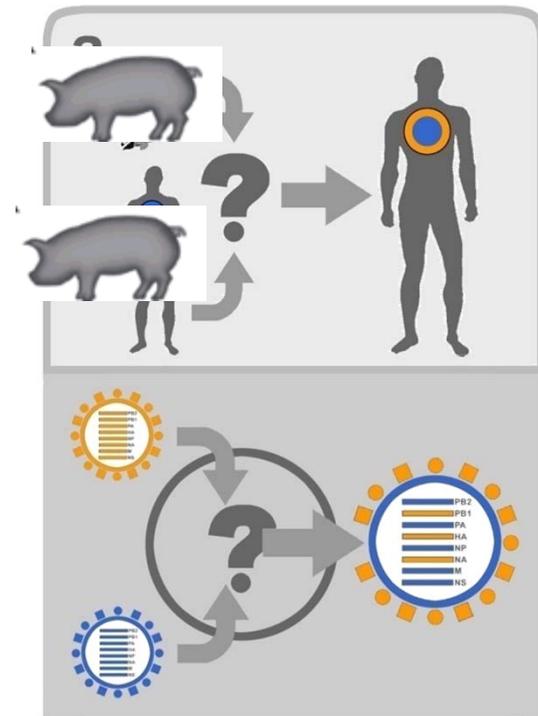
IMMUNE COURSE OF INFLUENZA INFECTION

- Influenza is initially controlled by antibody and CD8+ T cells
- Secondary infection with heterologous virus is cleared with CD8+ T cell activity much more rapidly
- Homologous infection can be prevented by antibody (sterilizing immunity)



PREDICTIONS OF THE 2009/H1N1 PANDEMIC

- The 2009 H1N1 pandemic emerged as a particularly novel threat: an antigenic shift event between two swine viruses, without the “human” virus component expected to be required
- The initial rapid spread bred fears of an equally high incidence of severe morbidity and mortality (~90,000 deaths in the US, ~1.8 million hospitalizations)



ORIGINAL ARTICLE

PRE-EXISTING CROSS-REACTIVE IMMUNITY TO 2009/H1N1

Cross-Reactive Antibody Responses to the 2009 Pandemic H1N1 Influenza Virus

Kathy Hancock, Ph.D., Vic Veguilla, M.P.H., Xiuhua Lu, M.D., Weimin Zhong, Ph.D., Eboneé N. Butler, M.P.H., Hong Sun, M.D., Feng Liu, M.D., Ph.D., Libo Dong, M.D., Ph.D., Joshua R. DeVos, M.P.H., Paul M. Gargiullo, Ph.D., T. Lynnette Brammer, M.P.H., Nancy J. Cox, Ph.D., Terrence M. Tumpey, Ph.D., and Jacqueline M. Katz, Ph.D.

Table 1. Cross-Reactive Microneutralization Antibody Response against Pandemic Influenza A (H1N1) Virus in Pediatric and Adult Recipients of Seasonal Trivalent Inactivated Influenza Vaccines.*

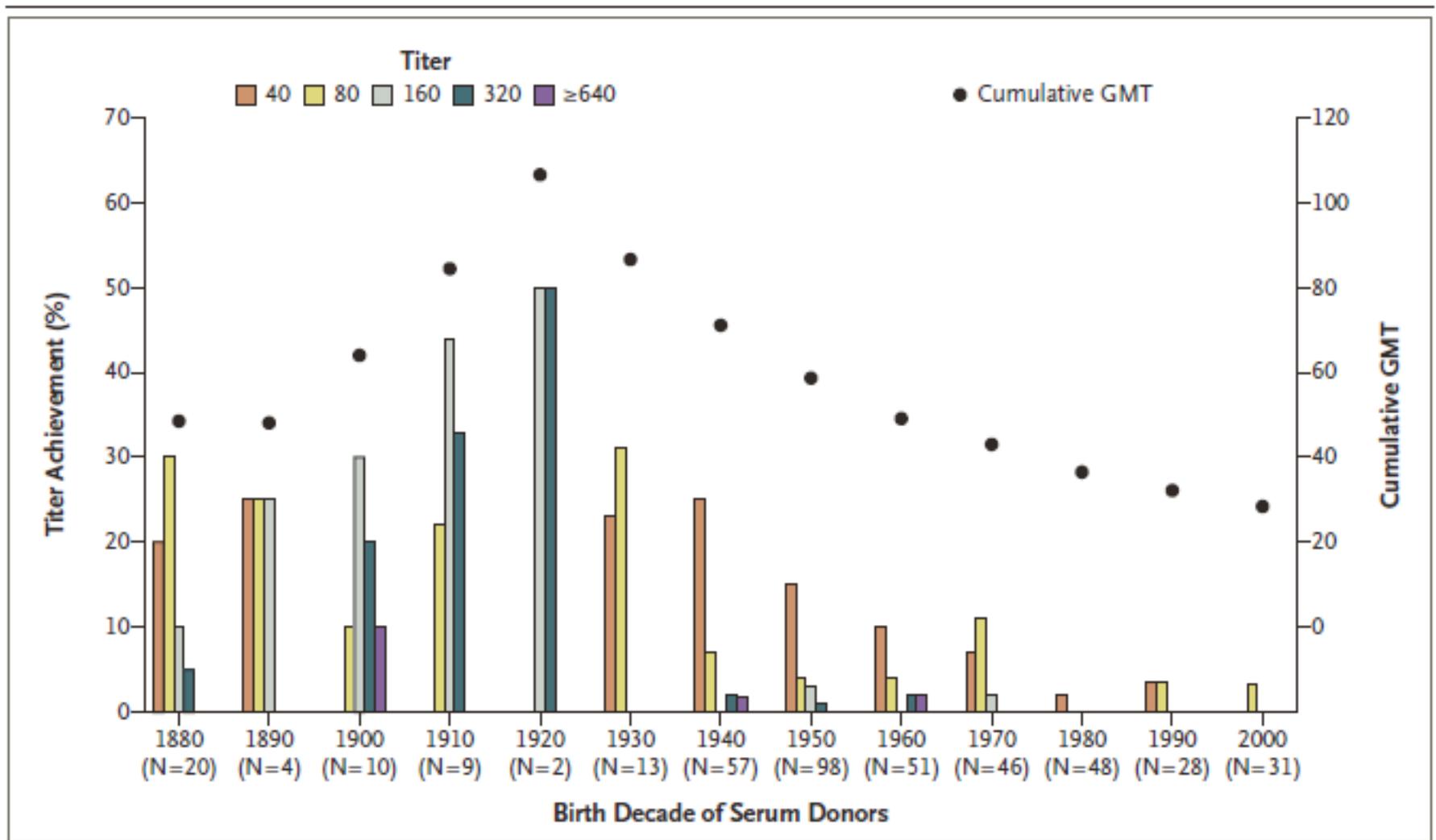
Type of Vaccine, Influenza Season, and Influenza Virus Used in Assay	Age Group	No. of Subjects	Increase in Antibody Titer by a Factor of ≥ 4	Geometric Mean Titer [†]		Microneutralization Titer of ≥ 40 for Children or ≥ 160 for Adults [‡]	
				Before Vaccination (95% CI)	After Vaccination (95% CI)	Before Vaccination	After Vaccination
			%			%	%
Children							
Trivalent inactivated influenza vaccine							
2005–2007	6 mo to 9 yr	33					
Seasonal H1N1			67	26 (16–40)	267 (171–418)	45	94
Pandemic H1N1			0	5 (5–6)	6 (5–6)	0	0
2007–2008	5 yr to 9 yr	13					
Seasonal H1N1			85	42 (22–80)	575 (303–1093)	54	100
Pandemic H1N1			0	10 (7–15)	12 (8–17)	8	15
2008–2009	6 mo to 23 mo	9					
Seasonal H1N1			100	5 (4–7)	285 (202–402)	0	100
Pandemic H1N1 [§]			0	5	5	0	0
Trivalent inactivated influenza vaccine with adjuvant							
2008–2009	6 mo to 59 mo	45 [¶]					
Seasonal H1N1			96	12 (8–18)	193 (134–280)	24	100
Pandemic H1N1			2	6 (5–7)	8 (7–9)	0	4



TABLE CONTINUED

		(%)	(%)	(%)	(%)	(%)
Adults						
Trivalent inactivated influenza vaccine						
2007–2008	18 yr to 64 yr	148				
Seasonal H1N1			75	48 (40–58)	598 (497–720)	29 93
Pandemic H1N1			22	25 (21–31)	54 (44–65)	7 25
2008–2009	18 yr to 40 yr	83				
Seasonal H1N1			78	29 (22–38)	546 (418–713)	20 88
Pandemic H1N1			12	11 (9–14)	21 (16–26)	6 7
Older adults						
Trivalent inactivated influenza vaccine						
2007–2008	≥60 yr	63				
Seasonal H1N1			54	31 (22–42)	143 (105–194)	14 54
Pandemic H1N1			5	92 (71–121)	97 (74–127)	33 43
2008–2009	≥60 yr					
Seasonal H1N1		49**	18	22 (17–28)	51 (39–66)	6 14
Pandemic H1N1		50**	0	47 (36–61)	51 (39–65)	8 8

PEOPLE BORN PRIOR TO 1940 HAVE "PROTECTIVE" LEVELS OF ANTIBODY TO 2009/H1N1



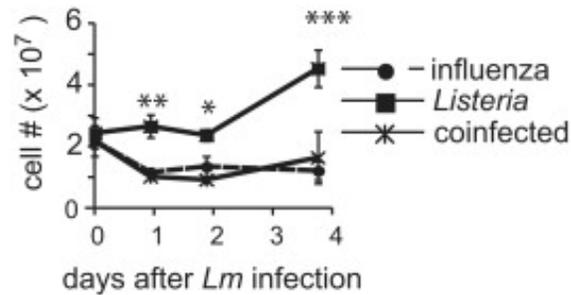
INFLUENZA MORTALITY IS ASSOCIATED WITH SECONDARY BACTERIAL INFECTIONS

- Between August 2009 and March 2010, 276 influenza-associated pediatric deaths were reported to the CDC
- 34% of the tested children had a bacterial co-infection
 - Strep-21%
 - Staph-34%
 - Rest apparently unidentified
- Several mechanisms for secondary infection susceptibility have been proposed, including action of the viral neuraminidase promoting bacterial colonization and immunological disruption

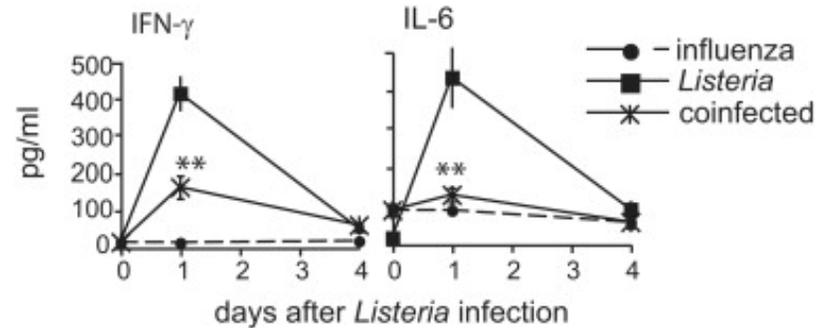


INFLUENZA INDUCED GLUCOCORTICOID MEDIATED SUPPRESSION?

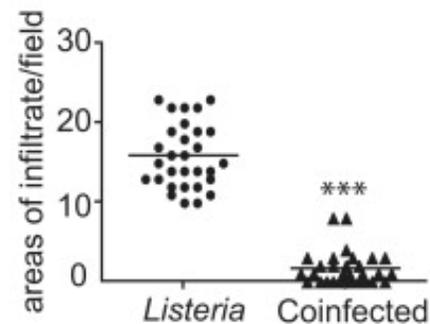
A SPLENOCYTE NUMBERS



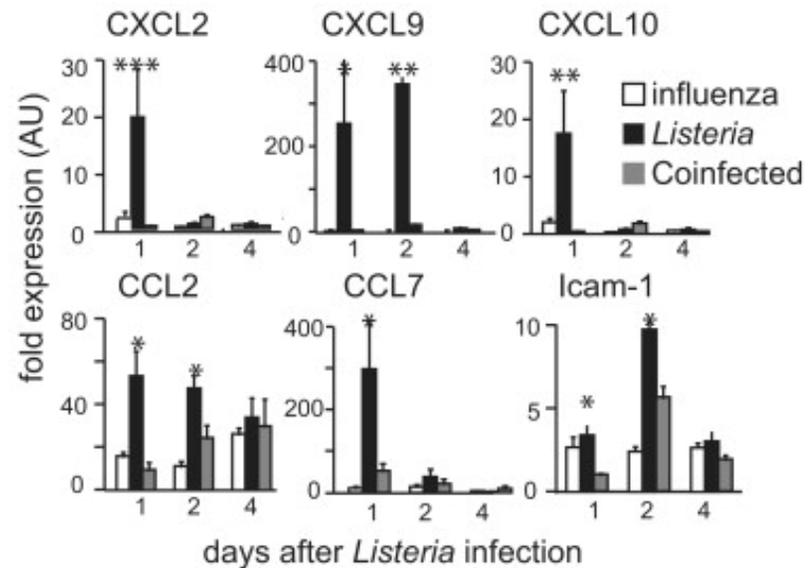
B CYTOKINES IN SERUM OF INFECTED MICE



C INFILTRATING CELLS IN THE LIVER



D EXPRESSION OF GENES IN THE LIVER



[Influenza virus-induced glucocorticoids compromise innate host defense against a secondary bacterial infection.](#)

Jamieson AM, Yu S, Annicelli CH, Medzhitov R.

Cell Host Microbe. 2010 Feb 18;7(2):103-14.

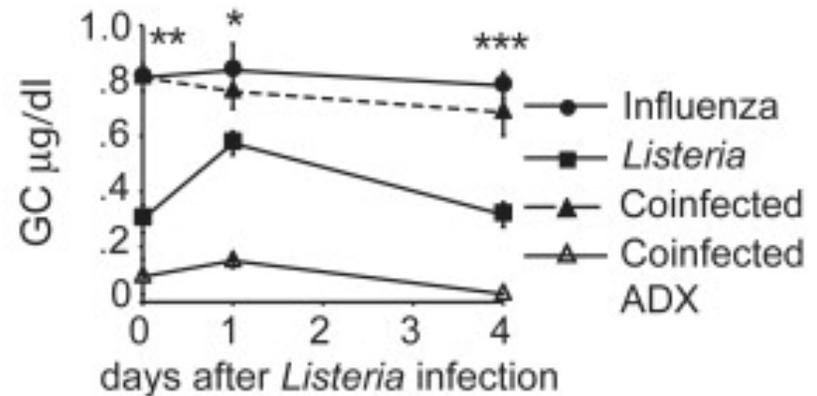


GLUCOCORTICOID MEDIATED BACTERIAL SUSCEPTIBILITY

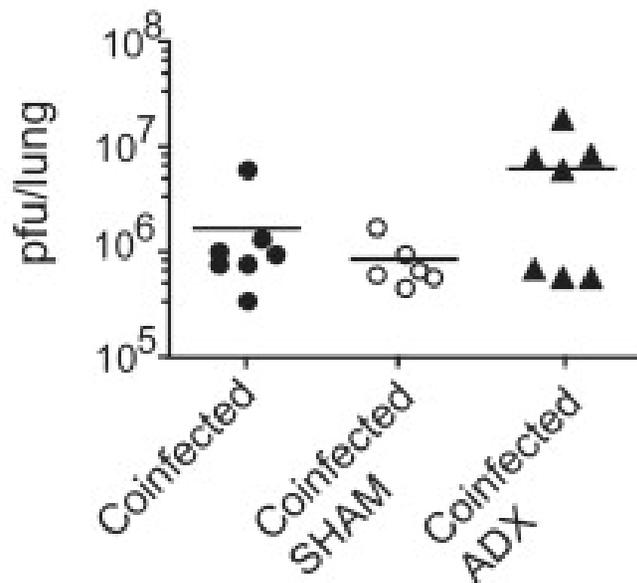
[Influenza virus-induced glucocorticoids compromise innate host defense against a secondary bacterial infection.](#)

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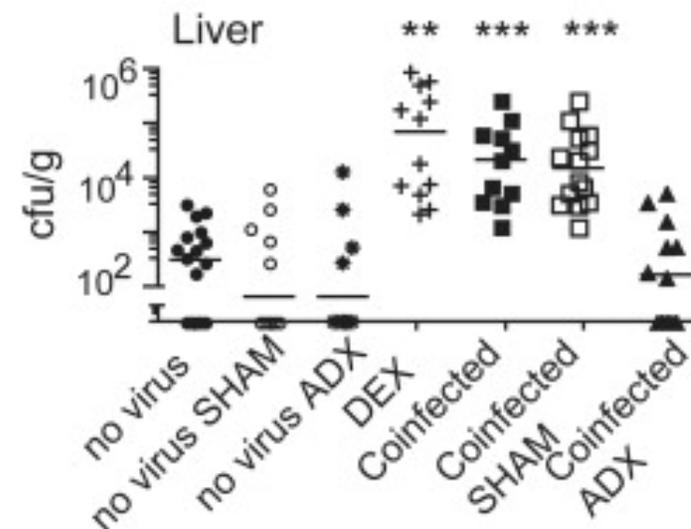
A SERUM GC LEVELS AFTER INFECTION



C VIRAL LOAD IN ADX MICE

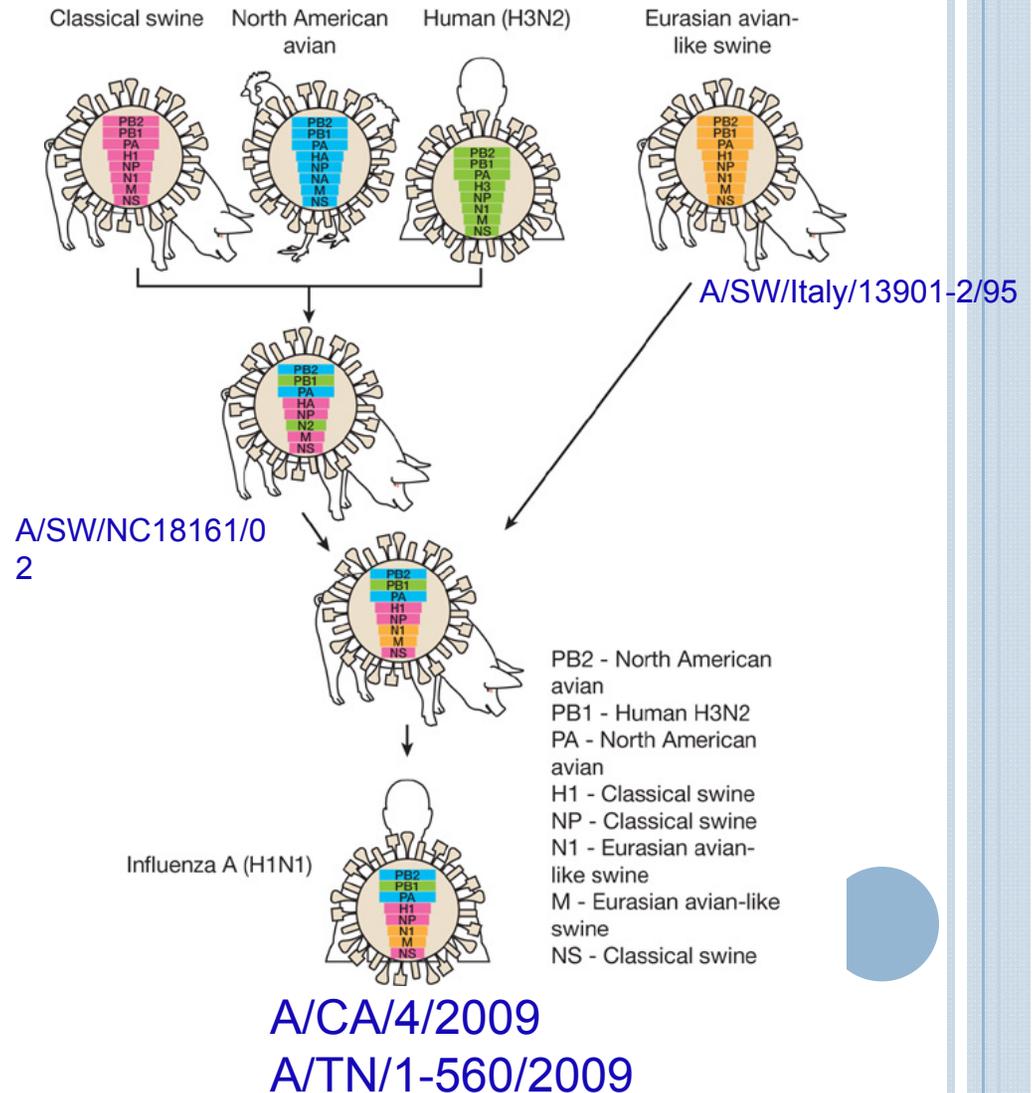


B GC INFLUENCE BACTERIAL BURDEN



2009 PANDEMIC H1N1

- 2009/H1N1 resulted from the recombination of two viruses (American and Eurasian Swine)
- The American Swine virus was itself a recombinant of three viruses that established itself in 1998
- These viruses are genetically distant from the human seasonal H1N1 (reference strain A/Brisbane/59/07)



EARLY PANDEMIC H₁N₁: APRIL – JULY 2009

Table 2. Estimates of pandemic (H1N1) 2009–related cases and rates of illness and hospitalization by age distribution of confirmed case-patients, United States, April–July 2009

Parameter	Estimated no. case-patients		Estimated rate/100,000*	
	Median	90% range	Median	90% range
Total no. case-patients by age group, y†	3,052,768	1,831,115–5,720,928	997	598–1,868
0–4	397,033	238,149–744,045	1,870	1,122–3,505
5–24	1,820,284	1,091,845–3,411,237	2,196	1,317–4,115
25–49	612,862	367,608–1,148,511	577	346–1,081
50–64	180,297	108,146–337,879	319	192–599
≥65	42,292	25,368–79,256	107	64–201
No. hospitalized case-patients by age group, y	13,764	9,278–21,305	4.5	3.0–7.0
0–4	2,768	1,866–4,285	13.0	8.8–20.2
5–24	4,991	3,364–7,725	6.0	4.1–9.3
25–49	3,440	2,319–5,324	3.2	2.2–5.0
50–64	1,912	1,289–2,959	3.4	2.3–5.2
≥65	654	441–1,012	1.7	1.1–2.6
Multiplier				
Hospitalized	2.7	1.7–4.5	–	–
Nonhospitalized	79	47–148	–	–
Through May 12	33	23–49	–	–
After May 12	84	50–163	–	–

*United States Population Estimates, 2009.

†Age distributions from line list and aggregate reports of laboratory-confirmed cases and hospitalizations to the Centers for Disease Control and Prevention through July 23, 2009.

HUMAN INFECTIONS WITH TRIPLE-REASSORTANT SWINE VIRUSES HAVE OCCURRED

Human Case of Swine Influenza A (H1N1) Triple Reassortant Virus Infection, Wisconsin

Alexandra P. Newman,¹ Erik Reisdorf, Jeanne Beinemann, Timothy M. Uyeki, Amanda Balish, Bo Shu, Stephen Lindstrom, Jenna Achenbach, Catherine Smith, and Jeffrey P. Davis

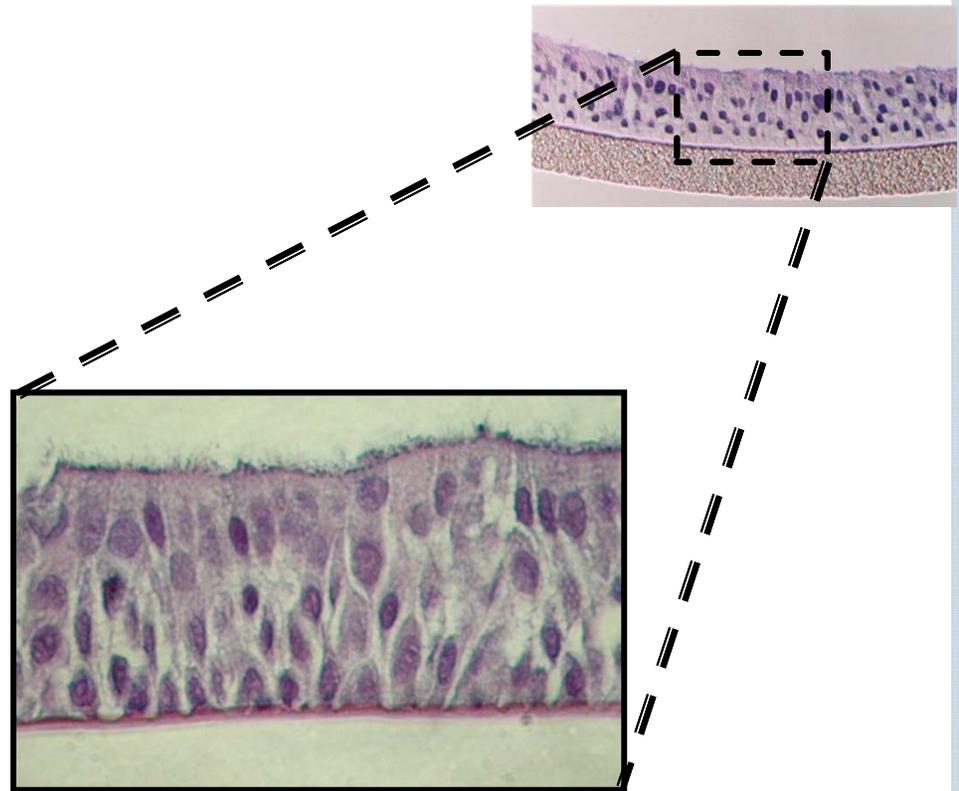
Zoonotic infections with swine influenza A viruses are reported sporadically. Triple reassortant swine influenza viruses have been isolated from pigs in the United States since 1998. We report a human case of upper respiratory illness associated with swine influenza A (H1N1) triple reassortant virus infection that occurred during 2005 following exposure to freshly killed pigs.

- "... custom slaughter house ..."
- "... helped hold and abduct the forelimbs of one (1) freshly killed pig while his brother eviscerated it."
- "No facial or respiratory protection was worn ..."
- "... father obtained a live chicken..."
- "...sacrificed during a ritual ceremony."
- "... patient was never within ten (10) feet of the chicken.."

H₁N₁ SWINE FLU STUDIES: RESPONSE IN HUMAN CELLS

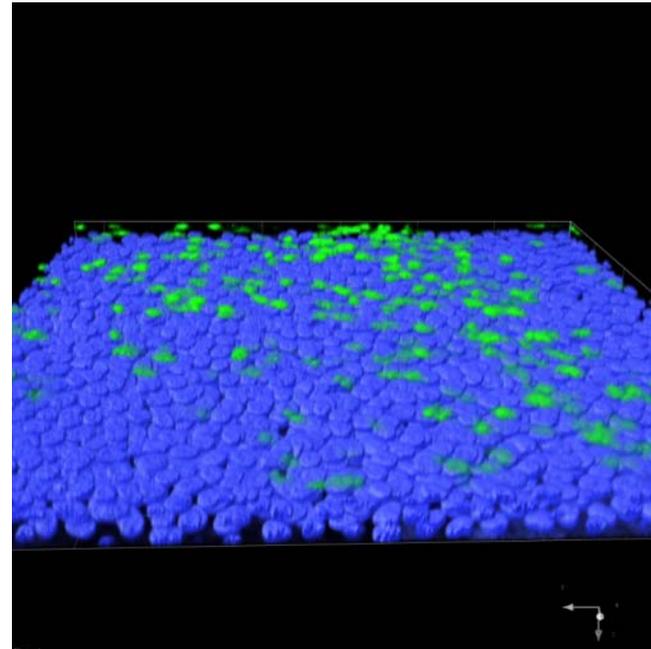
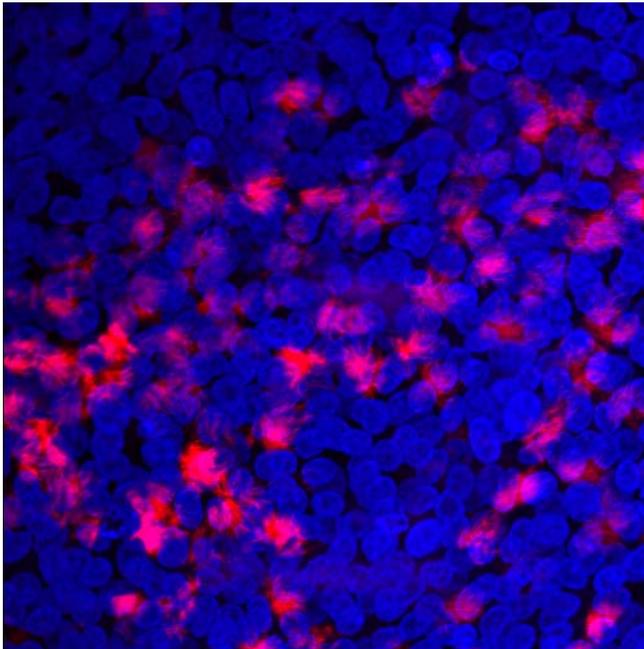
Measures:

- Infectivity and growth of virus (TCID₅₀, immunofluorescence)
- Secretion of inflammatory mediators from apical and basolateral surfaces (multiplexed immunoassay)
- Transcriptional response over the first 24 hours (Exon arrays, fluidigm analysis)
- Confirm results by “swapped viruses” made by reverse genetics



EpiAirway™,
MatTek

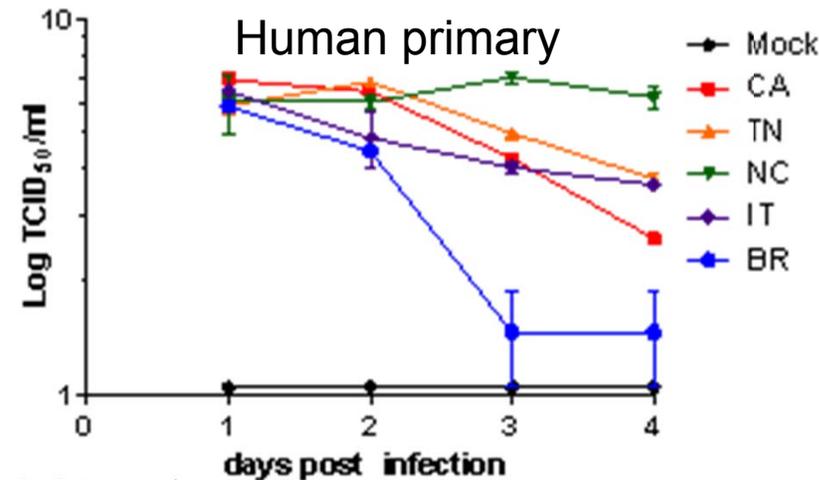
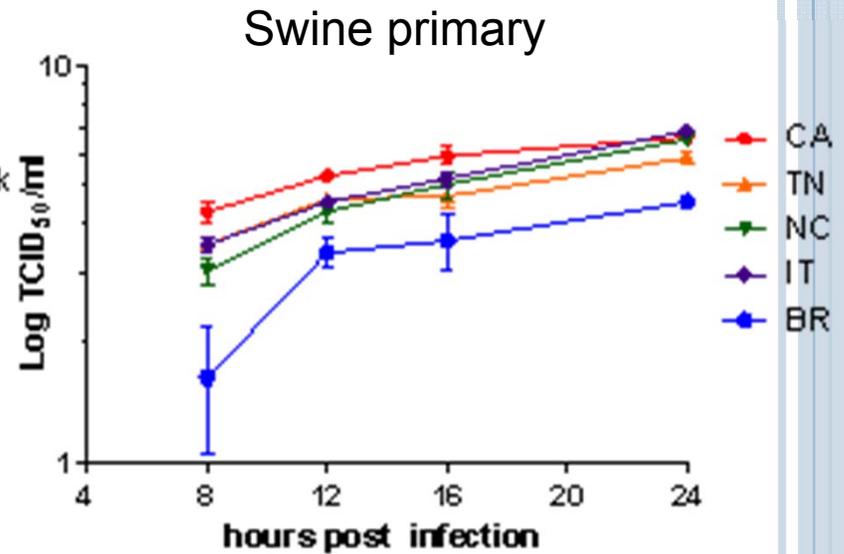
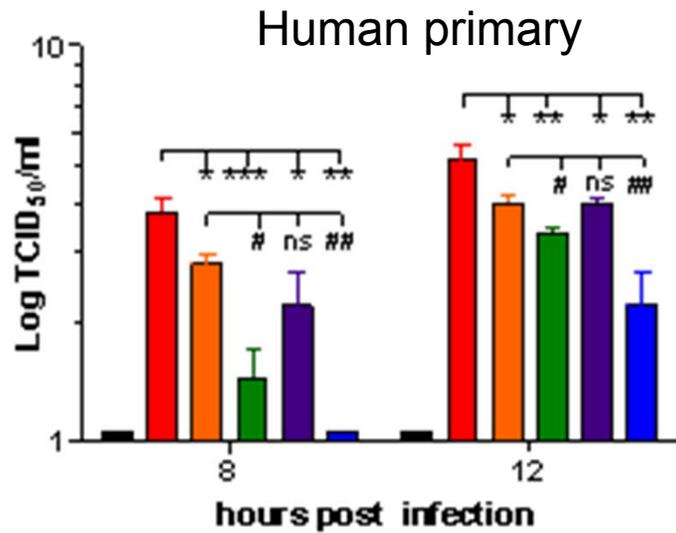
IN VITRO CULTURES OF HUMAN AIRWAY EPITHELIAL CELLS



cilia-red/green
Nucleus-blue



VIRAL GROWTH KINETICS IN HAE CELLS



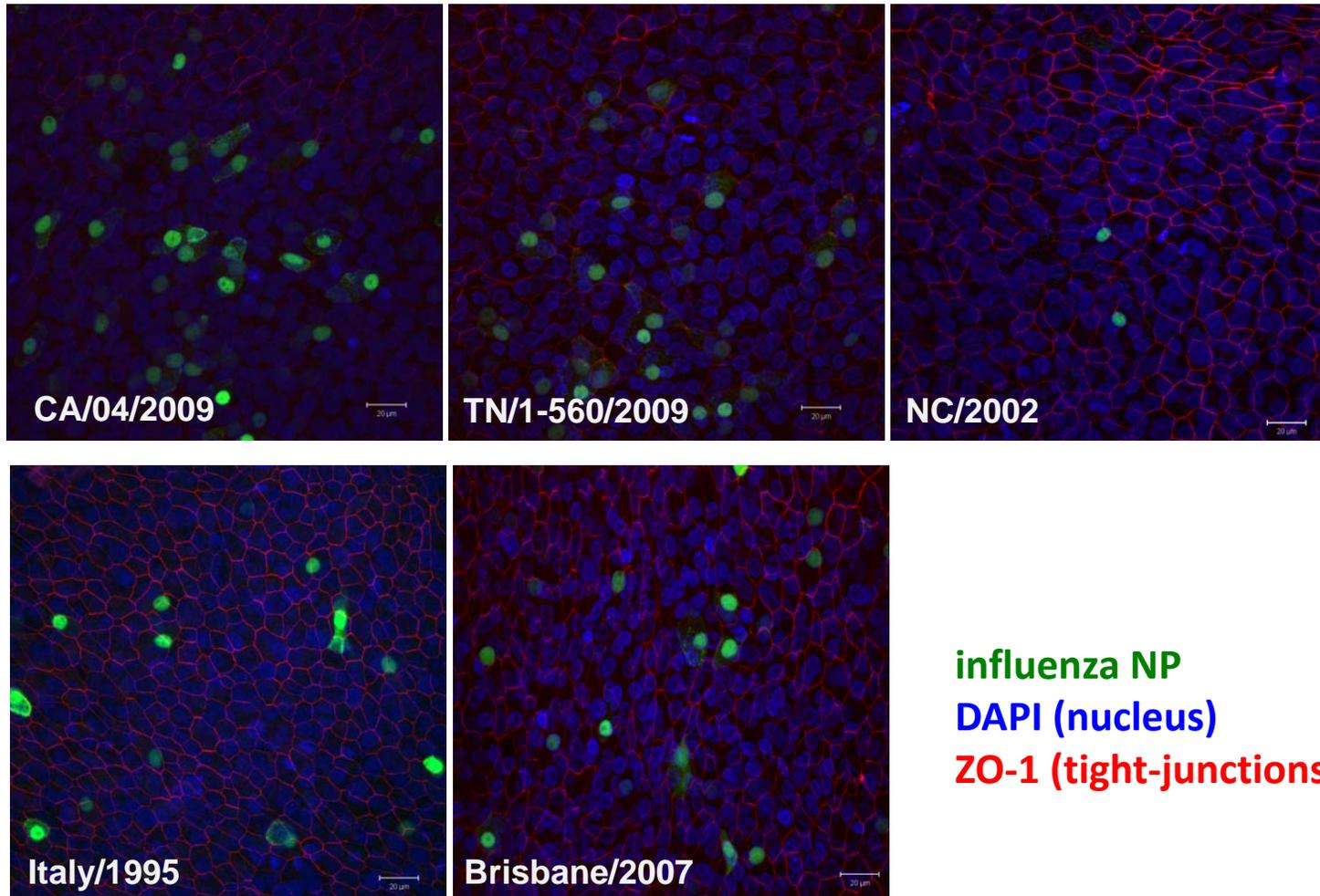
0.01 moi

All continued shedding from healthy monolayers for >3 weeks



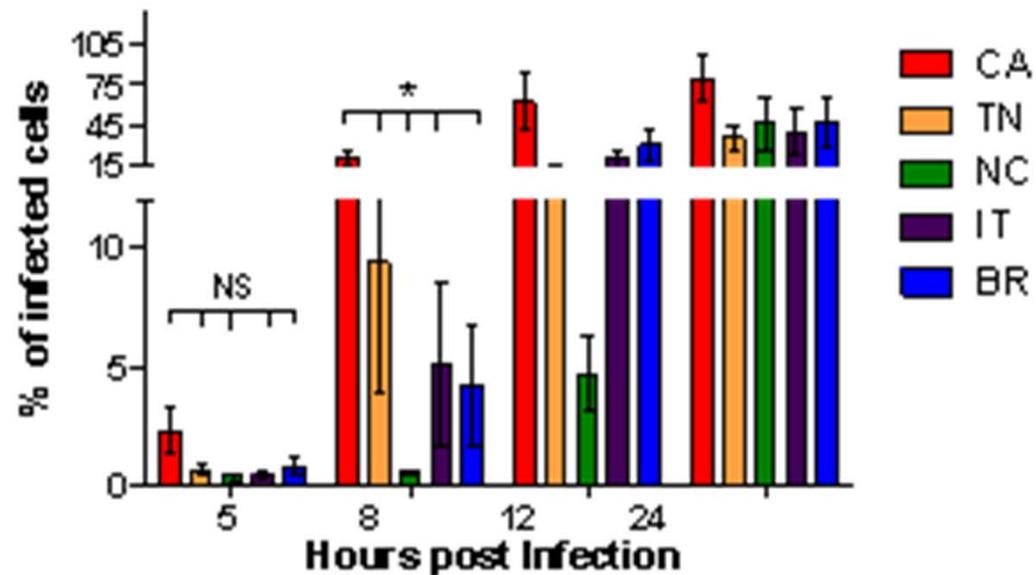
Influenza NP detection in 3D HAE cultures

viral growth kinetics in HAE cells



8 hr post infection- 0.01 moi

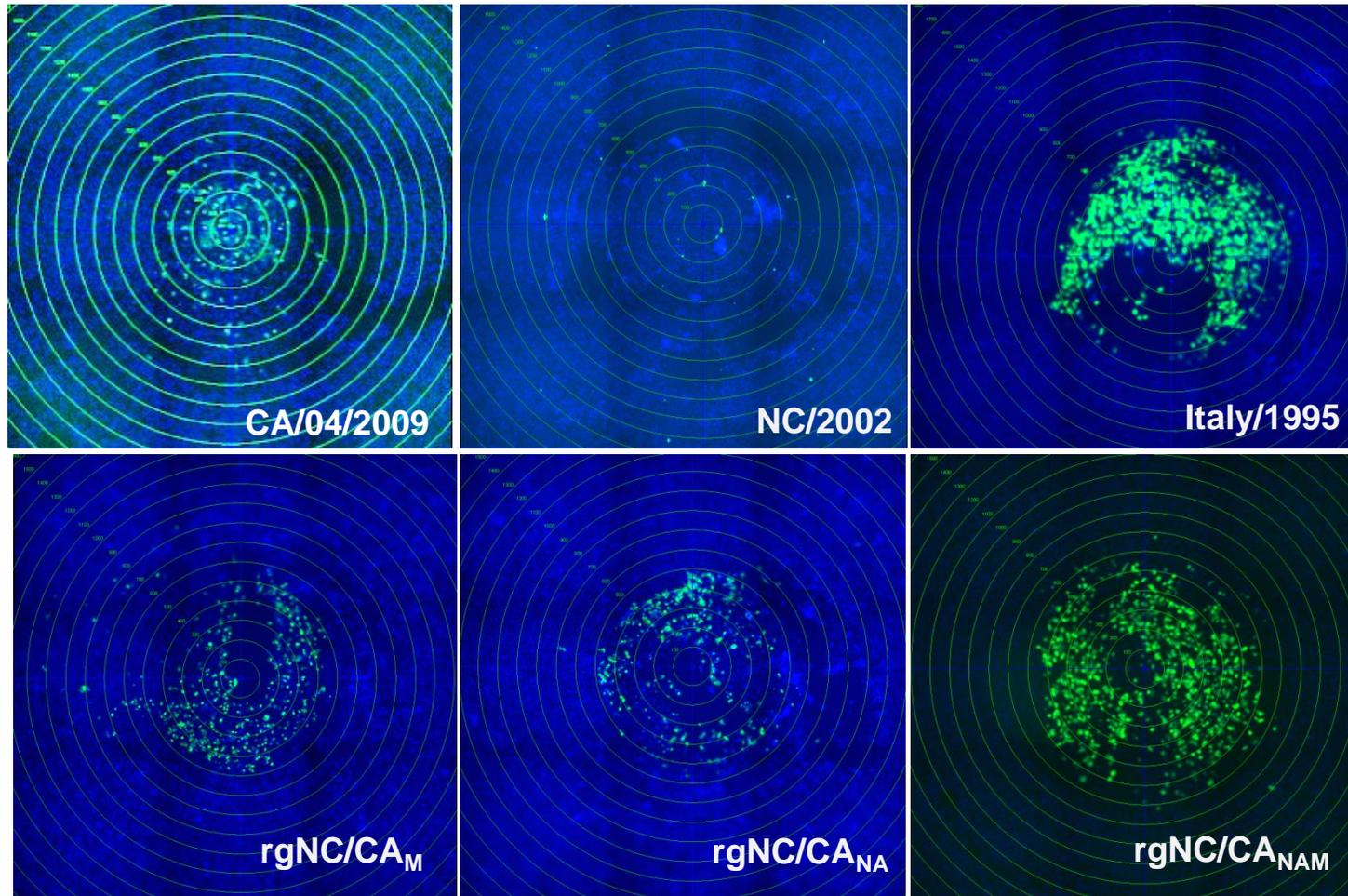
MORE RAPID COLONIZATION OF CULTURE BY PANDEMIC AND ESW VIRUS



By 12 hours, pandemic strains and Italy have infected ~50%-75% of the culture

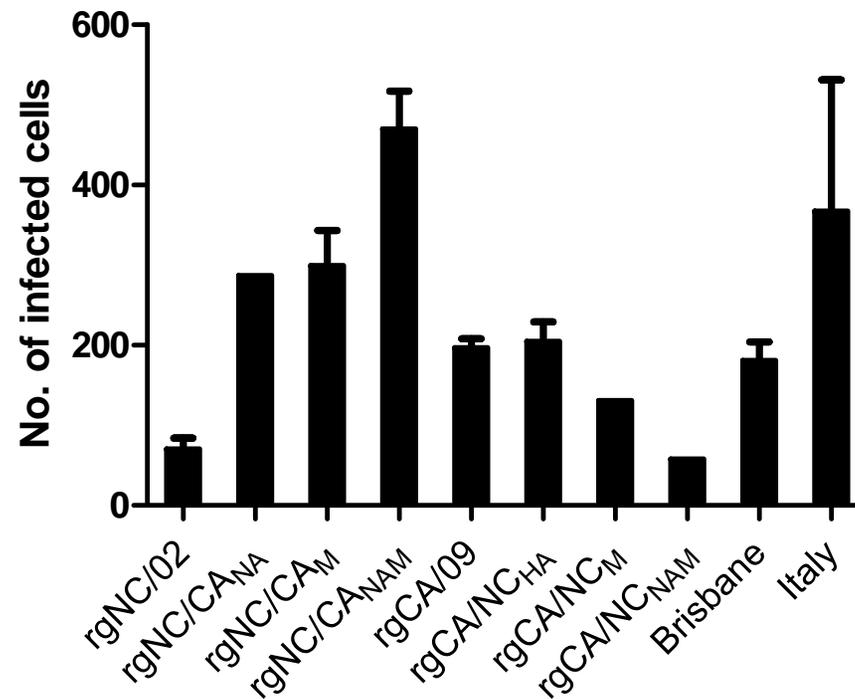


Viral efficiency of spread in A549



influenza NP
DAPI (nucleus)

SUMMARY DATA OF FOCAL INFECTION STUDY

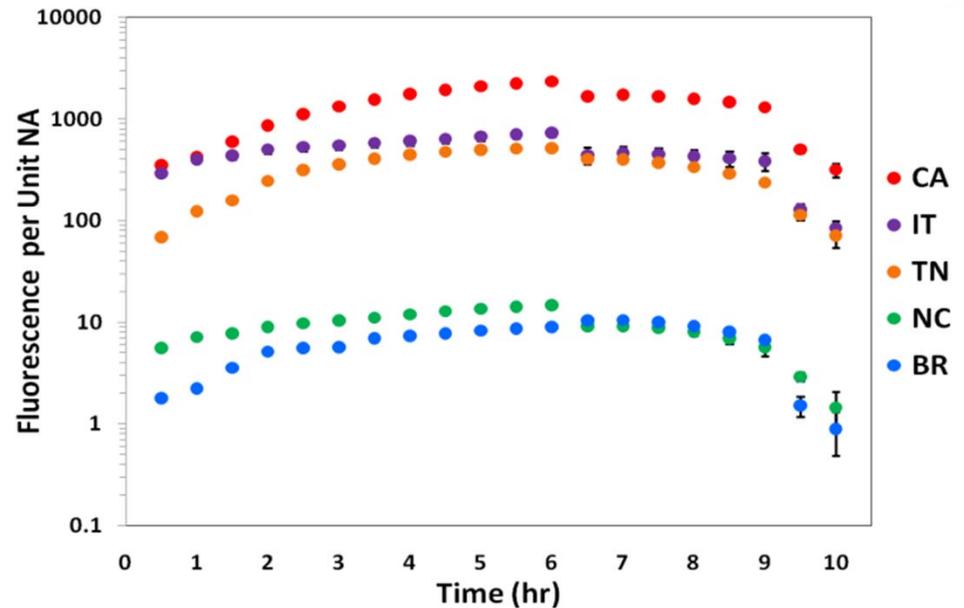


Both M and NA genes of CA/04/09 contribute to greater viral spread



HIGHER NA ACTIVITY IN PANDEMIC AND ESW

- NA activity measured as ability to convert sialic acid containing substrate
- Results normalized to functional viral titer, so NA activity/infectious virion
- Higher NA activity may relate to ability of virus to spread efficiently



GROWTH SUMMARY

- The pandemic virus acquired a rapid growth phenotype in human cells similar to the Esw virus
- This phenotype associates with both the NA and M of Esw virus
- The Esw virus transmits more efficiently in ferrets
- Titer and infected cell number can be de-coupled across infections/individuals



ODE MODEL OF INFLUENZA INFECTION—ANDREAS HANDEL, UGA

$$\frac{dU}{dt} = \lambda D - \frac{b}{1 + s_1 X} UV \quad \text{uninfected cells}$$

$$\frac{dE}{dt} = \frac{b}{1 + s_1 X} UV - \frac{g}{1 + s_3 X} E \quad \text{latent infected cells}$$

$$\frac{dI}{dt} = \frac{g}{1 + s_3 X} E - dI \quad \text{productively infected cells}$$

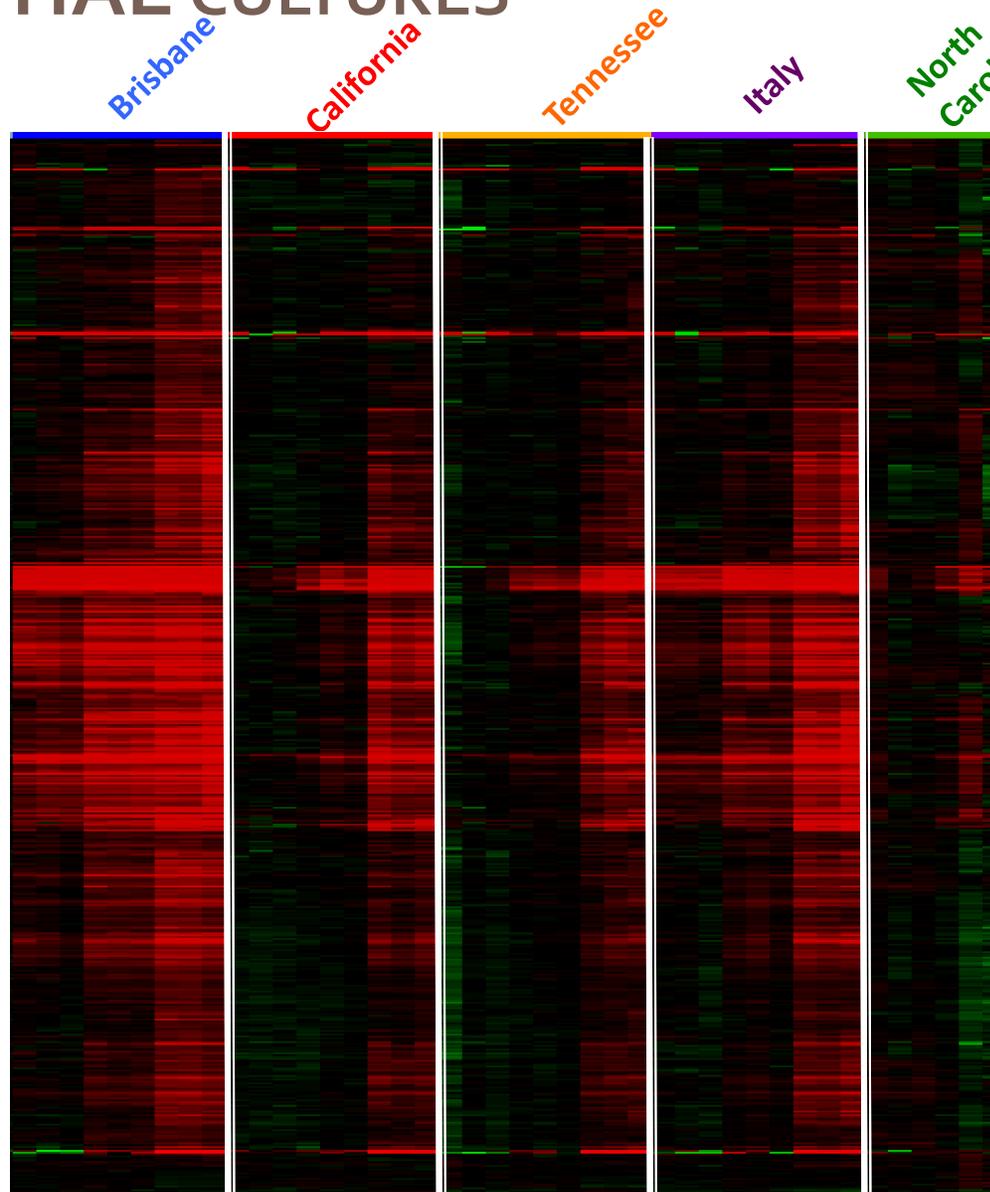
$$\frac{dD}{dt} = dI - \lambda D \quad \text{dead cells}$$

$$\frac{dV}{dt} = \frac{p}{1 + s_2 X} I - cV - \gamma \frac{b}{1 + s_1 X} VU \quad \text{free virus}$$

Why wasn't the Esw virus a pandemic?

TRANSCRIPTOME ANALYSIS OF PANDEMIC VIRUS INFECTED HAE CULTURES

mRNA expression in
hAE cultures
infected at
MOI=0.01

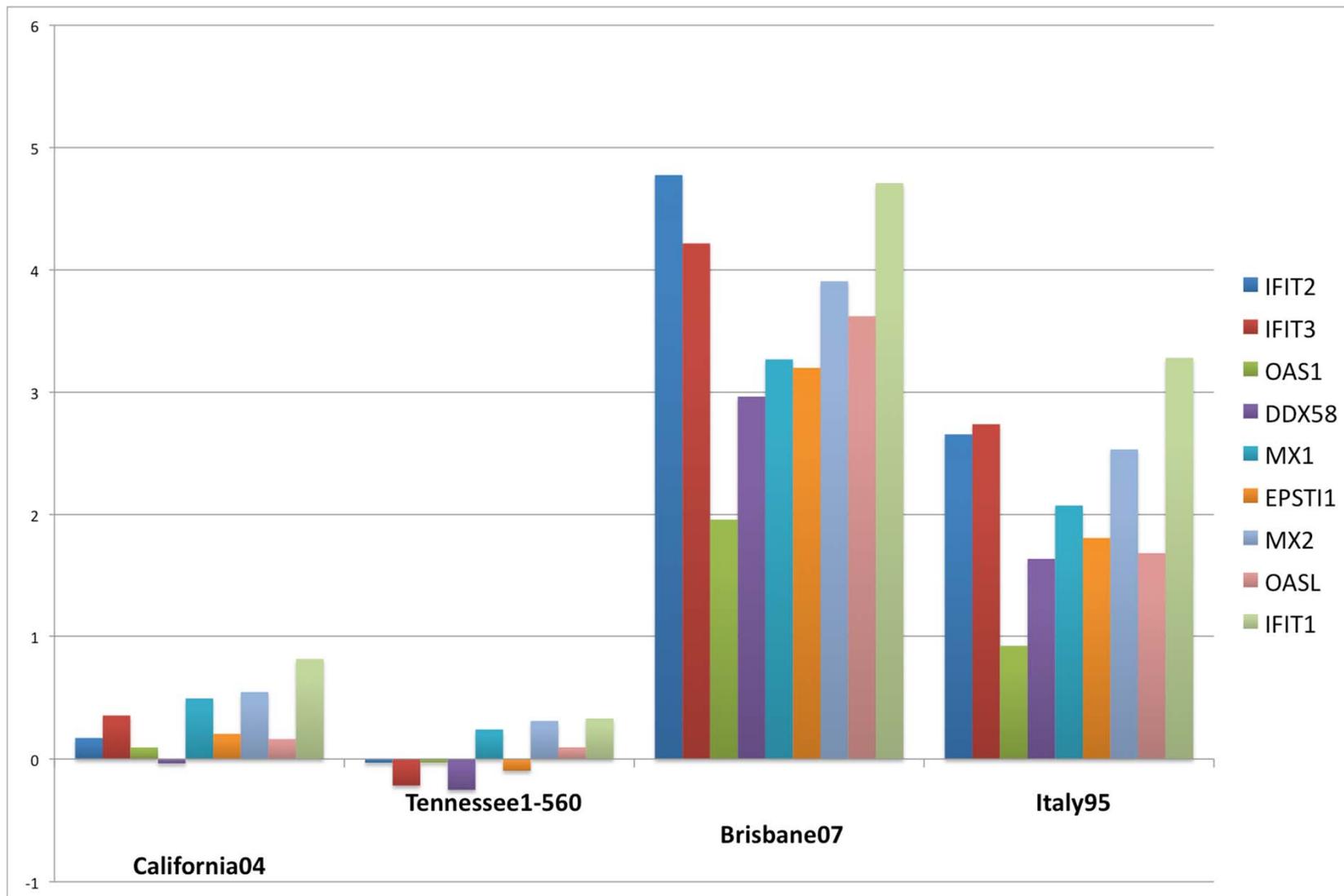


Time (hours p.i.) 12 16 24

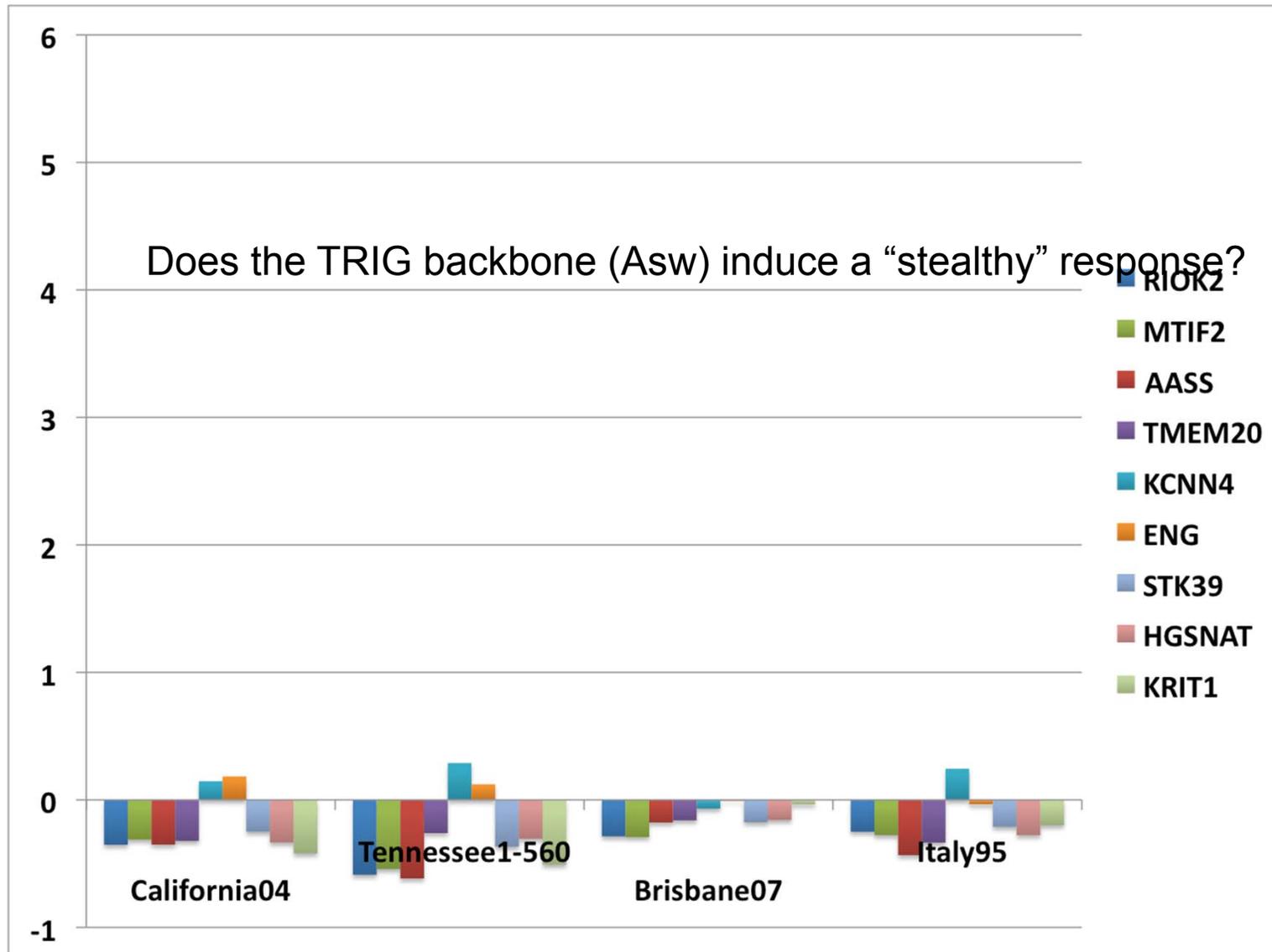
BIC applied to k-
means clustering:
2 clusters
271 upregulated in
all
24 downregulated
or differential



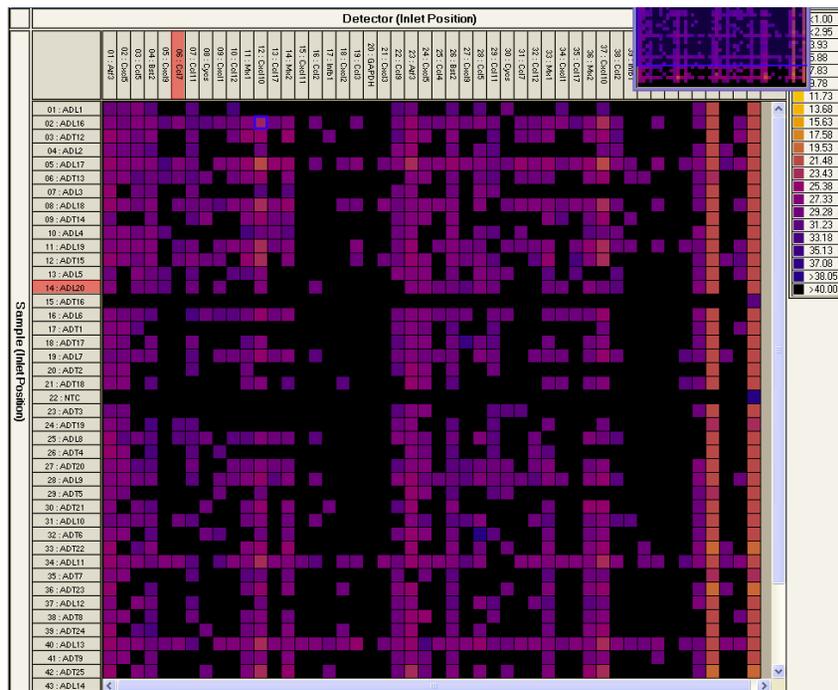
TOP 9 MOST SIGNIFICANT DIFFERENTIALLY EXPRESSED GENES 12 HOURS POST-INFECTION WITH A/BRISBANE/59/2007(H1N1)



TOP 9 MOST SIGNIFICANT DIFFERENTIALLY EXPRESSED GENES AT 12 HOURS POST-INFECTION WITH A/CALIFORNIA/04/2009(H1N1)



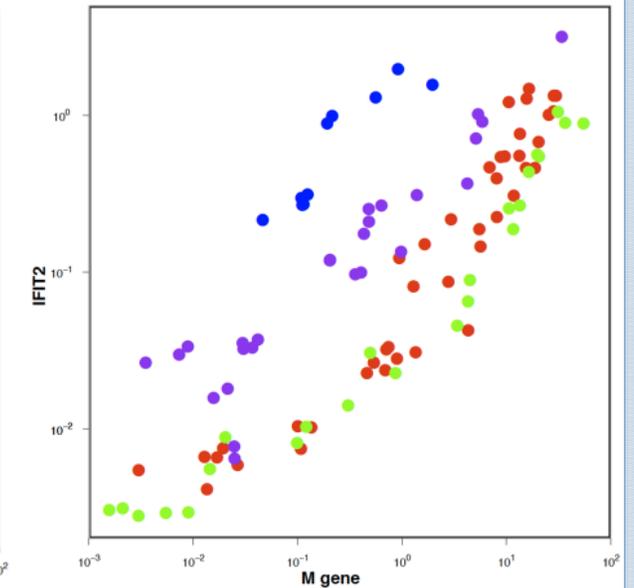
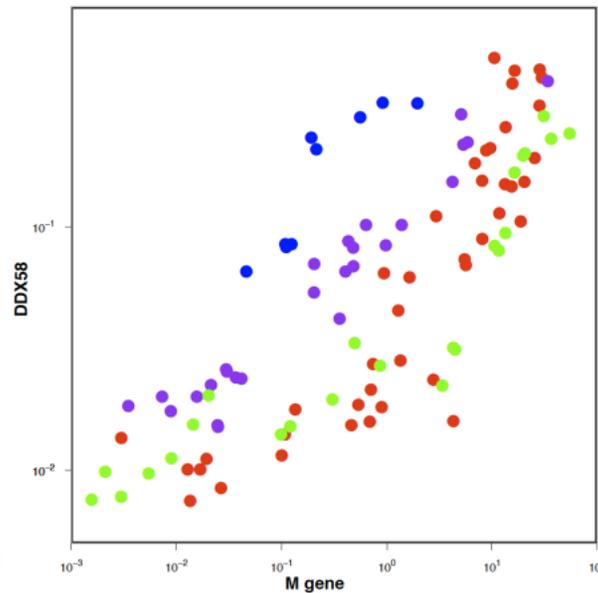
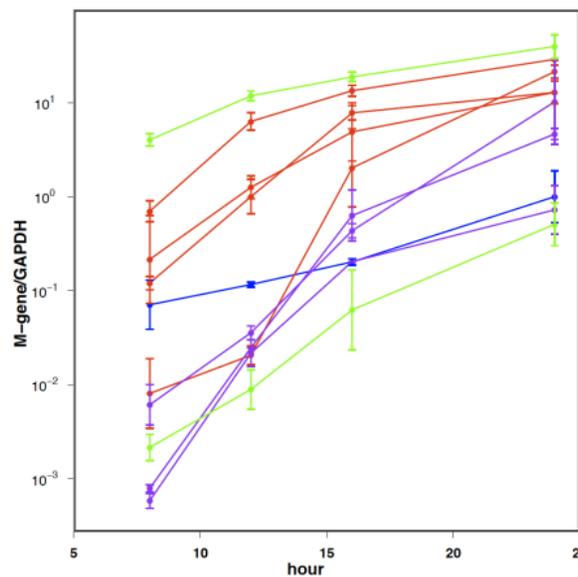
FLUIDIGM PLATFORM FOR ARRAY VERIFICATION



- Uses microfluidics to perform multiple qPCR reactions on one plate
- 48x48 (probes vs. targets) or 96x96 formats
- 1 48x48 plates=6 384 well plates
- Real time confirmation of arrays with a tiny amount of RNA
- For this project, selected 100 genes to track:
 - Most up- or down- regulated
 - Virus replication, IFN response, host response



HOST RESPONSE AS A FUNCTION OF VIRUS



Brisbane

California

Italy

North
Carolina

Fluidigm Real Time PCR from
primary human cell infections
(2 donors)

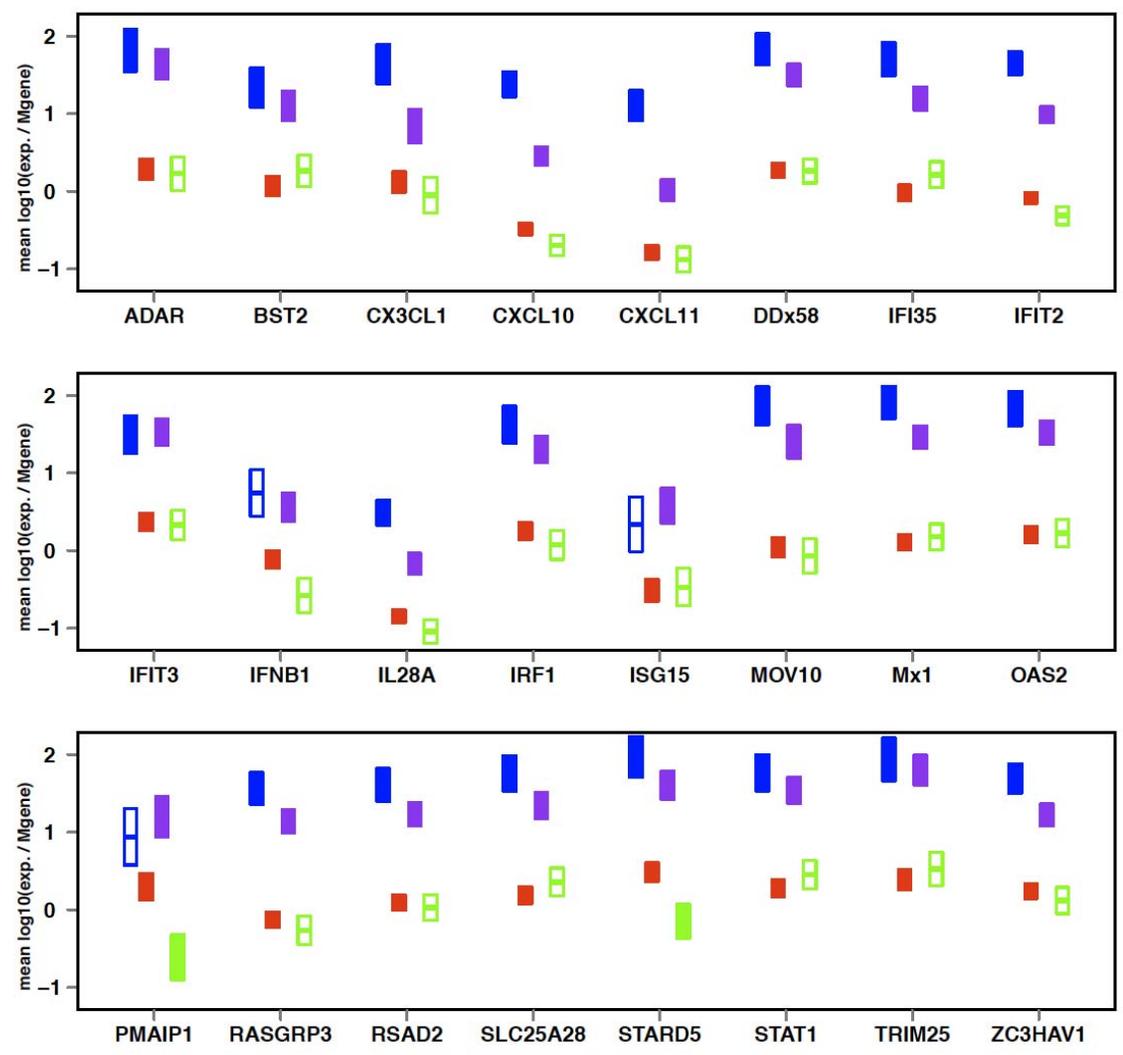


HOST RESPONSE AS A FUNCTION OF VIRUS II

Brisbane California
Italy North Carolina

$$\frac{\text{expression} - \text{expression}_{\text{mock}}}{\max(\text{expression})}$$

Mgene

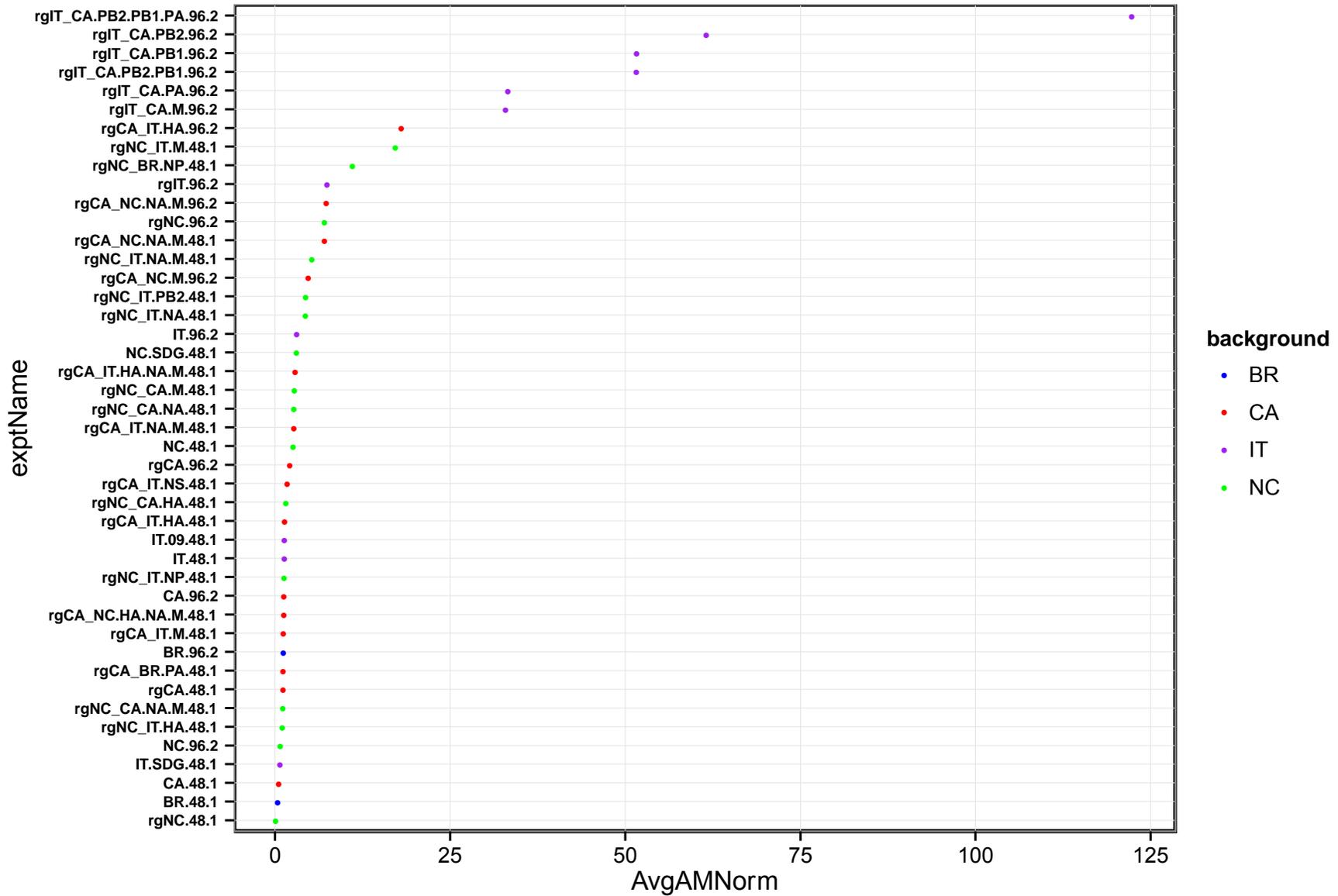


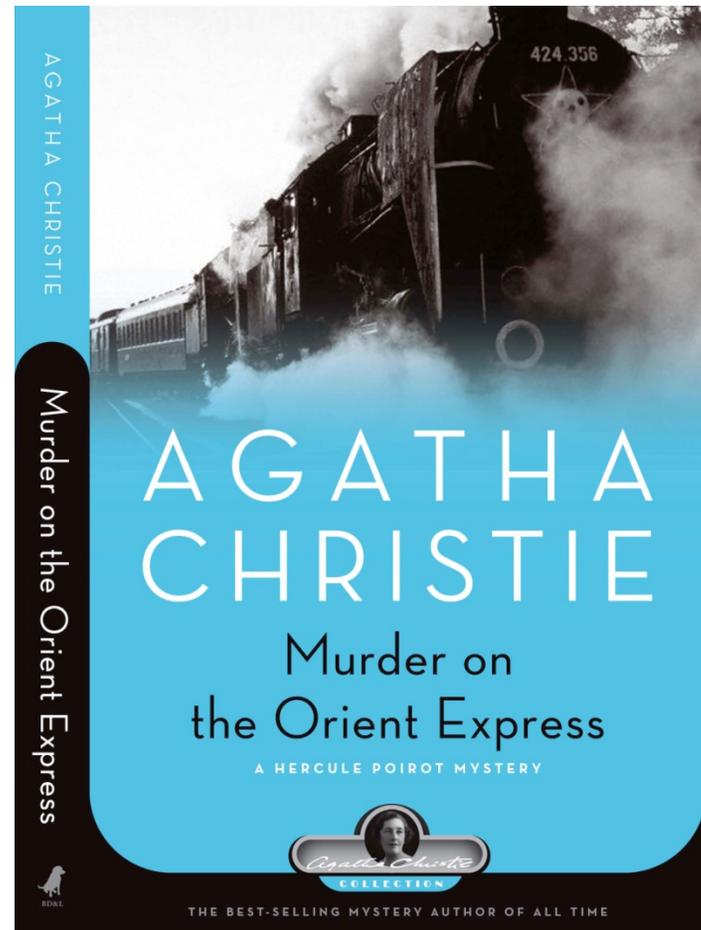
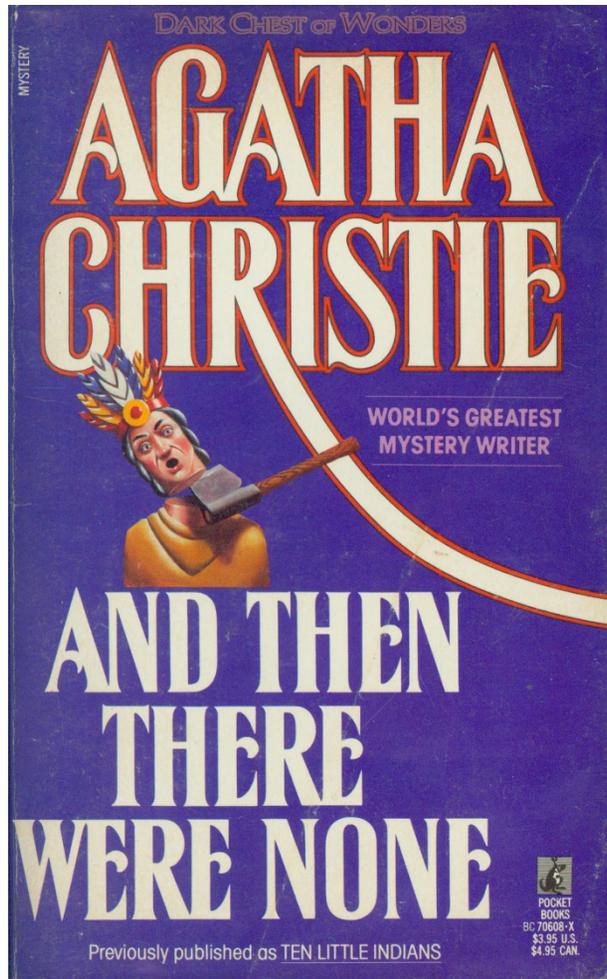
SWAPS

What's the mechanistic basis of the stealthy (or noisy) phenotype?



Average amplitude across all genes normalized to M-gene





THE PANDEMIC STRAIN IS EFFICIENT AND STEALTHY

- Rapid + stealthy growth = Pandemic

Morbidity and Mortality Weekly Report

Limited Human-to-Human Transmission of Novel Influenza A (H3N2) Virus — Iowa, November 2011

- The set of genes induced by diverse viruses is largely equivalent in the first 24 hours— “the flu program”
- The pandemic strategy is distinct from the well-adapted human seasonal virus
- Kinetic differences in the first ~18 hours of infection are critical to the quality and quantity of the later response
- The stealthy phenotype is mediated by contributions of the P-gene complex, with potential roles for NP and NS

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$$\frac{dD}{dt} = dI - \lambda D \quad \text{dead cells}$$

$$\frac{dV}{dt} = \frac{p}{1 + s_2 X} I - cV - \gamma \frac{b}{1 + s_1 X} VU \quad \text{free virus}$$

$$\frac{dX}{dt} = wI - \delta X \quad \text{innate immune response (IFN)}$$

AICc VALUES OF 8 DIFFERENT MODELS

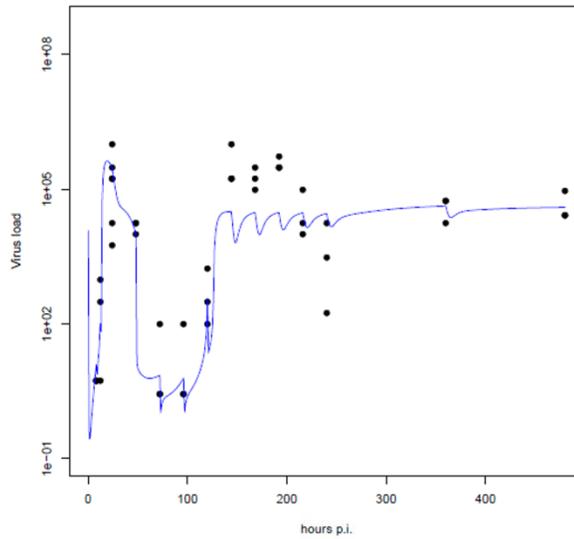
- | | | |
|----|---|--|
| 1. | No IR and no cell-regrowth | regrowth |
| 2. | No IR, with cell-regrowth | 6. With IR reducing virus production, with cell-regrowth |
| 3. | With IR reducing virus production, no cell-regrowth | 7. With IR reducing infection rate, with cell-regrowth |
| 4. | With IR reducing infection rate, no cell-regrowth | 8. With IR prolonging latency, with cell-regrowth |

Model	BB	CA	IT	NC
1	54.5	54.7	33.1	28.2
2	48.8	-22.6	0.8	28.5
3	52.8	24.8	17.0	30.3
4	59.9	33.2	38.3	33.6
5	53.2	32.1	24.6	31.7
6	-11.6	-17.6	-11.1	33.2
7	54.5	-17.7	6.1	29.3
8	56.1	-17.3	6.2	34.3

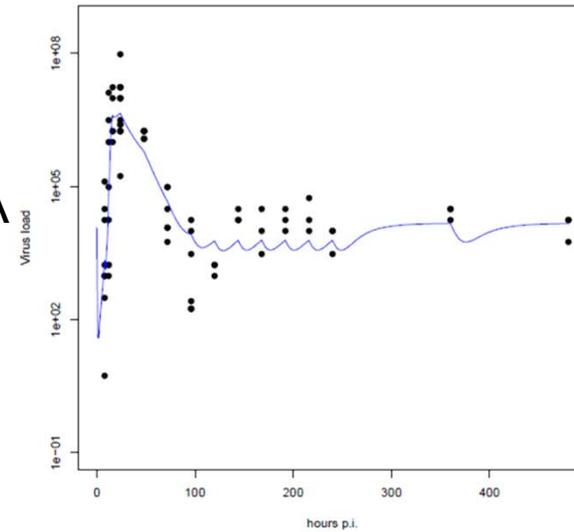


FITS FOR MODEL 6—IR REDUCES VIRUS PRODUCTION AND CELLS REGROW

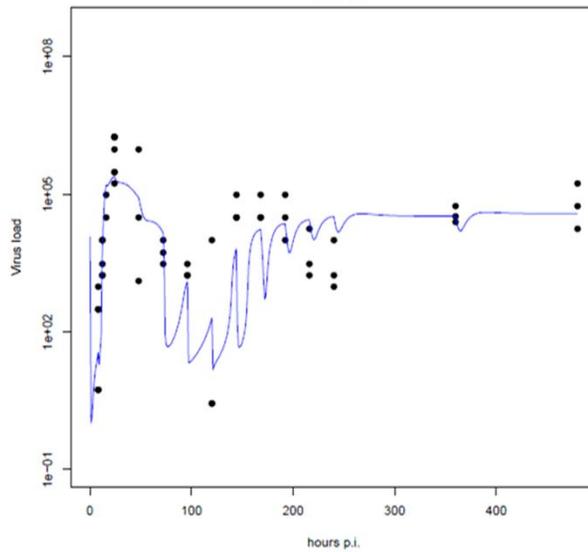
BR



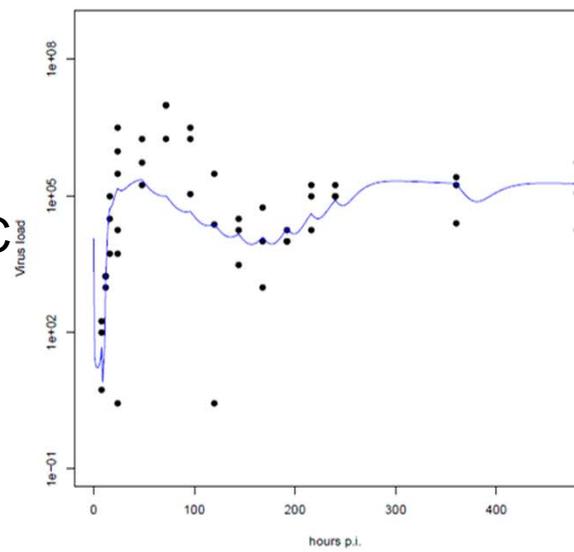
CA



IT

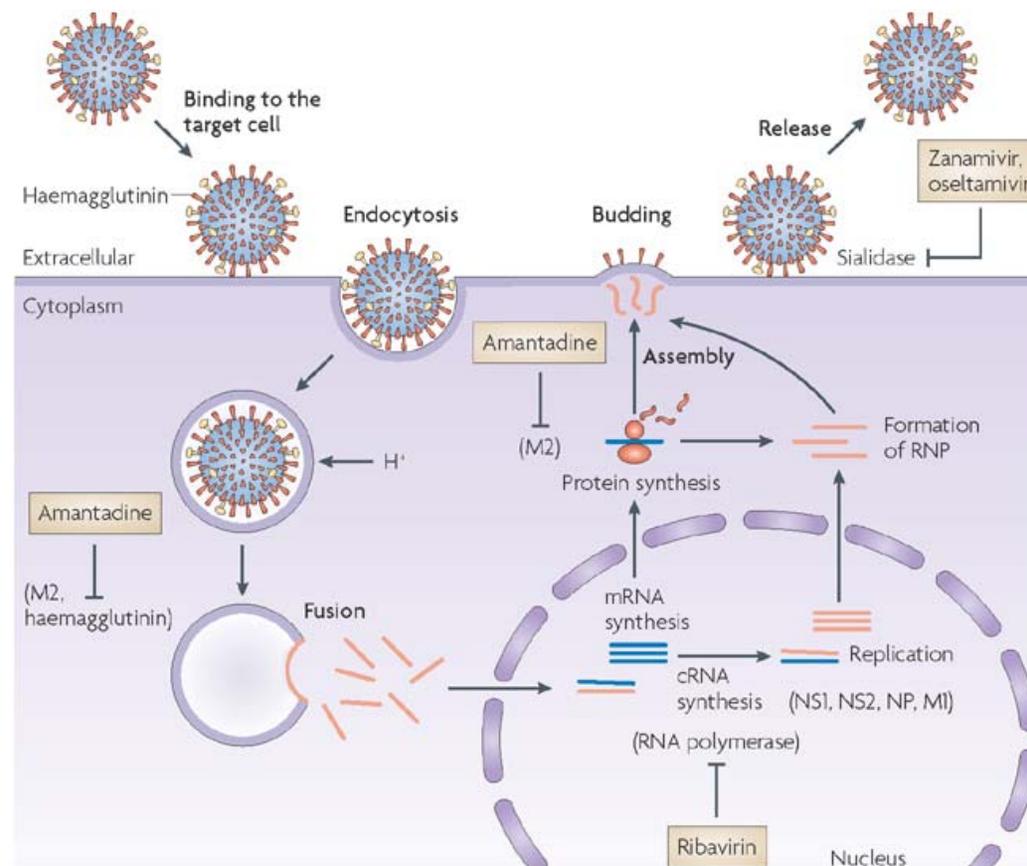


NC

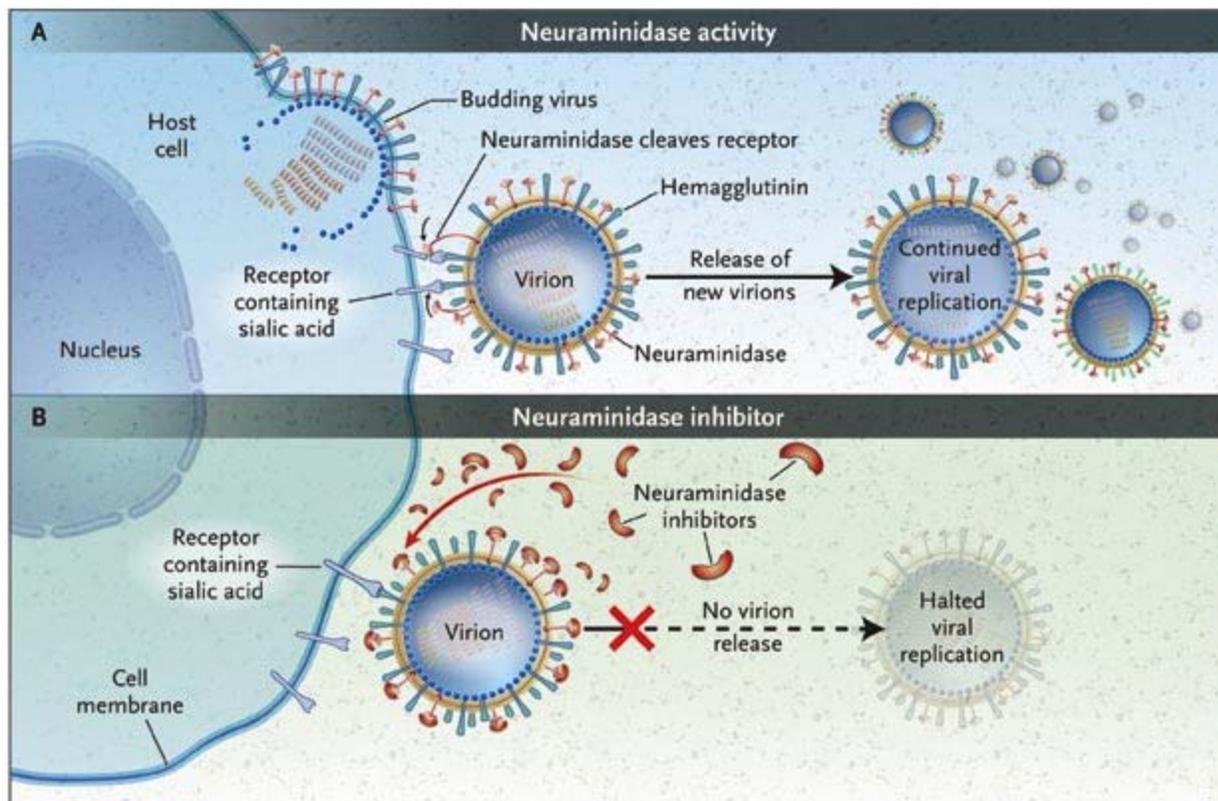


DRUG TARGETS FOR INFLUENZA TREATMENT

- The two approved drug families target the M2 ion channel and NA



NEURAMINIDASE INHIBITORS PREVENT VIRAL SPREAD



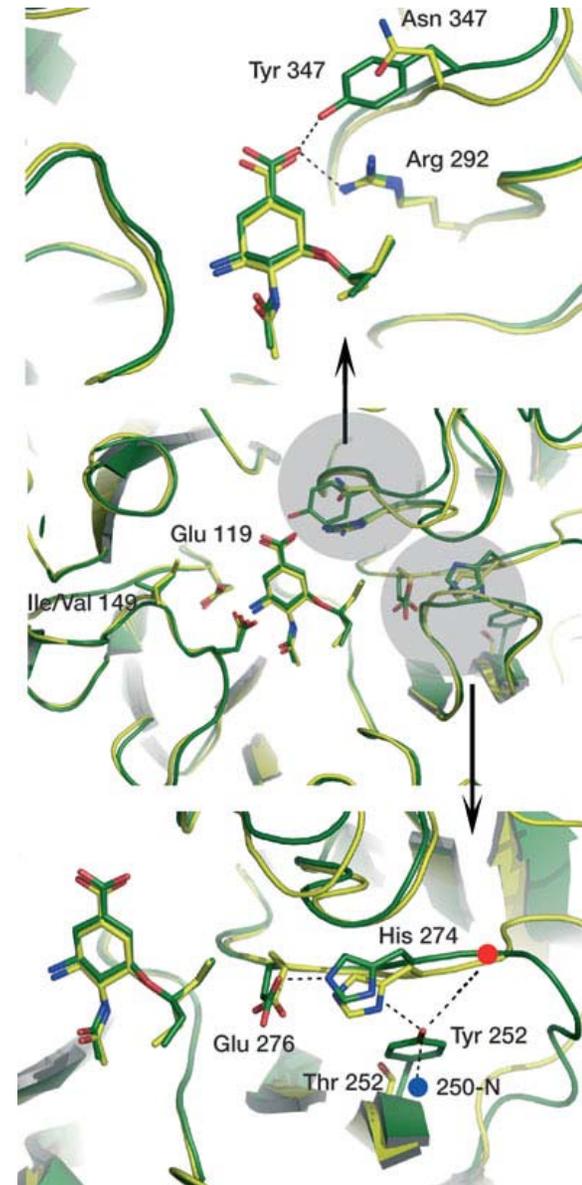
NEURAMINIDASE INHIBITOR RESISTANCE HAS EMERGED IN SEASONAL H₁N₁ INFLUENZA, AMANTADINE RESISTANCE IN H₃N₂

	Isolates tested (n)	Resistant Viruses, Number (%)	Isolates tested (n)	Resistant Viruses, Number (%)	
		Oseltamivir	Zanamivir		Adamantanes
Seasonal Influenza A (H ₁ N ₁)	825	820 (99.4%)	0 (0)	832	4 (0.5%)
Influenza A (H ₃ N ₂)	132	0 (0)	0 (0)	141	141 (100%)
Influenza B	403	0 (0)	0 (0)	N/A*	N/A*
Novel Influenza A (H ₁ N ₁)	68	0 (0)	0 (0)	96	96 (100%)



MUTATIONS ASSOCIATED WITH NA INHIBITOR RESISTANCE

- Group 1 (green) and group 2 (yellow) neuraminidase inhibitors and their associated mutations
 - Top panel: Group 2 mutation Arg 292 Lys (Group 1 viruses still have the Tyr₃₄₇ interaction to stabilize)
 - Bottom panel: Group 1 mutation His₂₇₄Tyr disrupts interaction with Tyr₂₅₂



POINTS FOR DISCUSSION

- How would a “cellular based vaccine” work and what types of effects would it have across a population?
- NA Inhibitor resistance mutations often need to be balanced by changes in the HA—change in NA activity requires matching change in HA activity
 - Can restrict the ability of the NA to mutate, but can also make mutations more “cryptic”—if the HA has acquired the necessary changes the NA resistance mutations may actually be favored (likely the case in the previous seasonal H1N1 situation)
- Points for modeling: how early does anti-viral treatment need to be given to stop spread? What is the appropriate use of prophylaxis given symptoms follow the contagious period?

