Immunity to Influenza Infection

Unit 7

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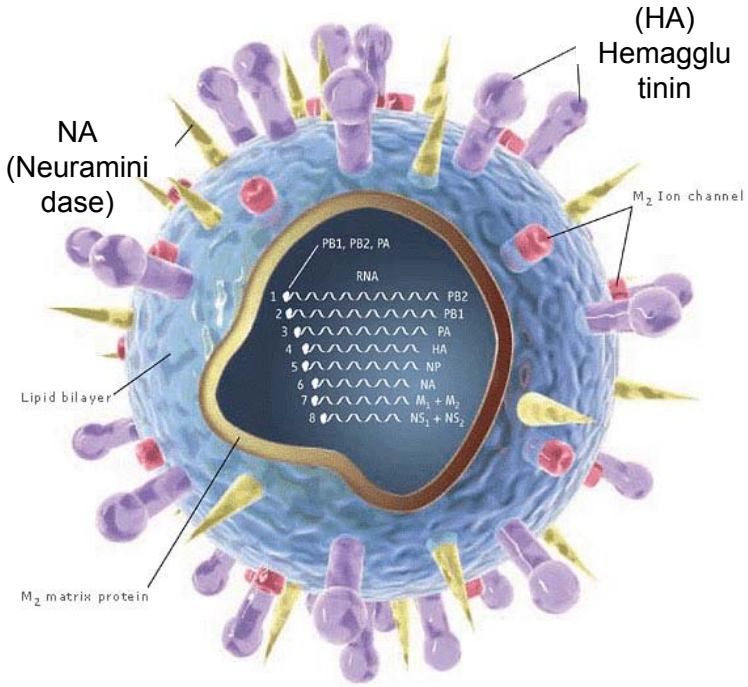
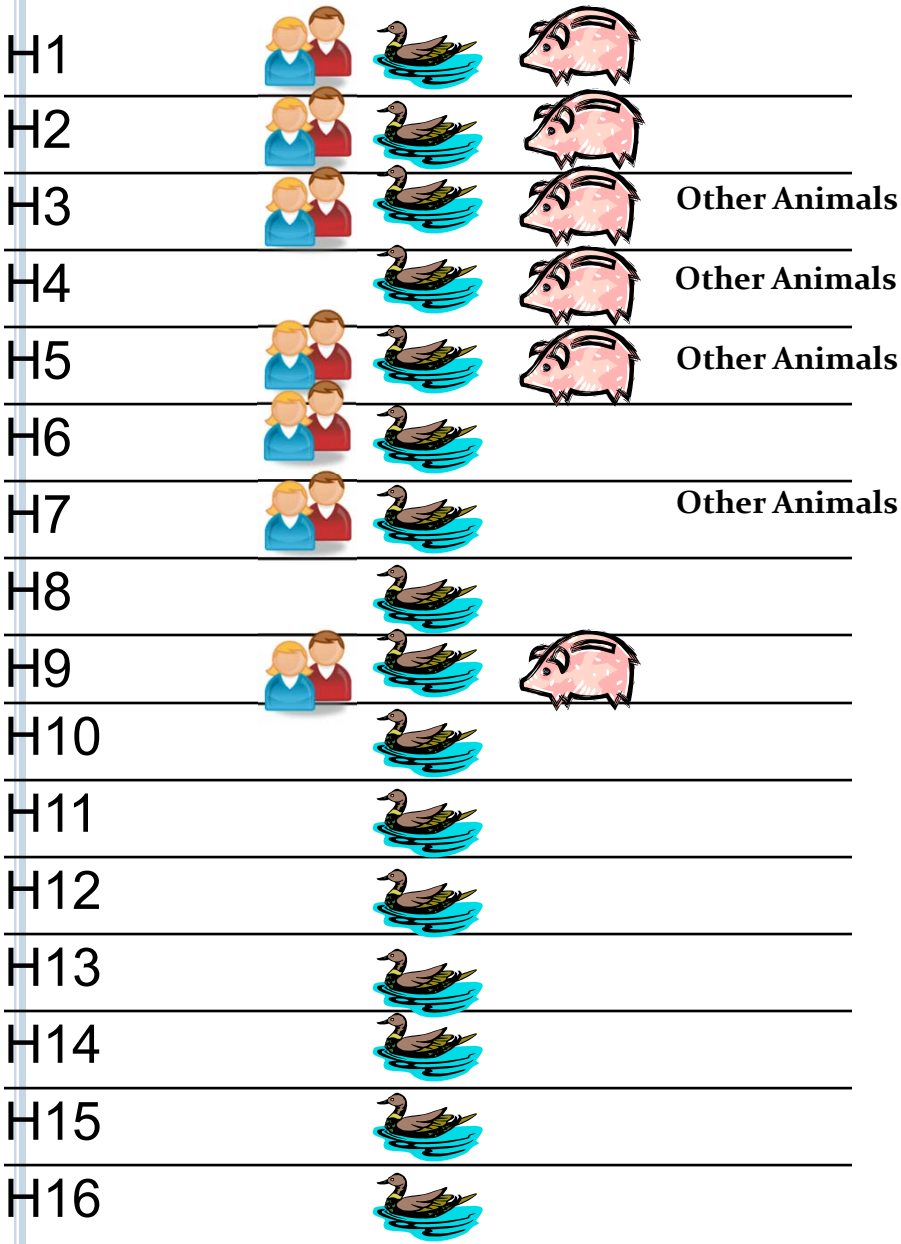
Influenza A Virus

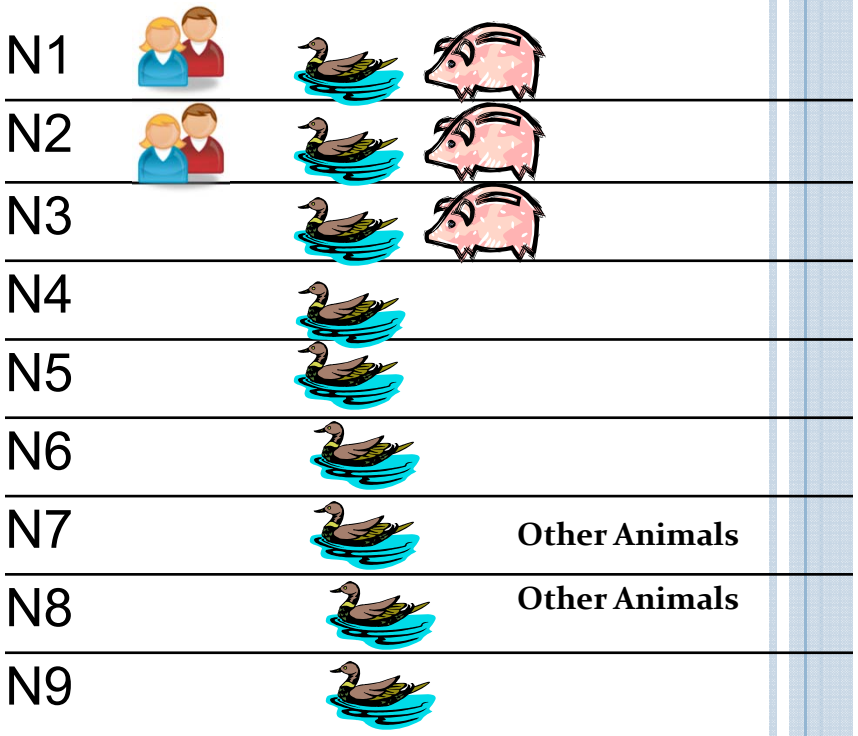
- Negative sense, segmented RNA virus
- *Orthomyxoviridae*
- Eight genes, 11 proteins (three alternate reading frames)
- Two non-structural proteins (NS1 and PB1-F2)
- Surface proteins HA and NA determine serotype



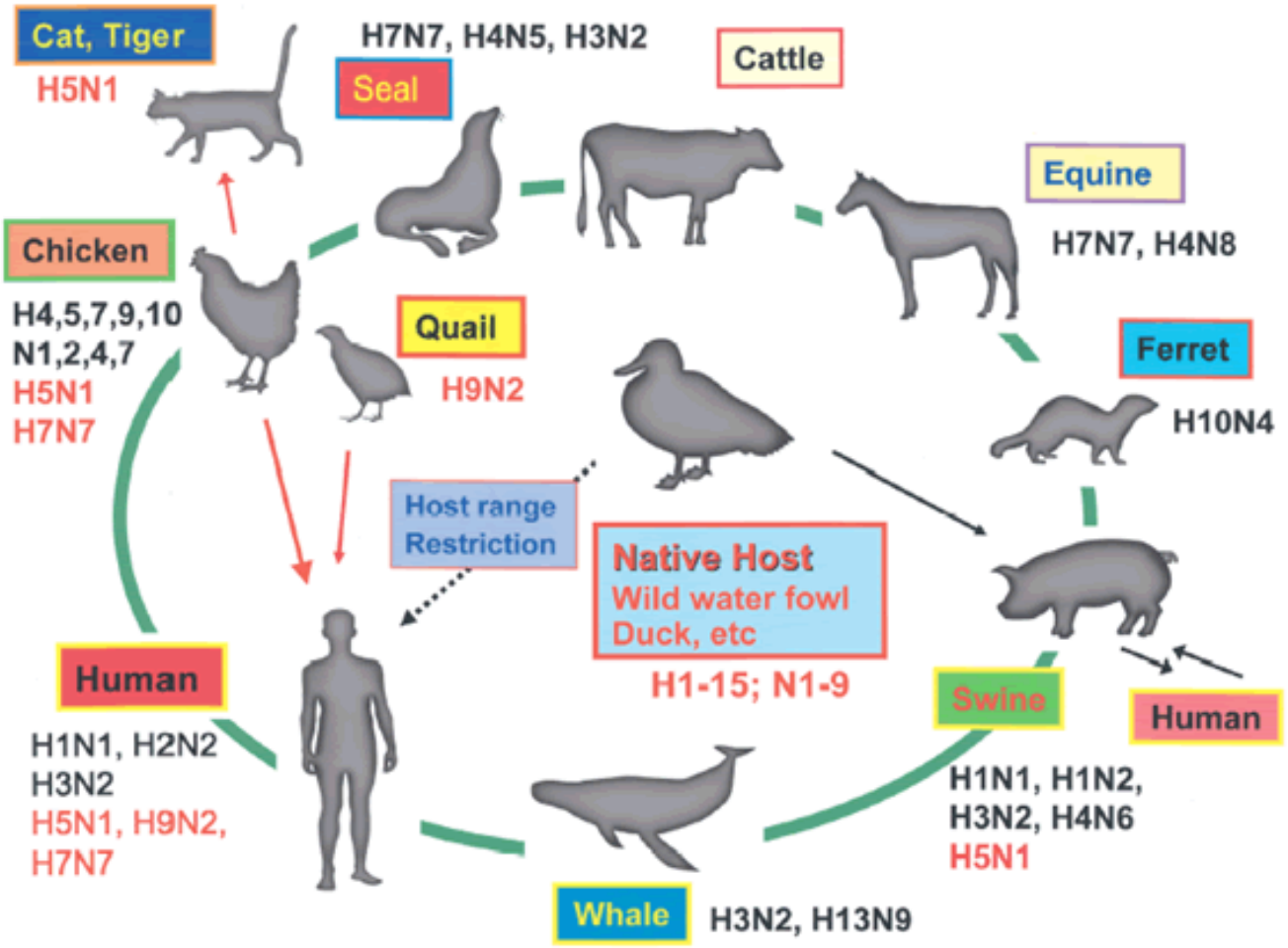
Modified from: Kaiser. *Science* 2006, 312:380-382.

Influenza A HA and NA Subtypes

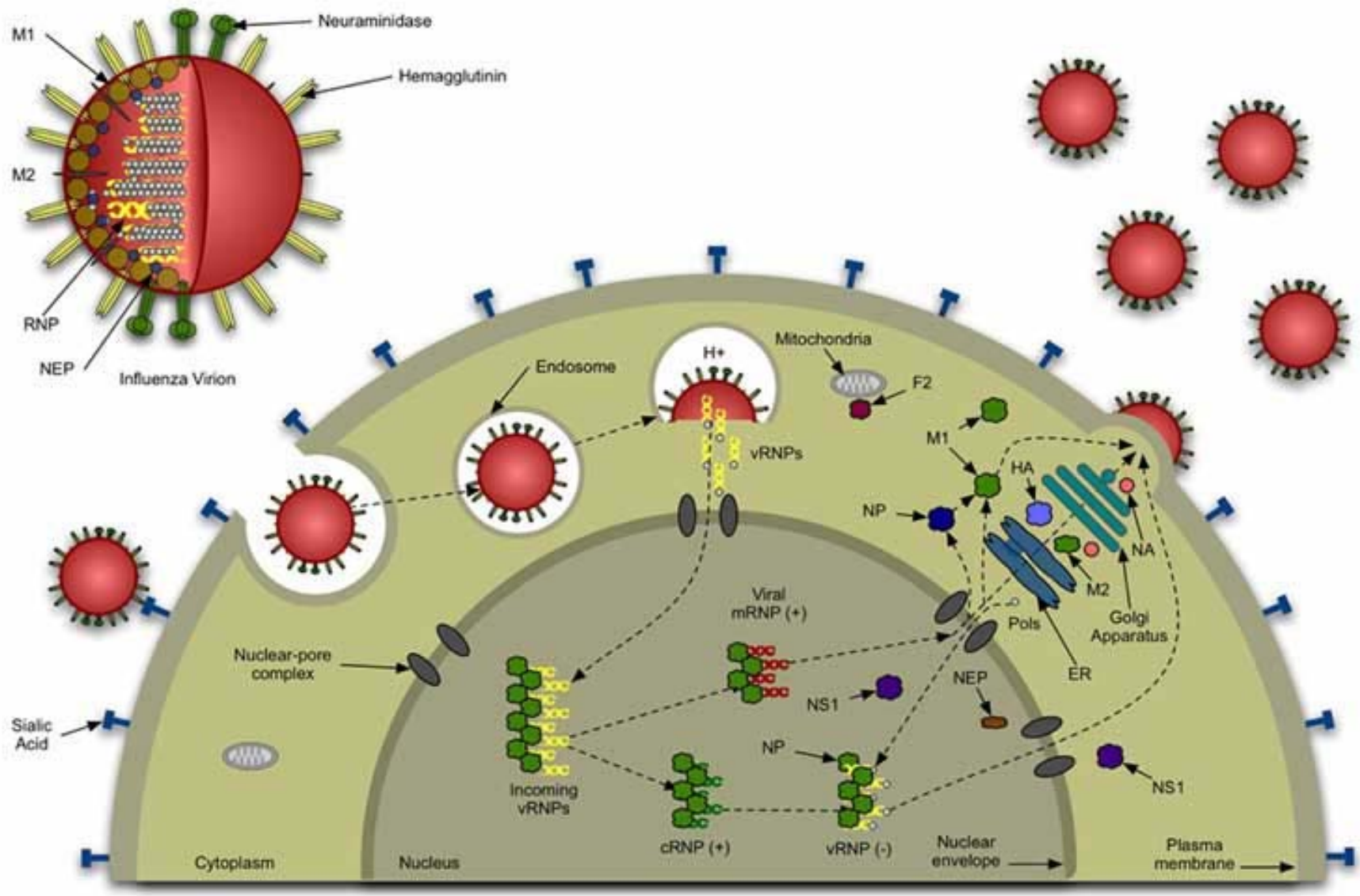




Diverse Host tropism allows restriction and recombination

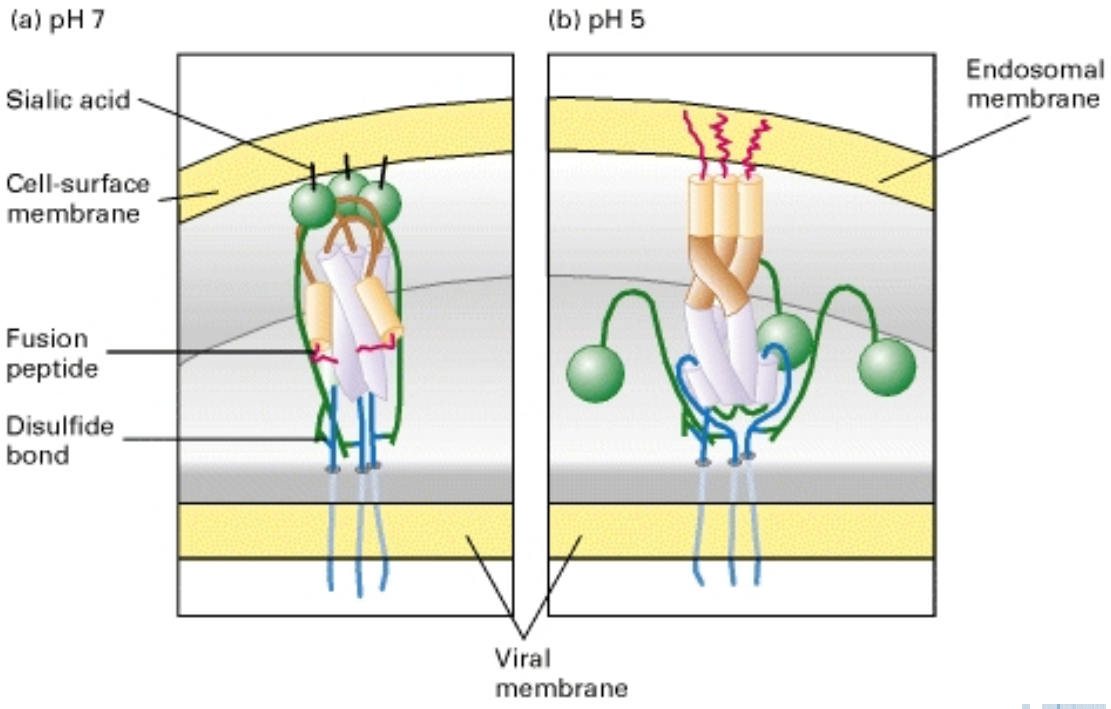


INFLUENZA LIFE CYCLE



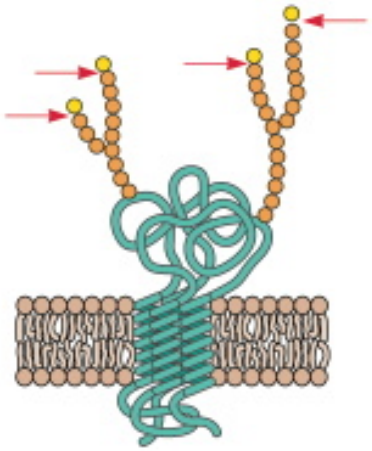
HA is required for Cell Entry

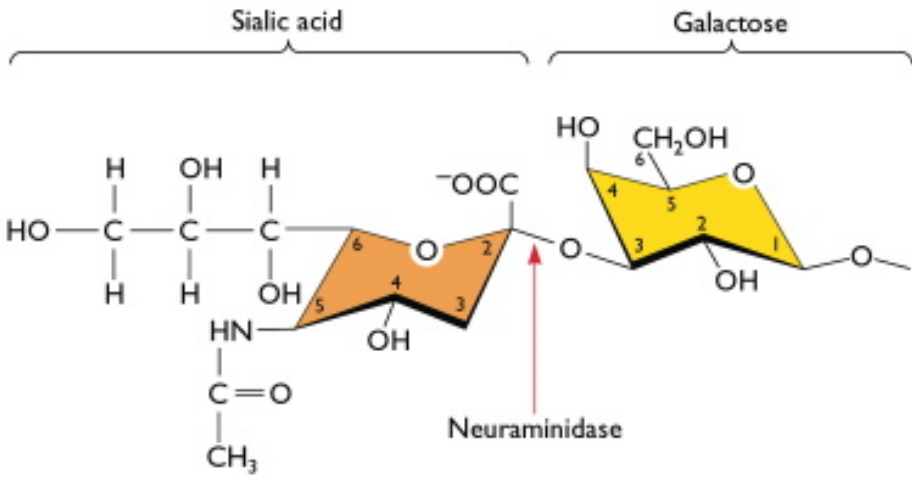
- HA binding to sialic acid on the surface of cells mediates initial attachment
- Virus is endocytosed, where the endosome is acidified
- This triggers a conformational change in the virus, resulting in membrane fusion
- For HA to be active, it needs to be cleaved by a protease into two pieces—this protease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



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

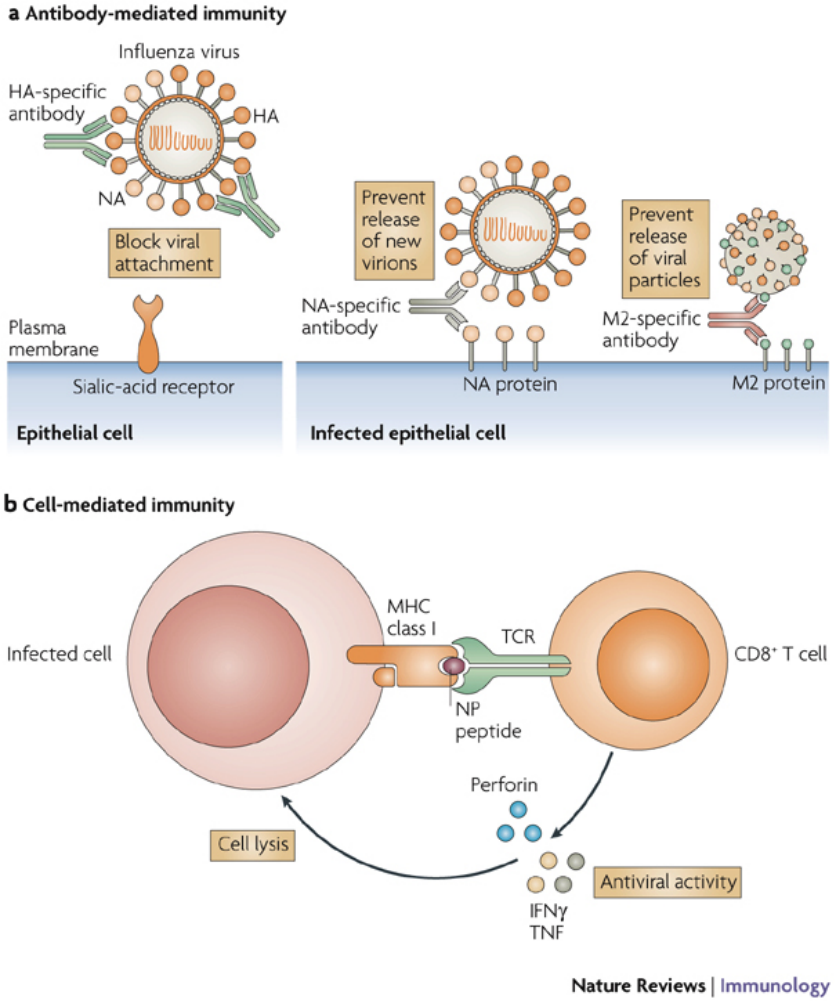
- IAV must balance the binding and entry activity of HA with the sialic acid cleavage activity of NA so that virus efficiently enters and buds from the cell surface—thus HA and NA are often "matched" for activity





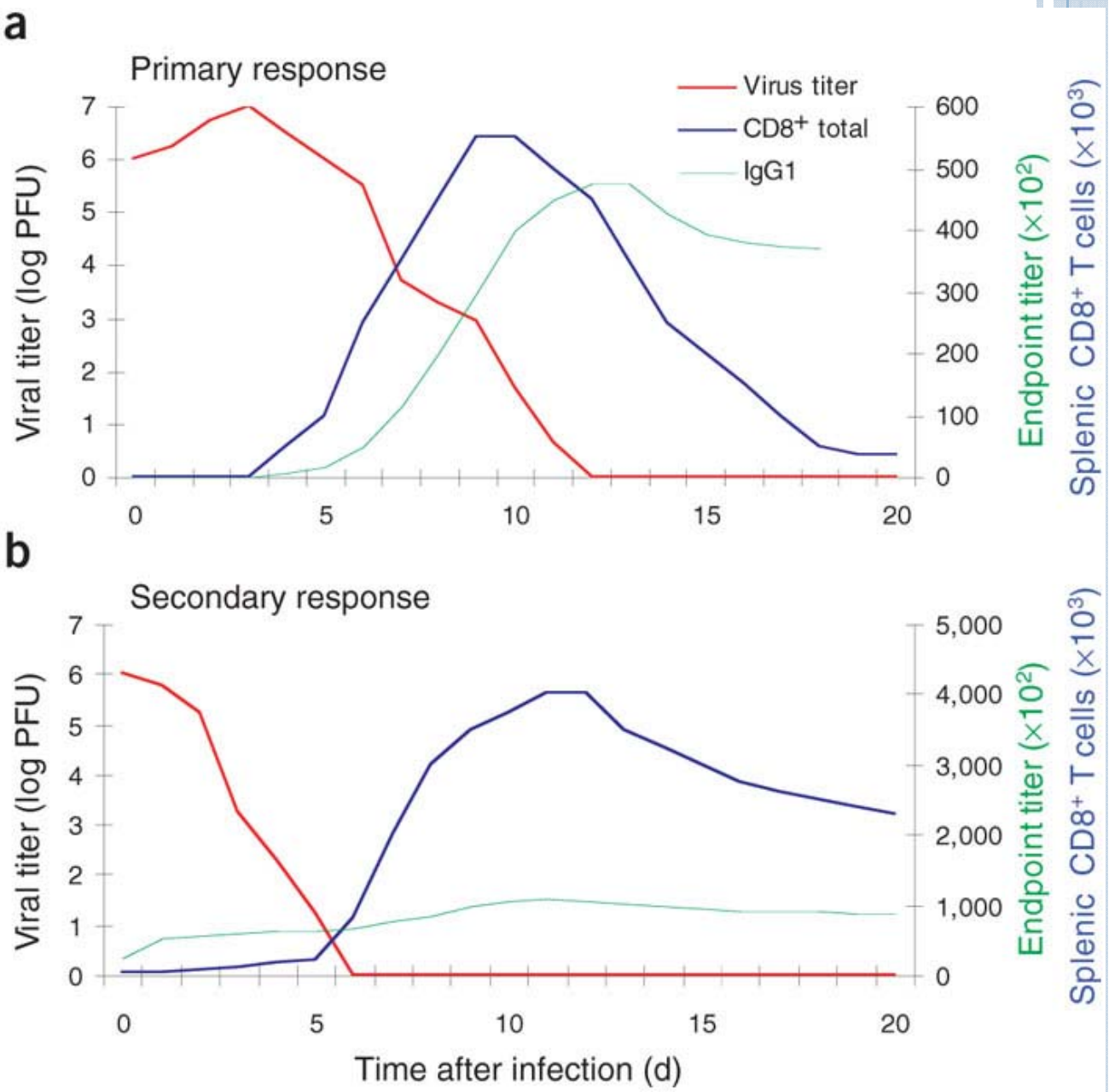
Immune Mechanisms of Protection

- 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

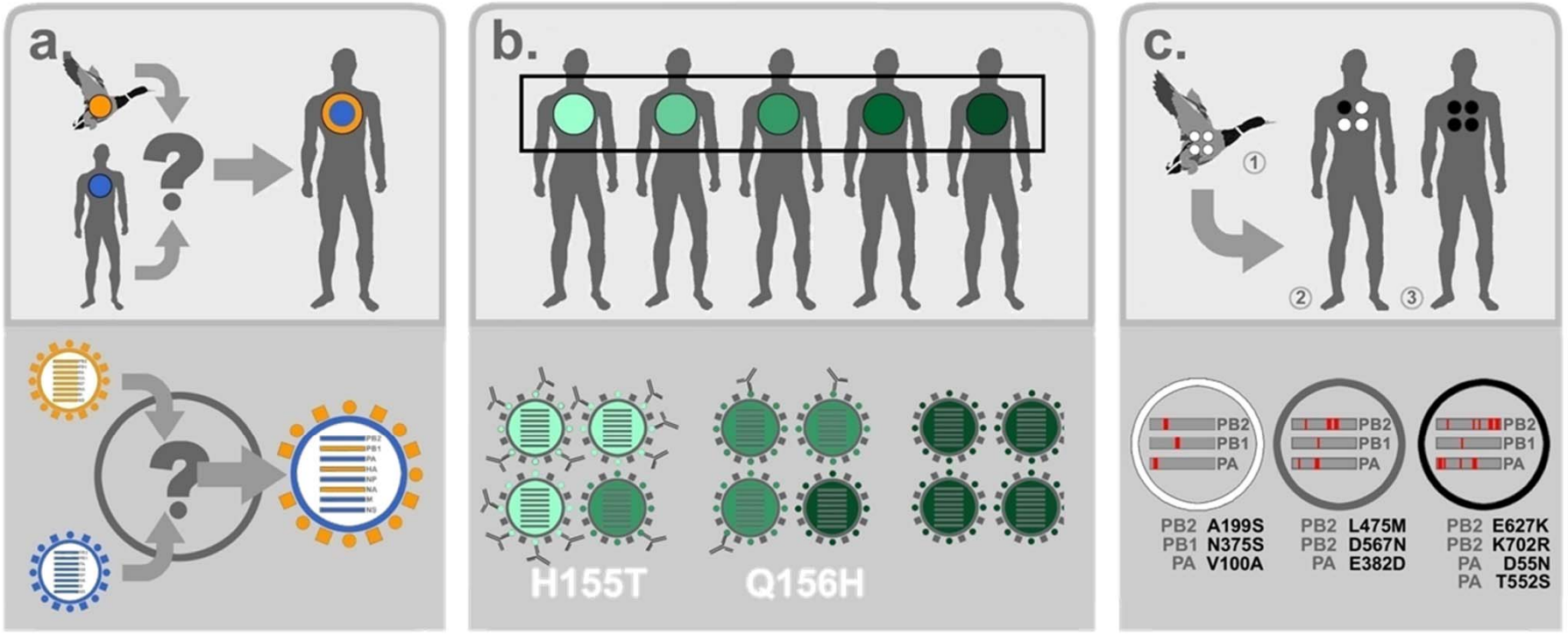


IMMUNE COURSE OF INFLUENZA INFECTION

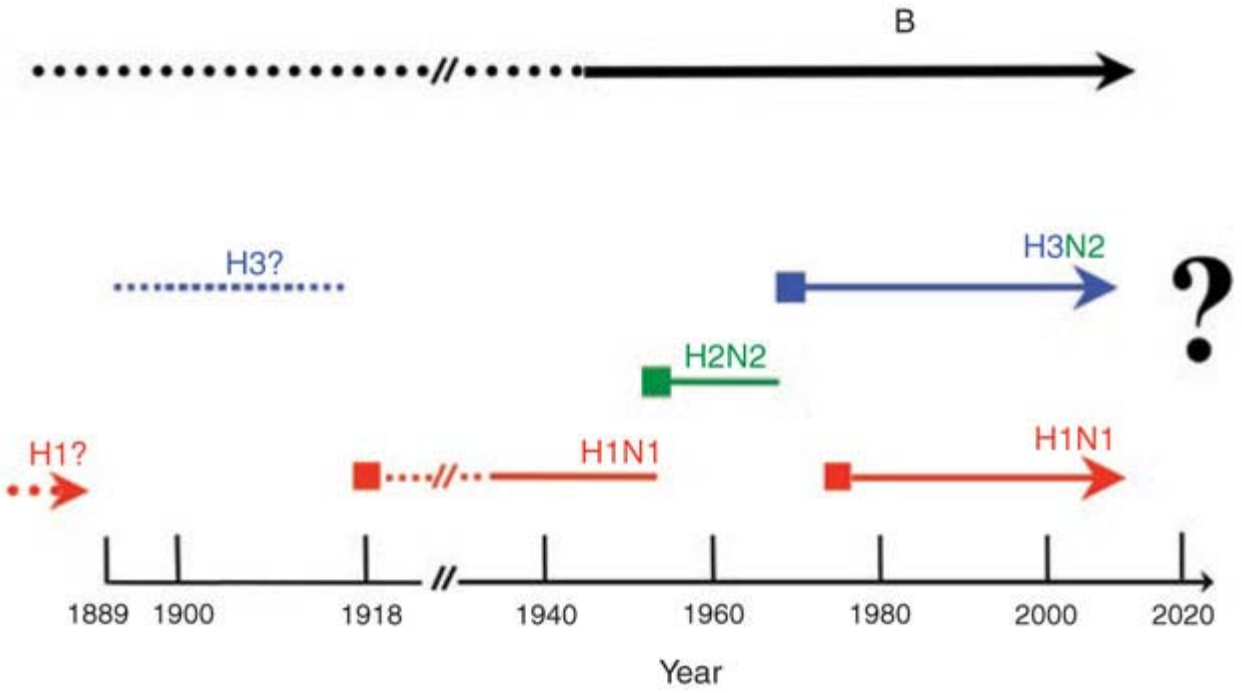
- Influenza is initially controlled by antibody and CD8+ T cells
- Secondary infection with heterologous virus is cleared with CD8+ T cell activity much more rapidly
- Homologous infection can be prevented by antibody (sterilizing immunity)



INFLUENZA EVOLUTION

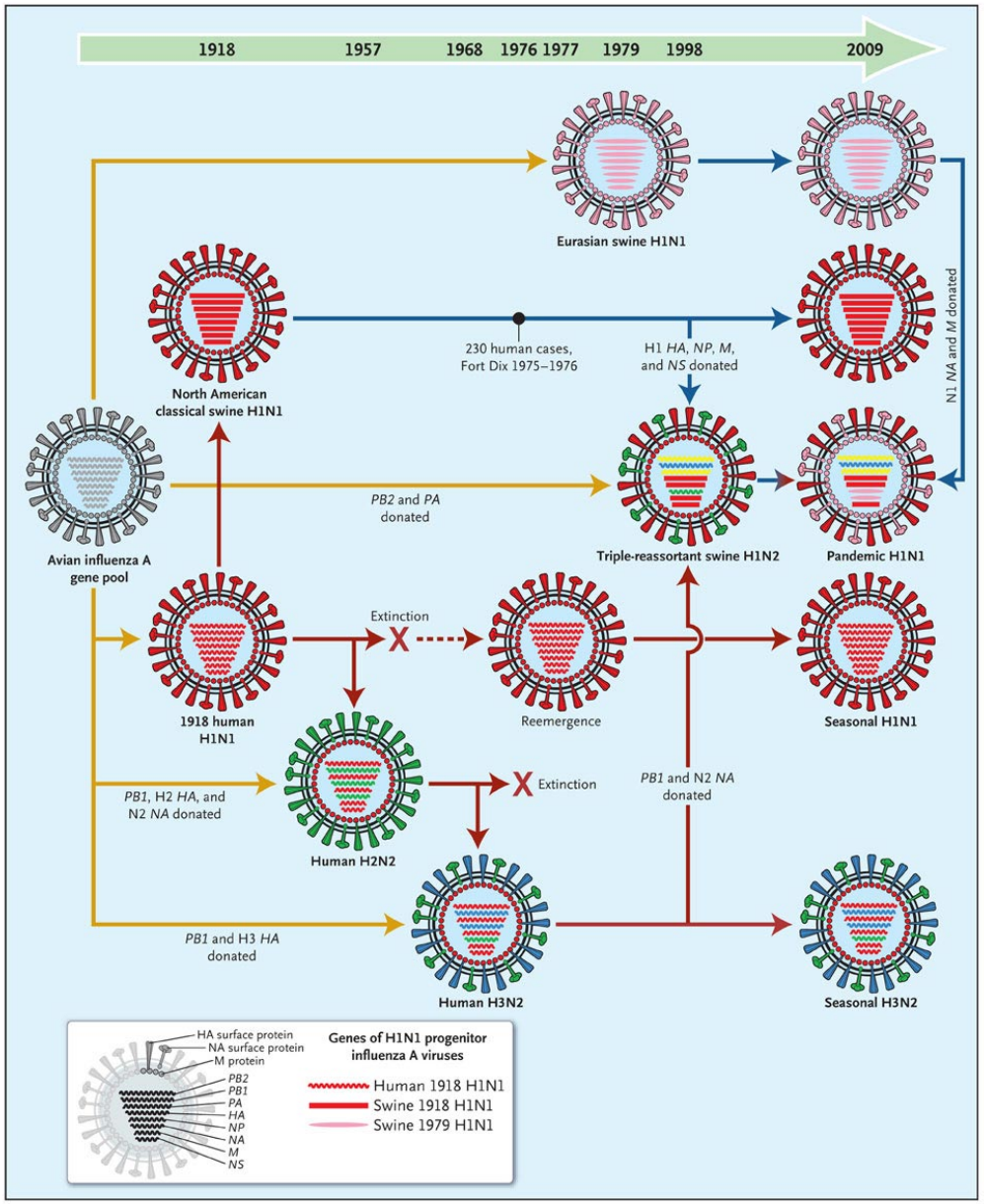


HUMAN INFLUENZA PANDEMICS

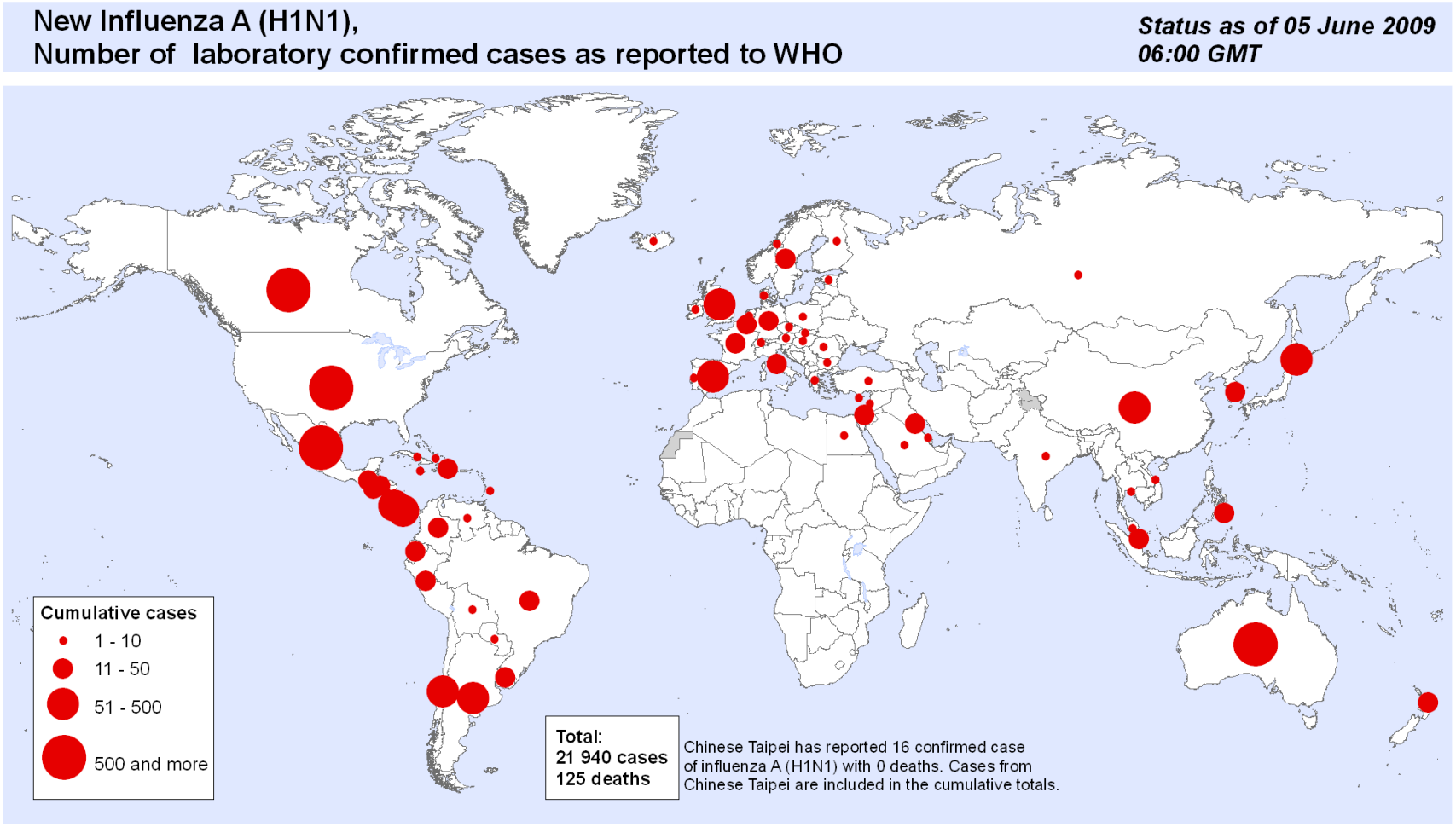


Evolution of Human Influenza from 1918

- 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



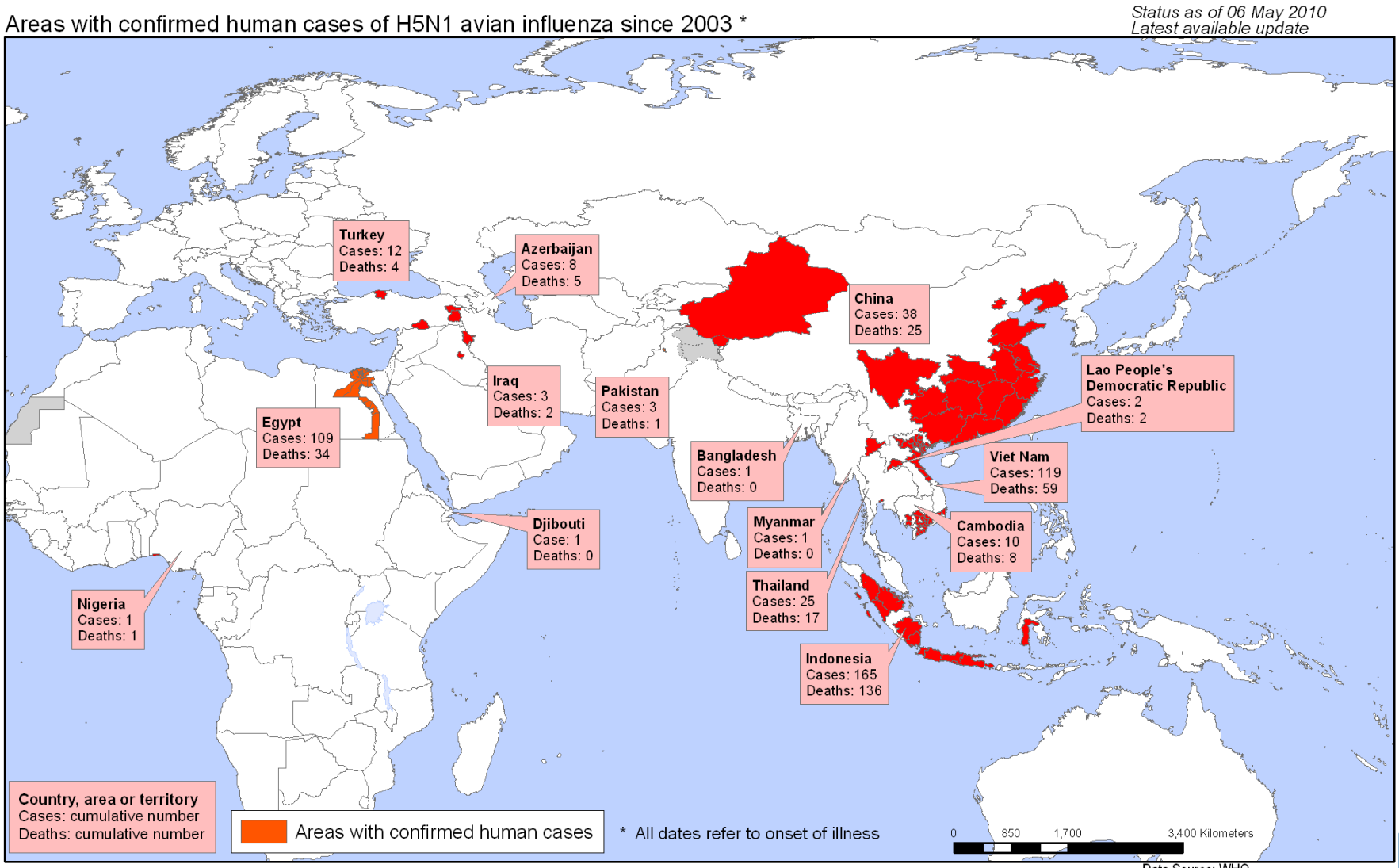
The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers 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

World Health Organization

H5N1 Incidence



World Health Organization

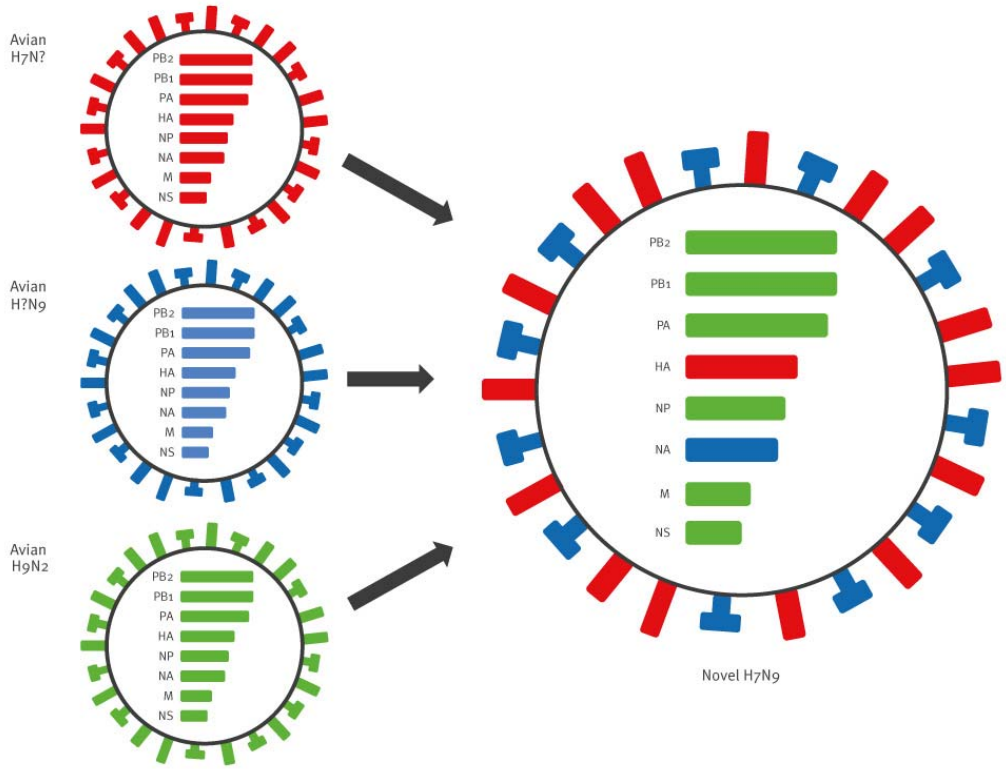
Data Source: WHO
Map Production: Public Health Information and Geographic Information System (GIS)
World Health Organization

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

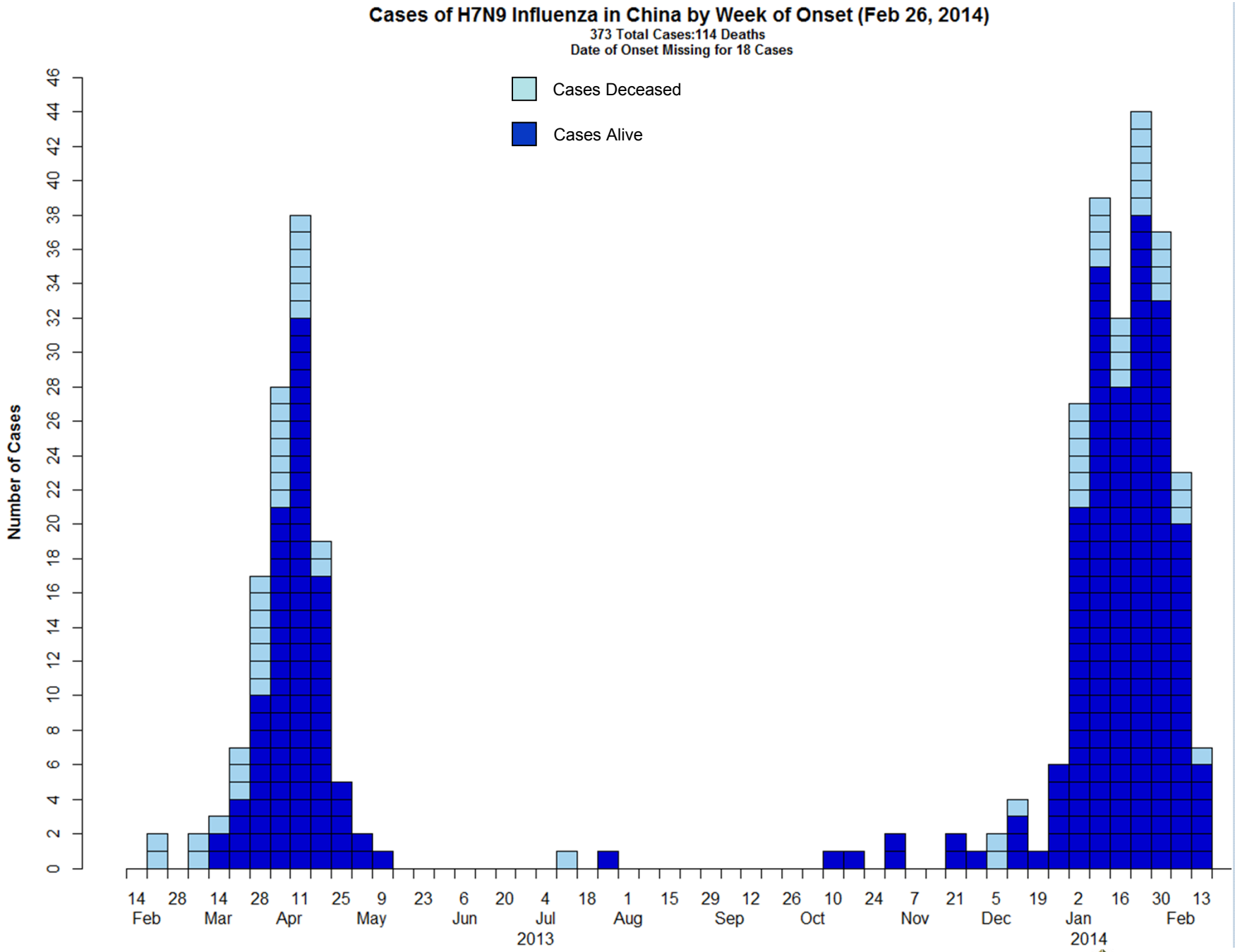
FIGURE 3

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.



Note: Total cases include an asymptomatically infected child in Beijing

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

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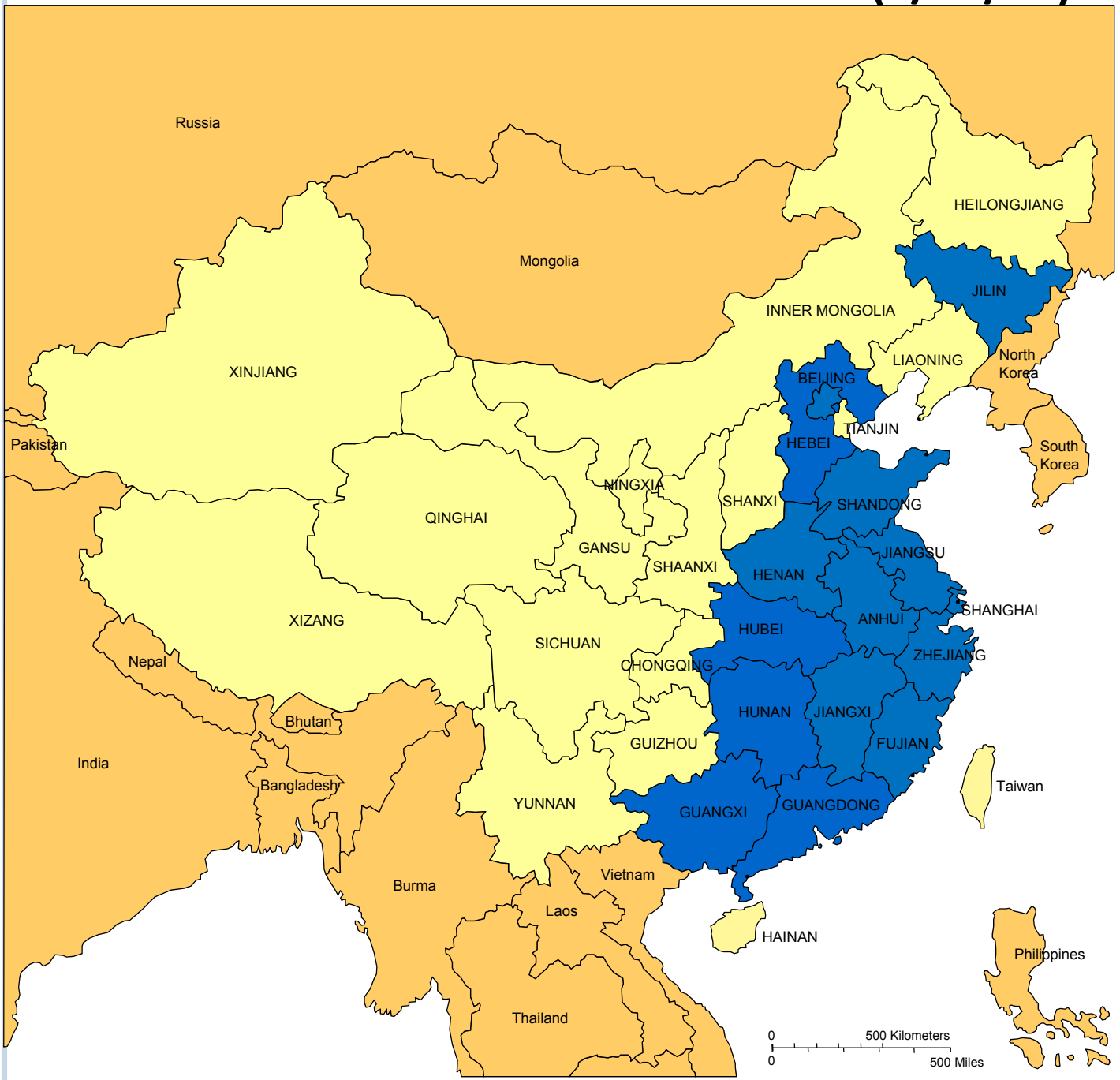
Location of H7N9 Influenza in China (2/26/14)*



*373 total cases/114 deaths

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

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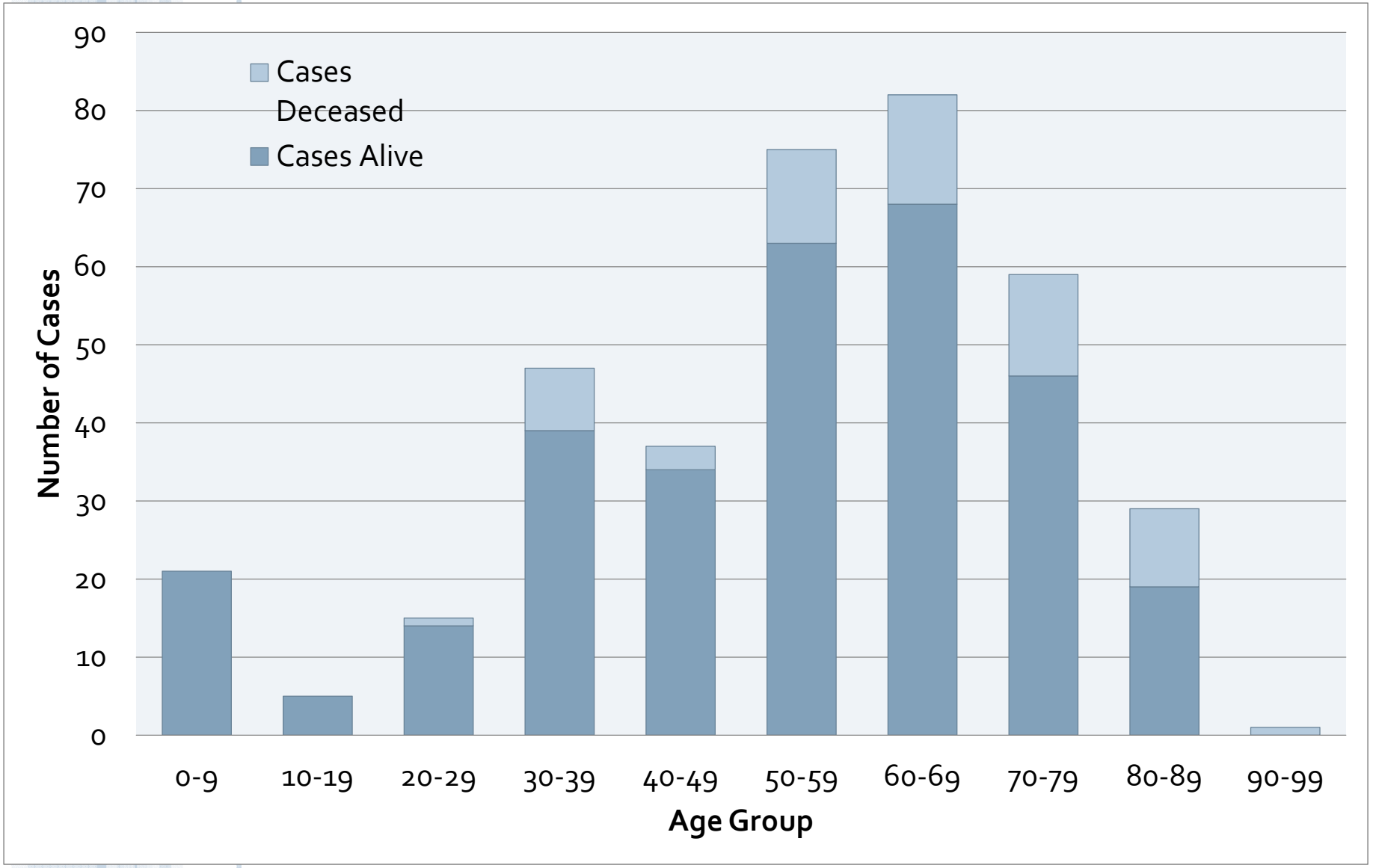
Cases of H7N9 Influenza in China by Age-Group (2/26/14)*



*Total cases = 373

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

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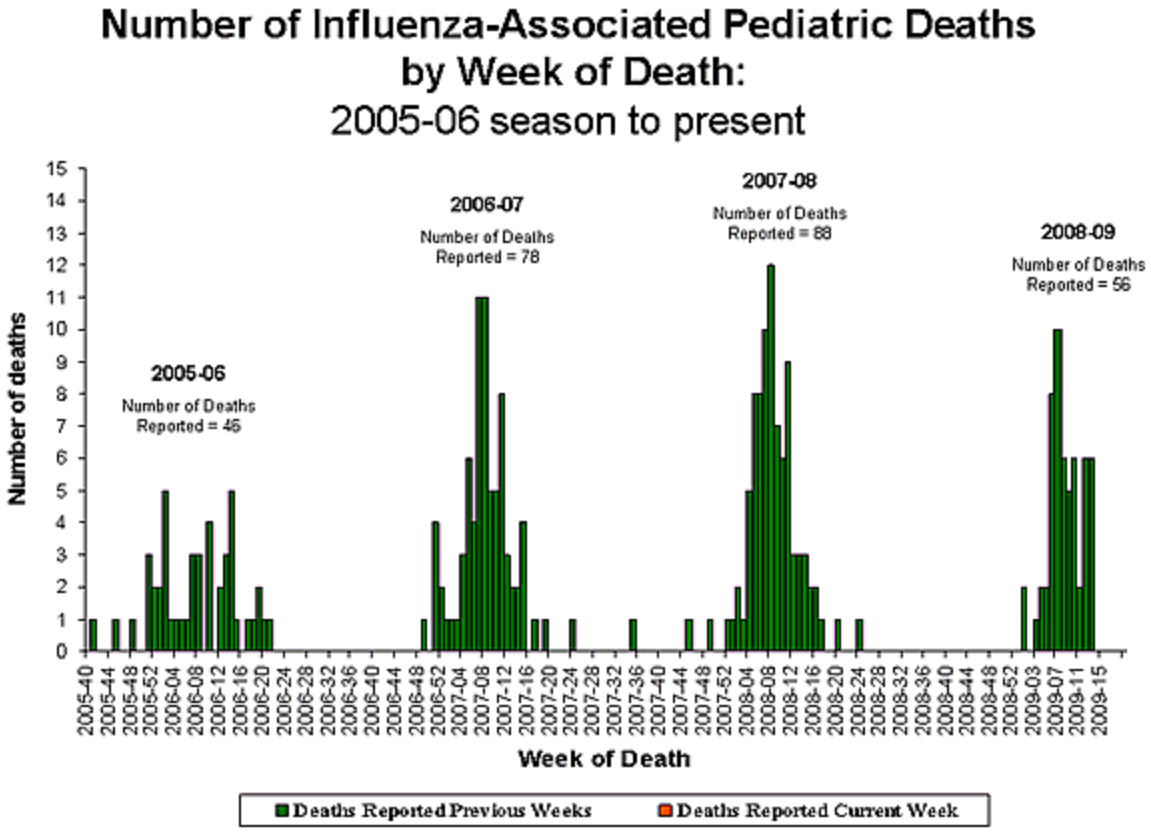
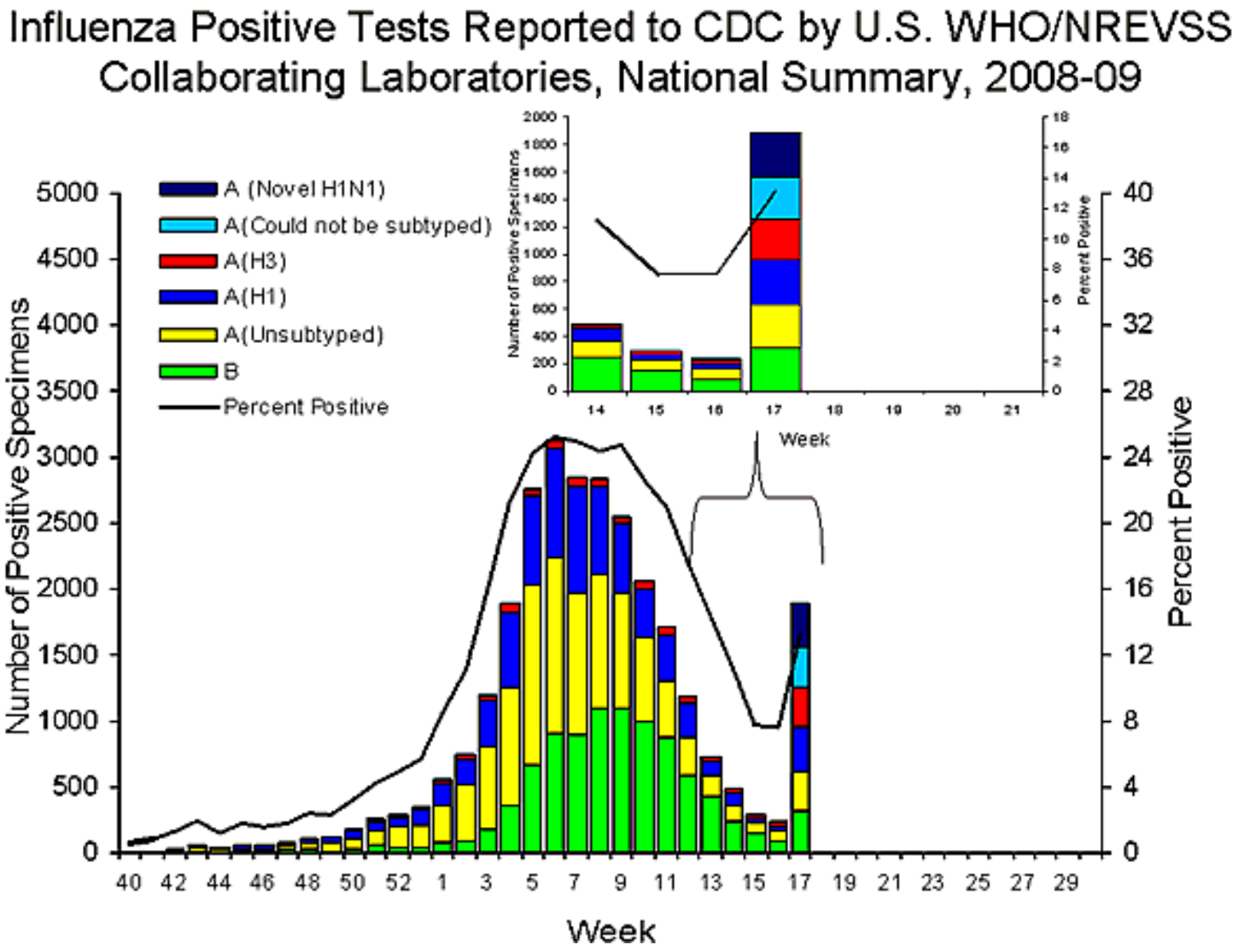
ANNUAL SEASONAL INCIDENCE, U.S.



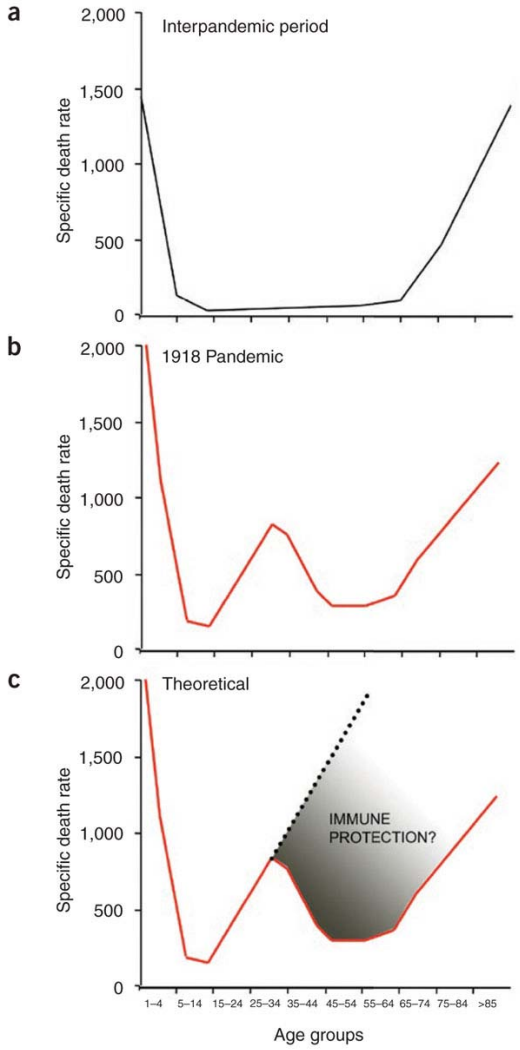
Annual number of deaths within the U.S. attributable to influenza: 41,400 (as of 2004)

Emergence of the 2009 Pandemic



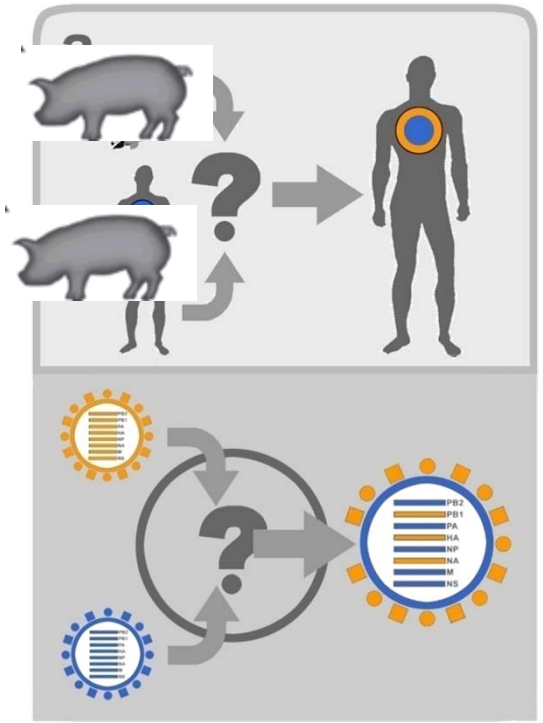
1918 (AND POSSIBLY SWORH1N1) MORTALITY CURVES SUGGEST PREVIOUS EXPOSURE

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



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)



Pre-Exisitng Cross-reactive Immunity to 2009/H1N1

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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.

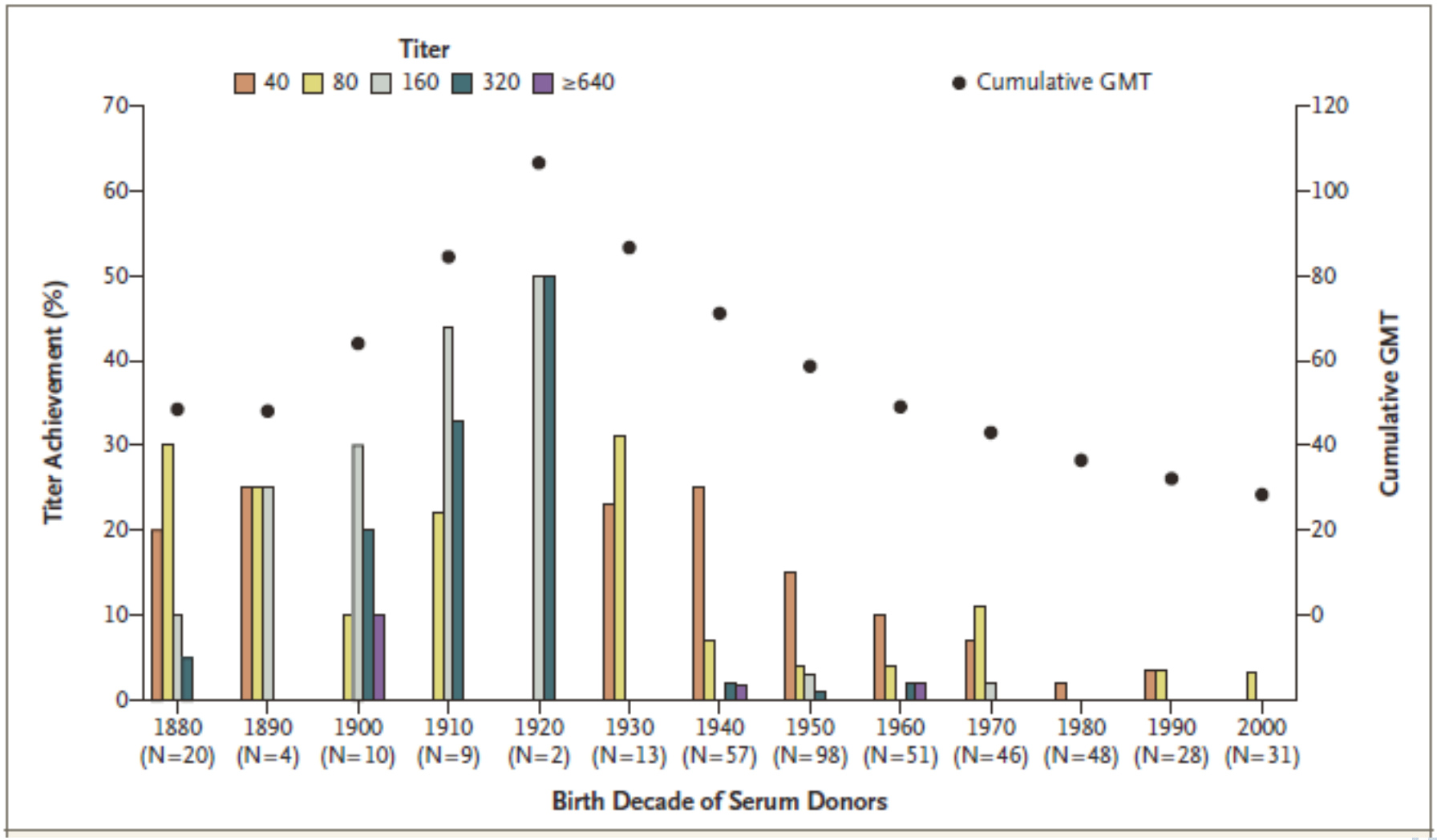
Table 1. Cross-Reactive Microneutralization Antibody Response against Pandemic Influenza A (H1N1) Virus in Pediatric and Adult Recipients of Seasonal Trivalent Inactivated Influenza Vaccines.*

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



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

	Estimated no. case-patients		Estimated rate/100,000*	
Parameter	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	–	–

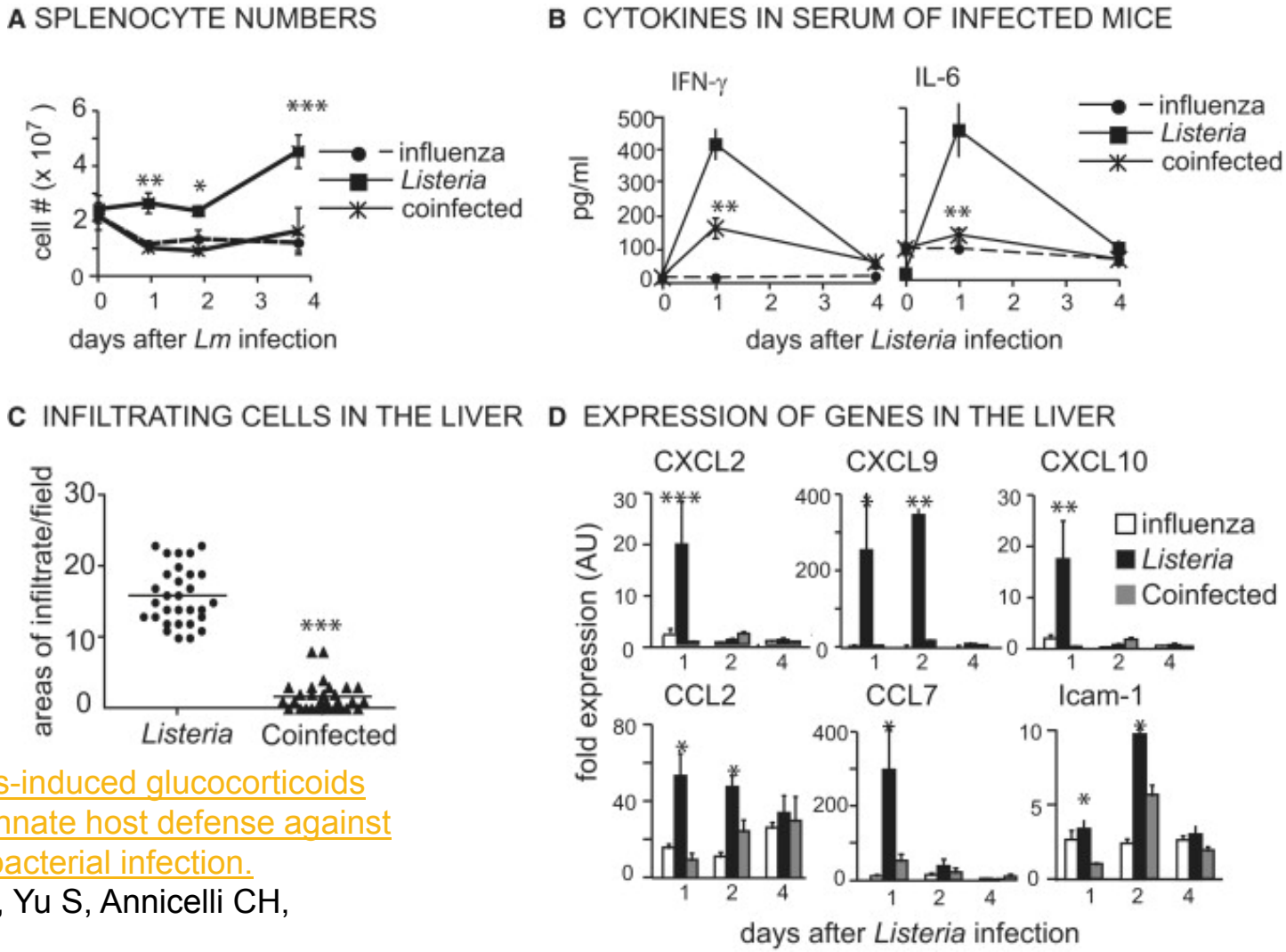
*United States Population Estimates, 2009.
†Age distributions from line list and aggregate reports of laboratory-confirmed cases and hospitalizations to the Centers for Disease Control and Prevention through July 23, 3009.

Reed C, Angulo FJ, Swerdlow DL, Lipsitch M, Meltzer MI, Jernigan D, et al. Estimates of the prevalence of pandemic (H1N1) 2009, United States, April–July 2009. *Emerg Infect Dis.* **15** (12): 2004-7.

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

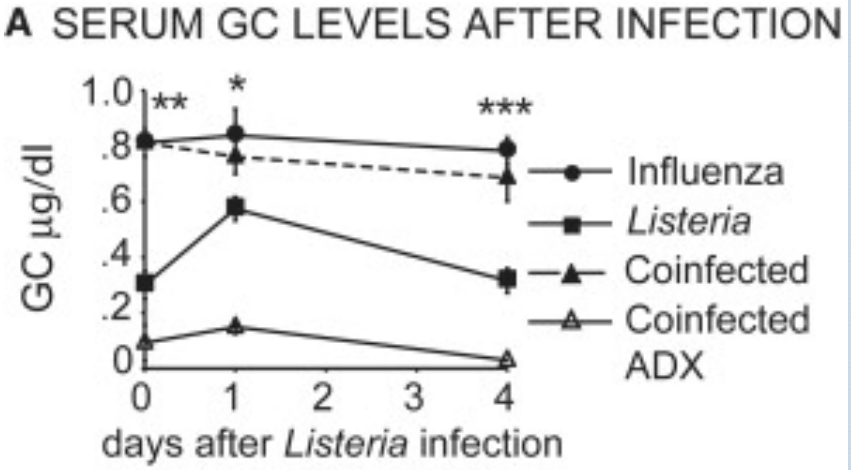
Influenza induced Glucocorticoid mediated suppression?

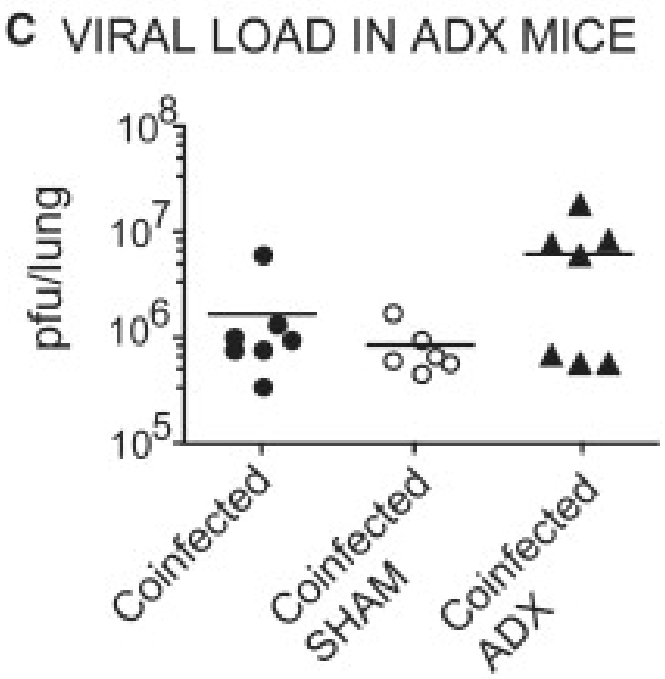


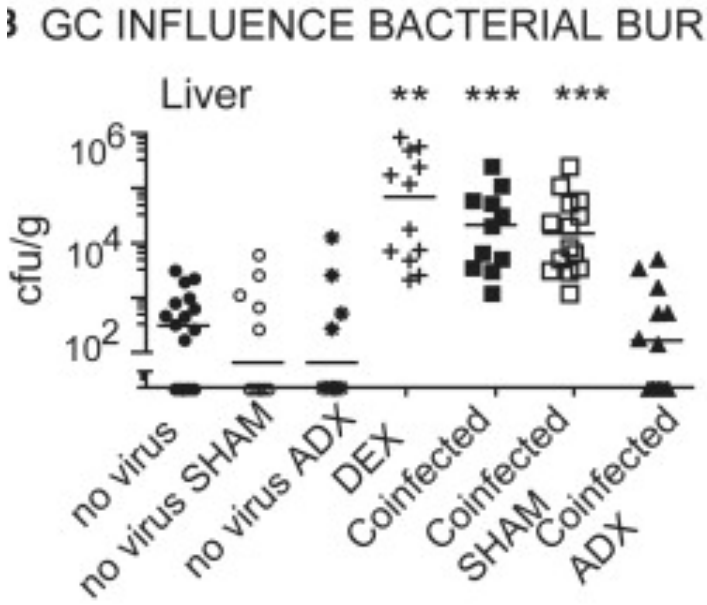
Influenza virus-induced glucocorticoids compromise innate host defense against a secondary bacterial infection.
Jamieson AM, Yu S, Annicelli CH, Medzhitov R.
Cell Host Microbe. 2010 Feb 18;7(2):103-14.

Glucocorticoid mediated Bacterial susceptibility

Influenza virus-induced glucocorticoids compromise innate host defense against a secondary bacterial infection.
Jamieson AM, Yu S, Annicelli CH, Medzhitov R.
Cell Host Microbe. 2010 Feb 18;7(2):103-14.

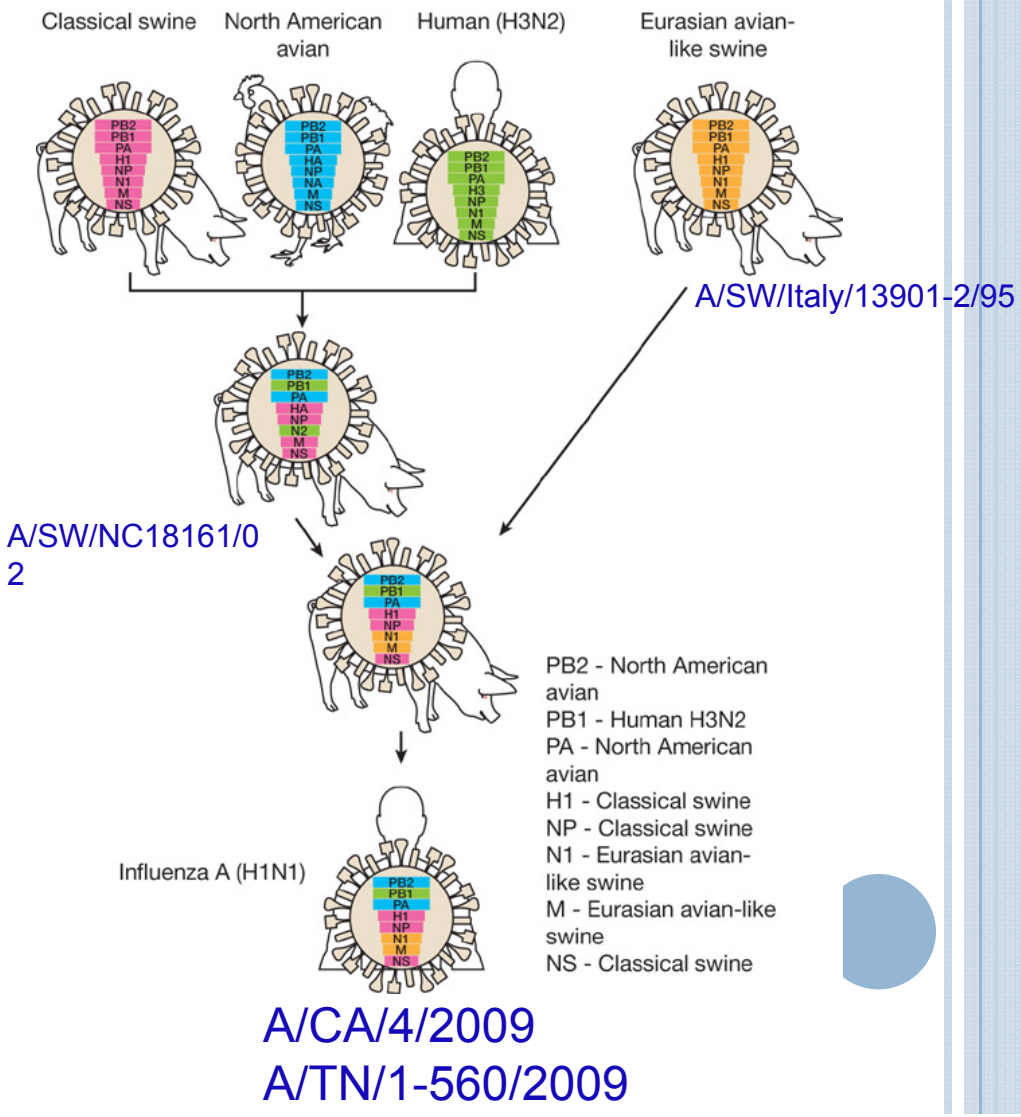




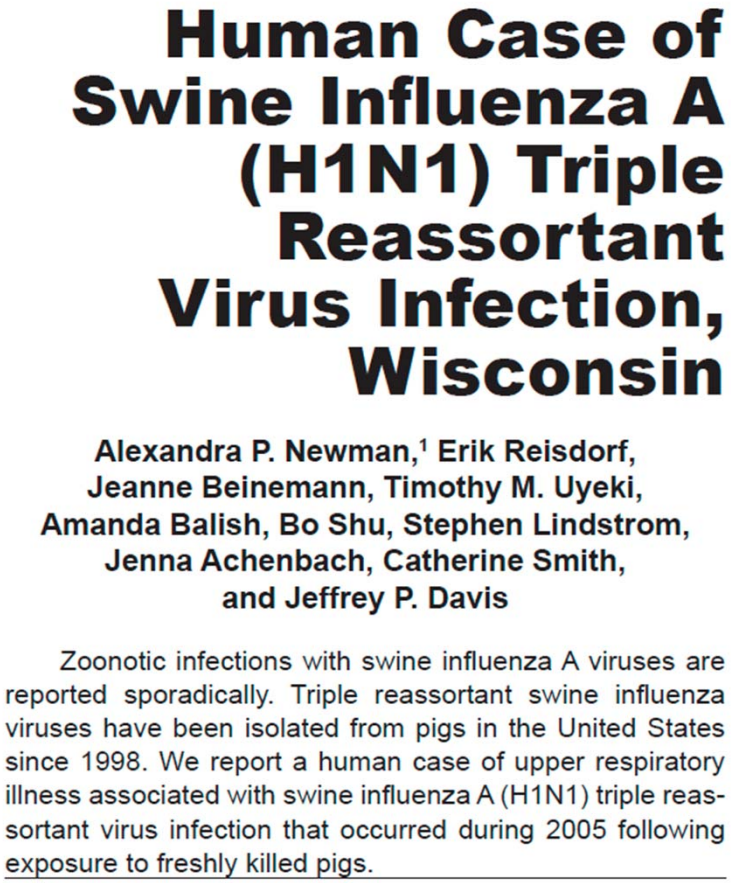


2009 PANDEMIC H1N1

- 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)



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.

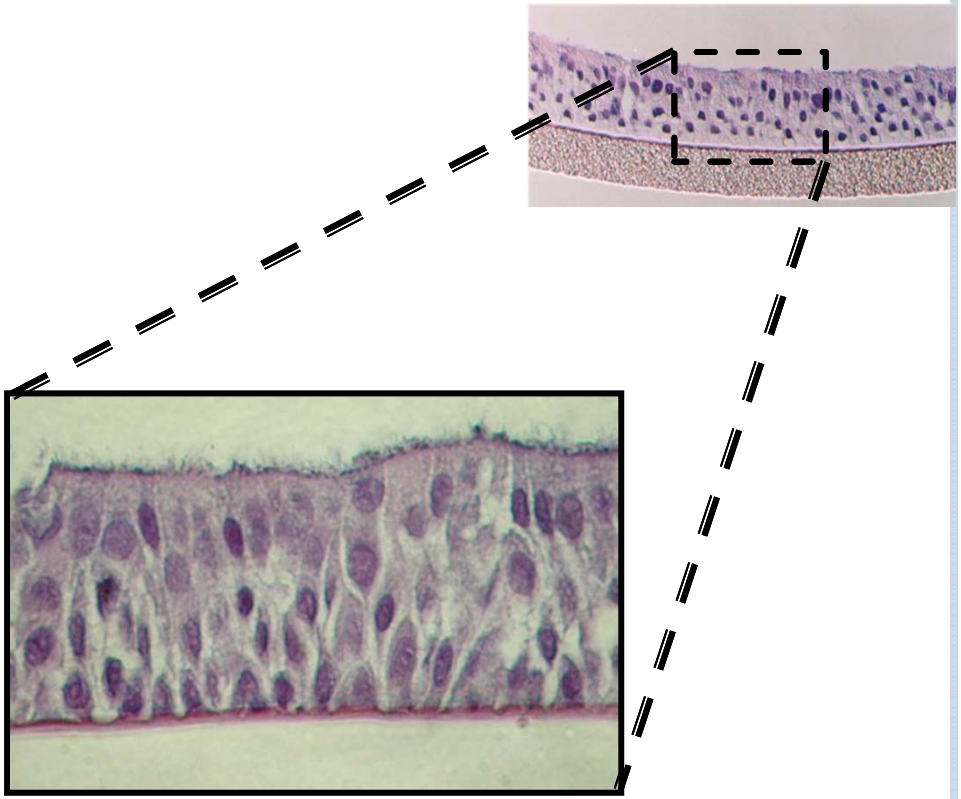
- "… custom slaughter house …"
- "… helped hold and abduct the forelimbs of one (1) freshly killed pig while his brother eviscerated it."
- "No facial or respiratory protection was worn …"
- "… father obtained a live chicken…"
- "…sacrificed during a ritual ceremony."
- "… patient was never within ten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

Newman *et al.*, *Emerging Infectious Diseases*, 2008

H1N1 Swine Flu studies: response in human cells

Measures:

- Infectivity and growth of virus ($TCID_{50}$, 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

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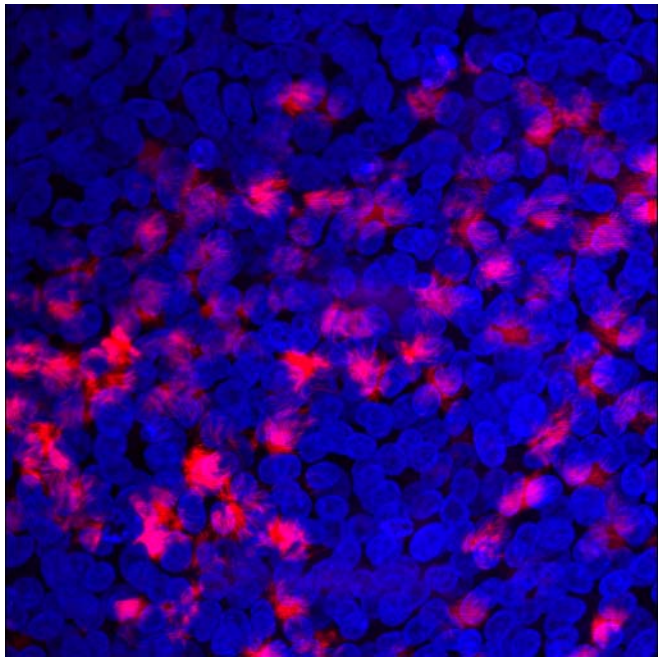
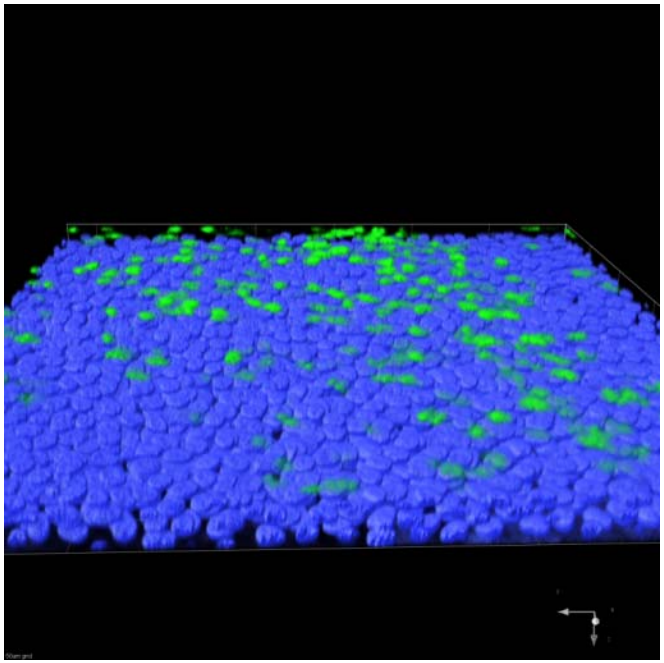
In vitro cultures of Human airway epithelial cells

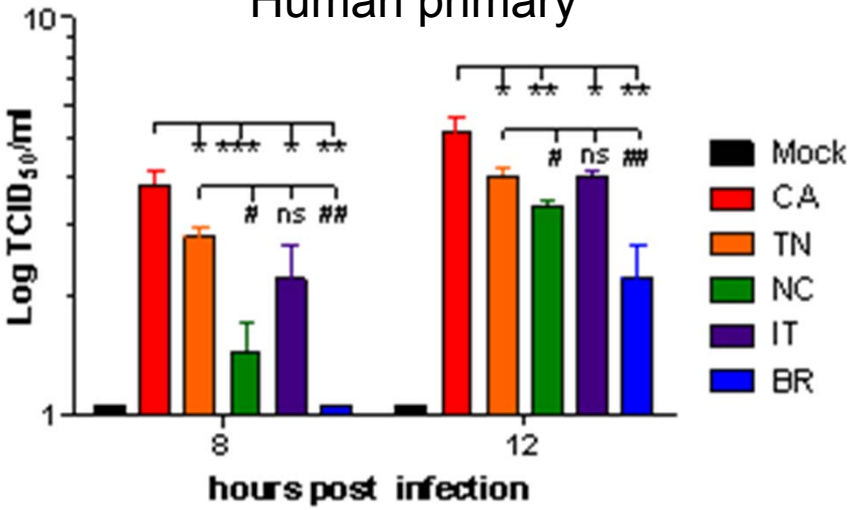




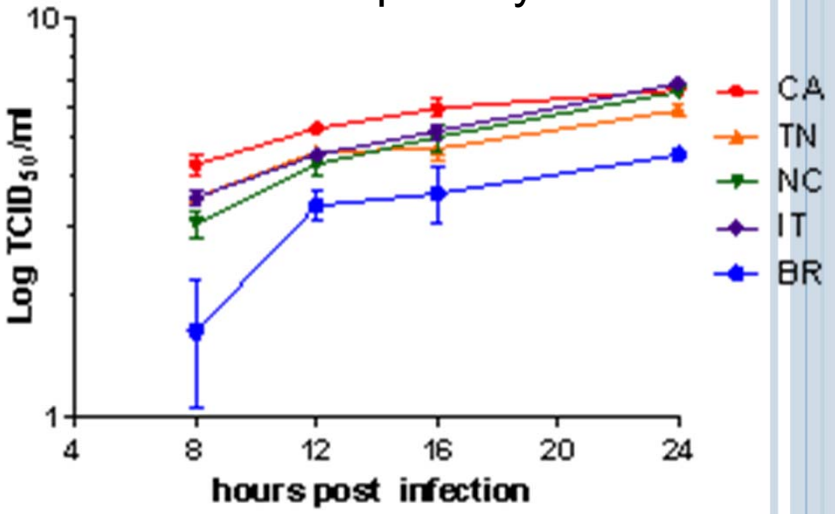
cilia-red/green
Nucleus-blue

VIRAL GROWTH KINETICS IN HAE CELLS

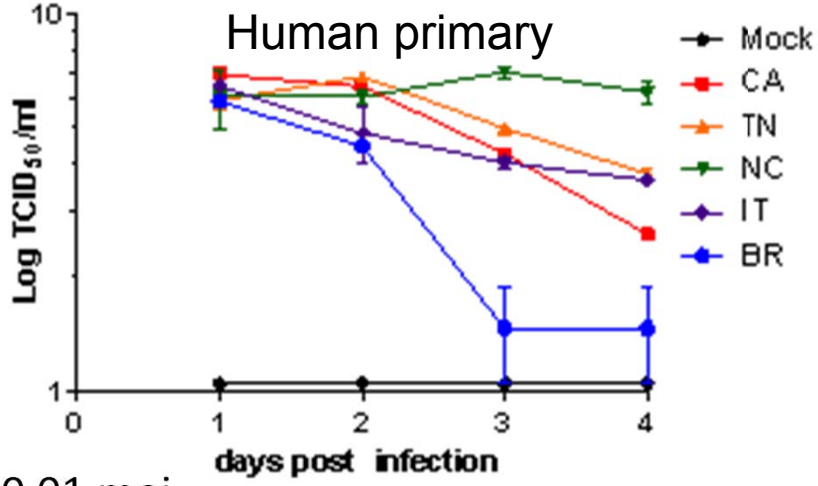
Human primary



Swine primary



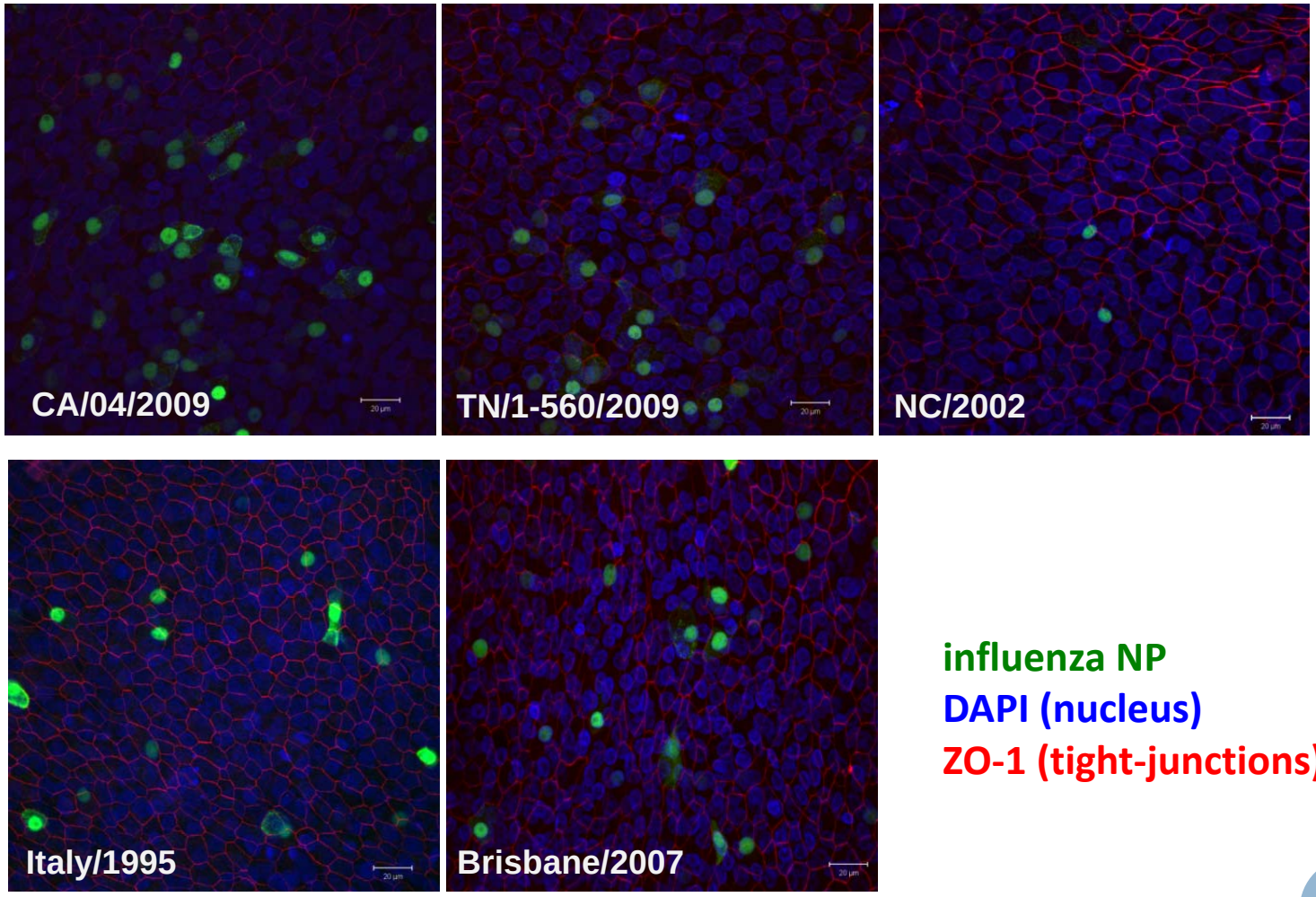
Human primary



0.01 moi

All continued shedding from healthy monolayers for >3 weeks

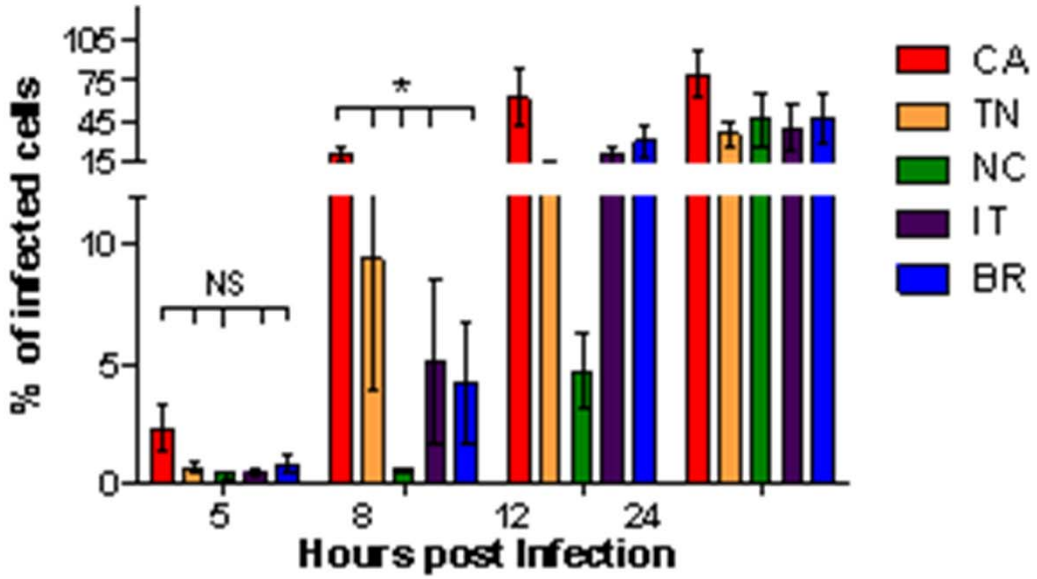
Influenza NP detection in 3D HAE cultures viral growth kinetics in HAE cells



influenza NP
DAPI (nucleus)
ZO-1 (tight-junctions)

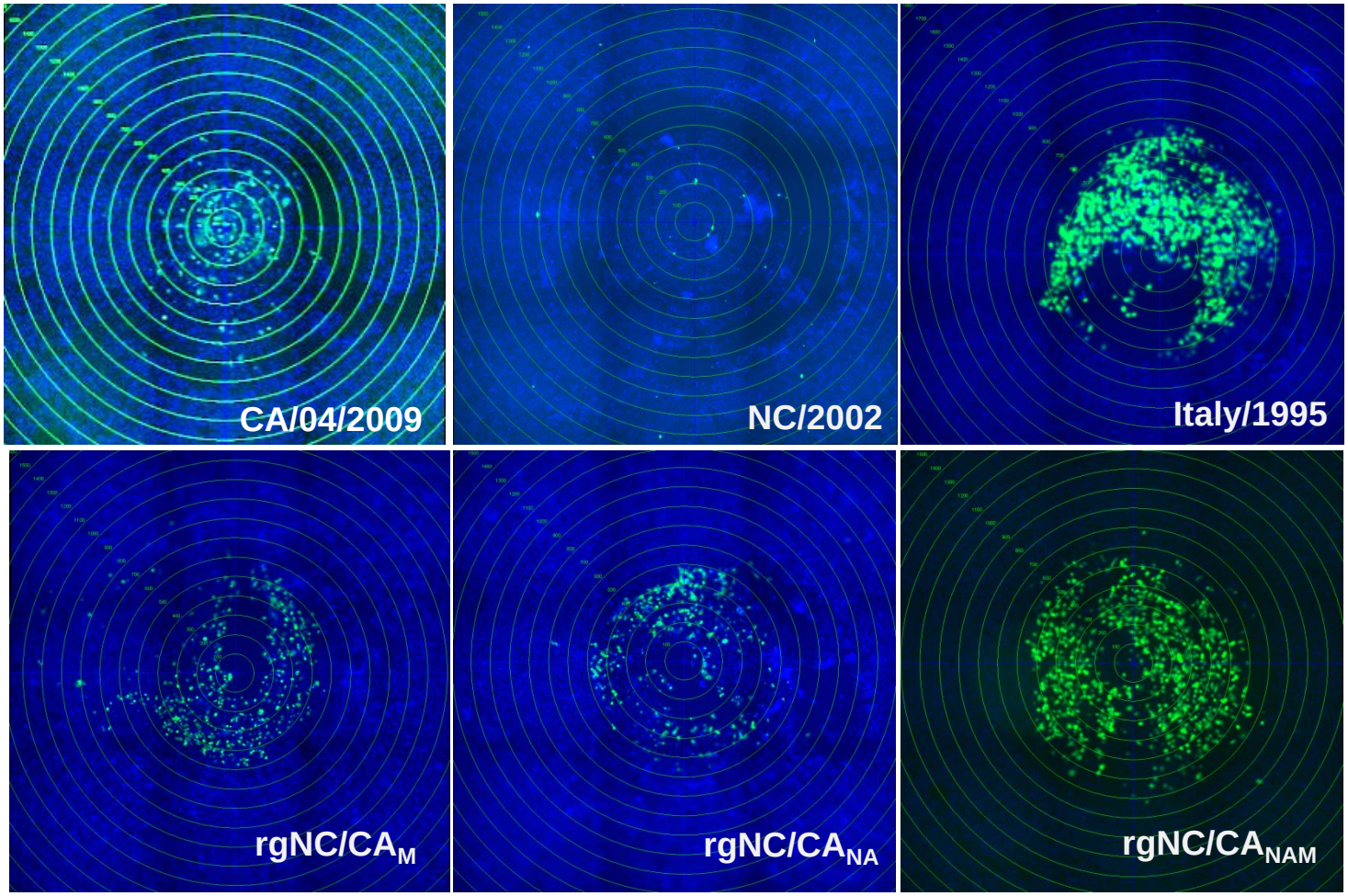
8 hr post infection- 0.01 moi

More rapid colonization of culture by Pandemic and Esw virus



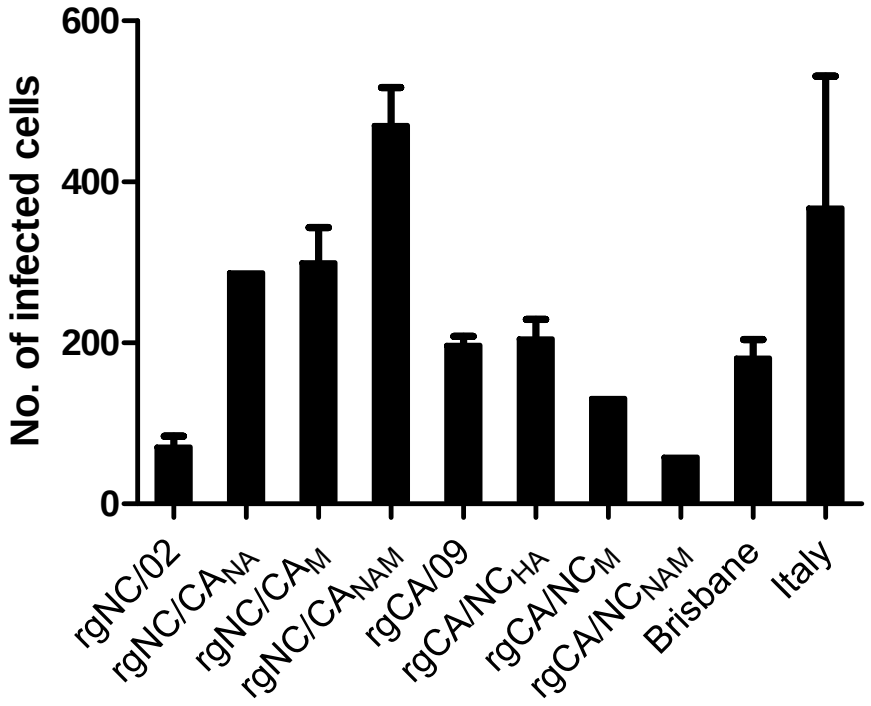
By 12 hours, pandemic strains and Italy have infected ~50%-75% of the culture

Viral efficiency of spread in A549



influenza NP
DAPI (nucleus)

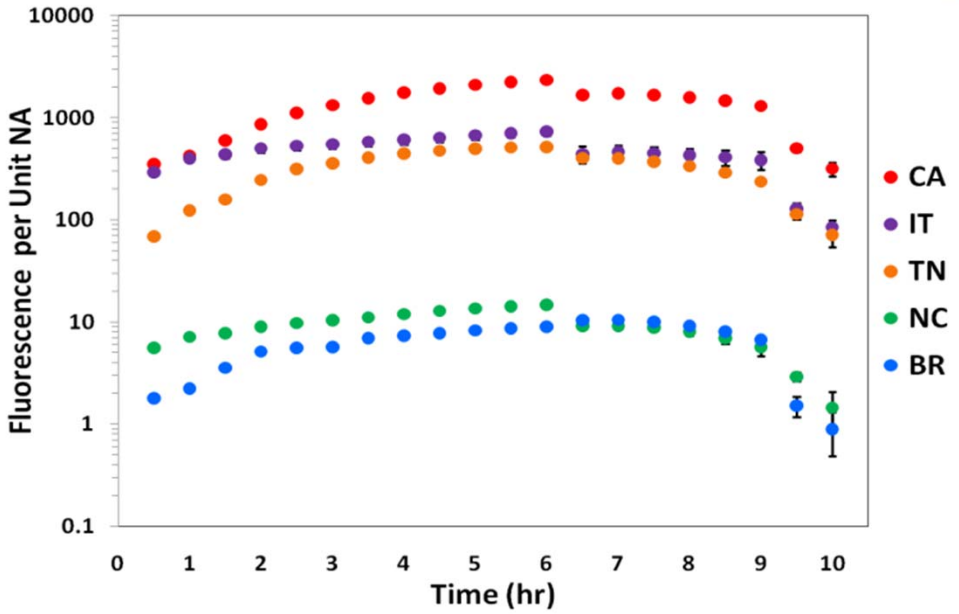
Summary Data of Focal infection study



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

$$\frac{dU}{dt} = \lambda D - \frac{b}{1+s_1X}UV \qquad \text{uninfected cells}$$

$$\frac{dE}{dt} = \frac{b}{1+s_1X}UV - \frac{g}{1+s_3X}E \qquad \text{latent infected cells}$$

$$\frac{dI}{dt} = \frac{g}{1+s_3X}E - dI \qquad \text{productively infected cells}$$

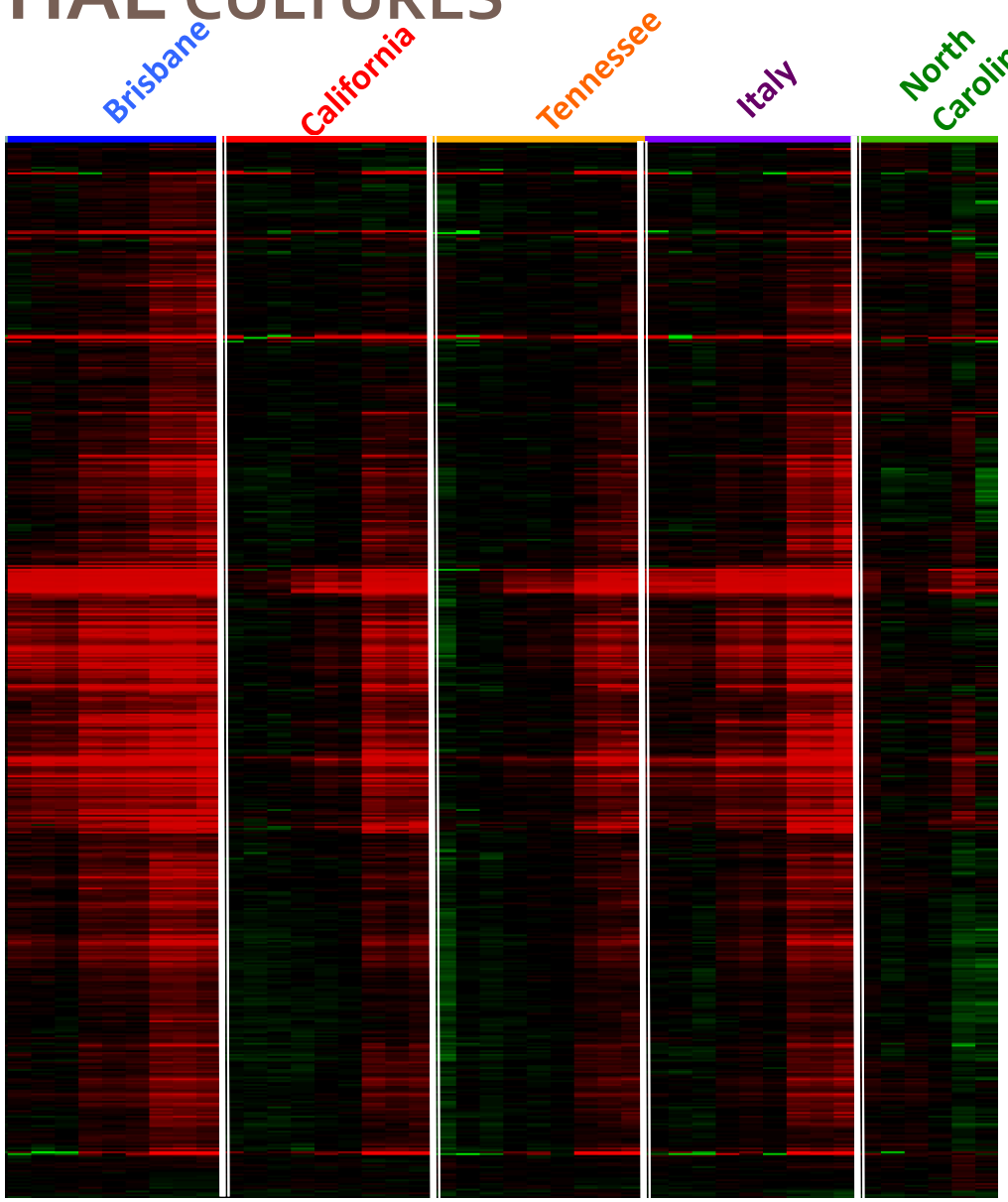
$$\frac{dD}{dt} = dI - \lambda D \qquad \text{dead cells}$$

$$\frac{dV}{dt} = \frac{p}{1+s_2X}I - cV - \gamma\frac{b}{1+s_1X}VU \qquad \text{free virus}$$

Why wasn't the Esw virus a pandemic?

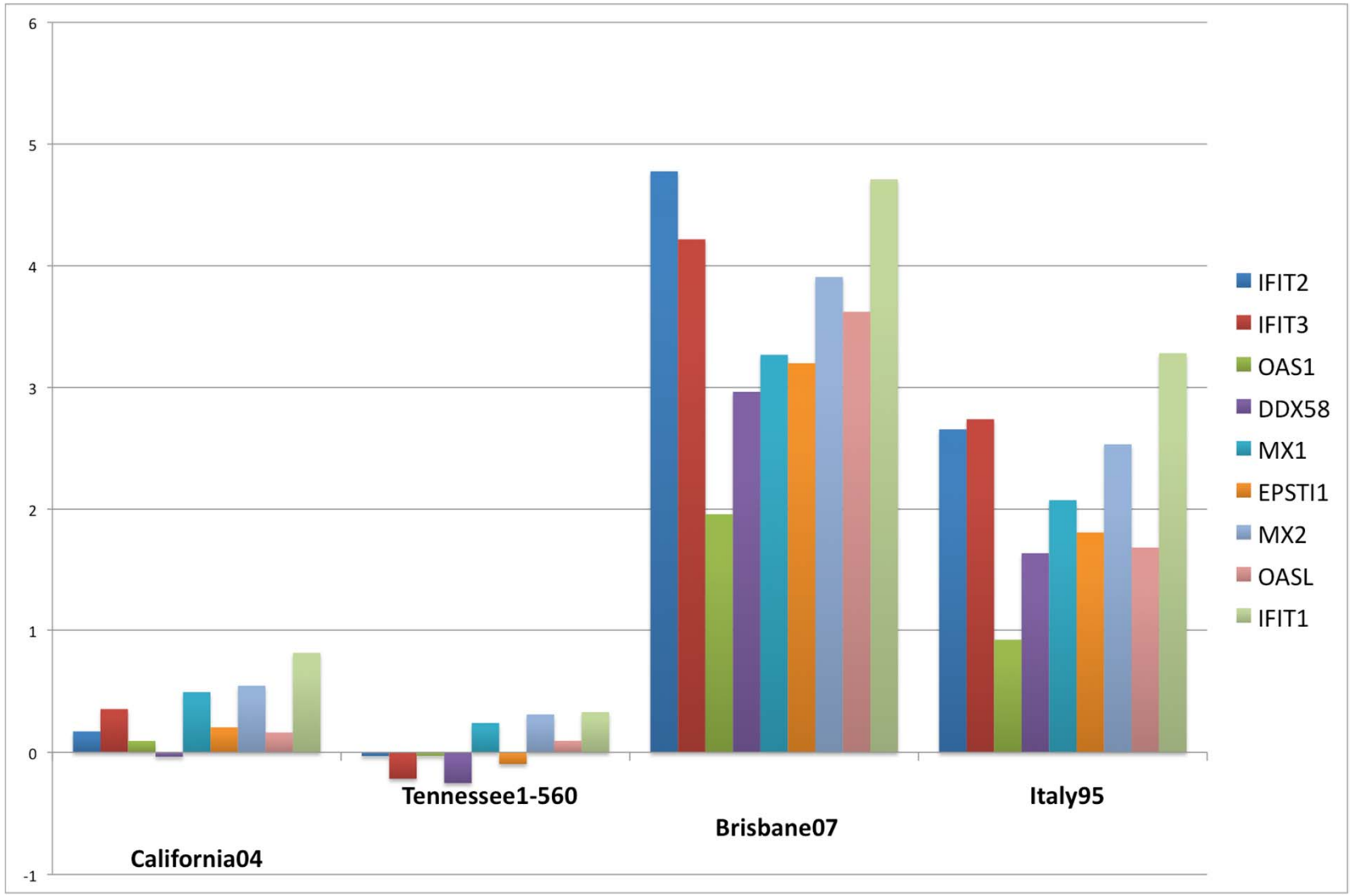
TRANSCRIPTOME ANALYSIS OF PANDEMIC VIRUS INFECTED HAE CULTURES

mRNA expression in hAE cultures infected at MOI=0.01



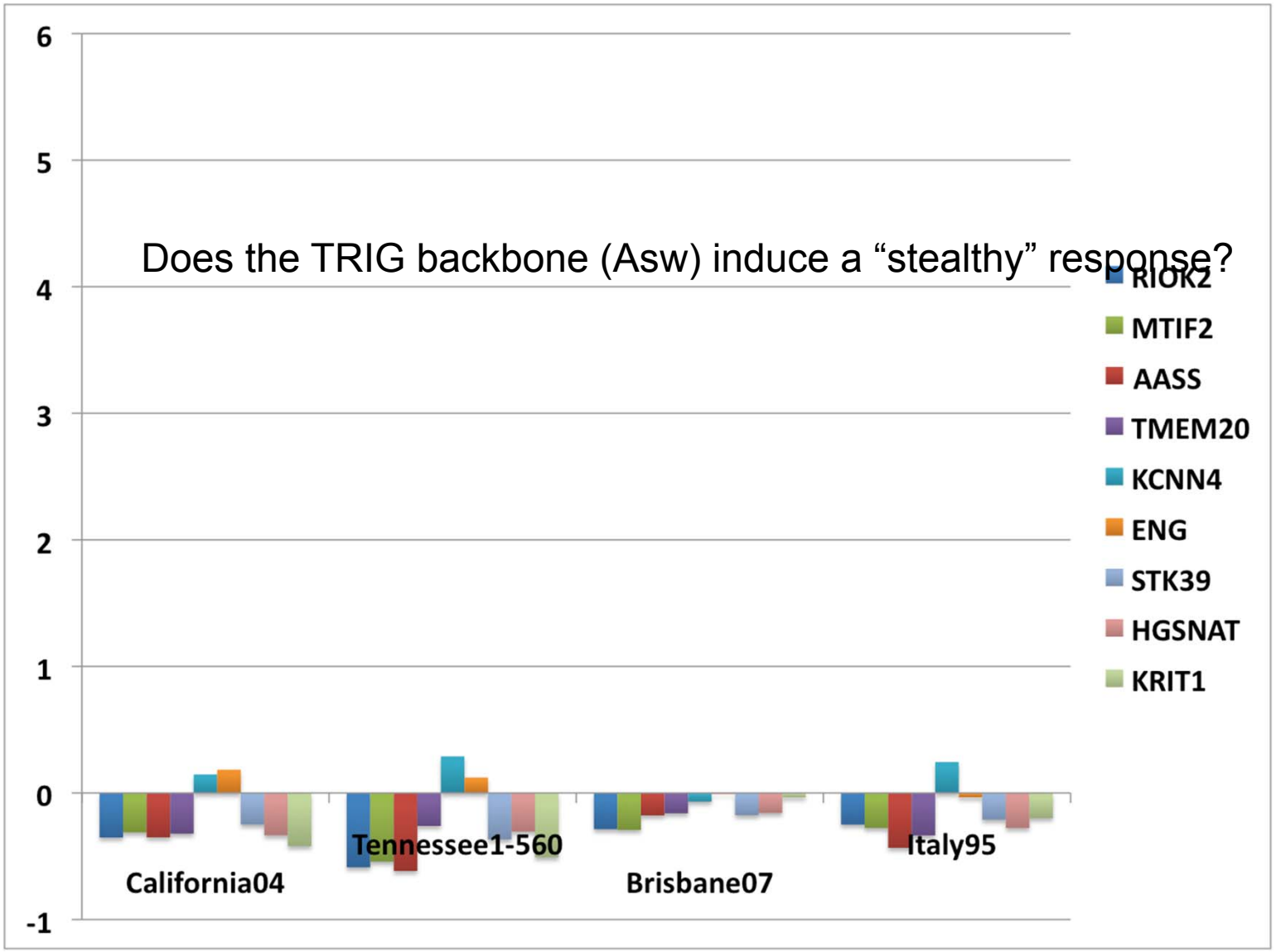
BIC applied to k-means clustering:
2 clusters
271 upregulated in all
24 downregulated or differential

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



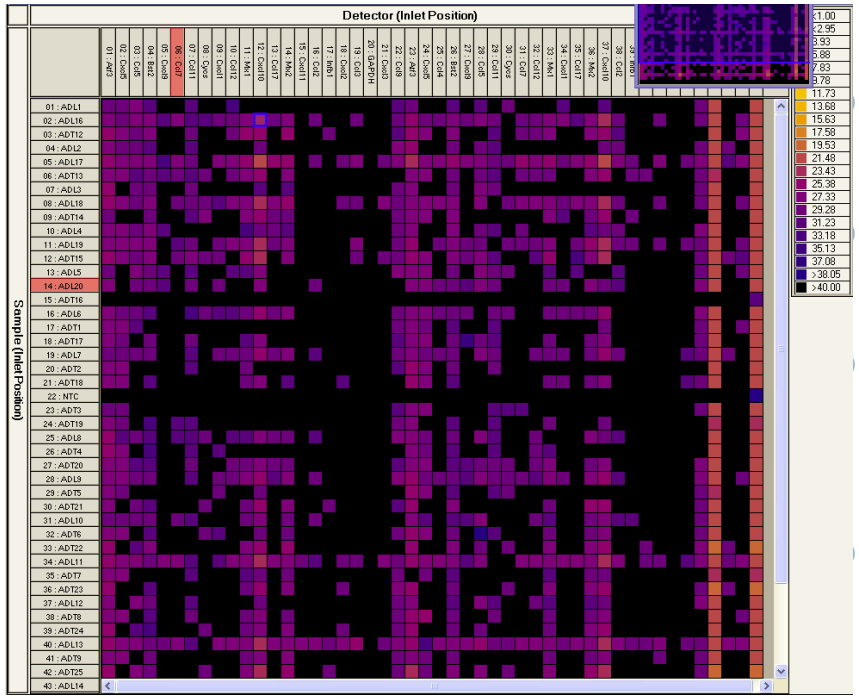
A

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



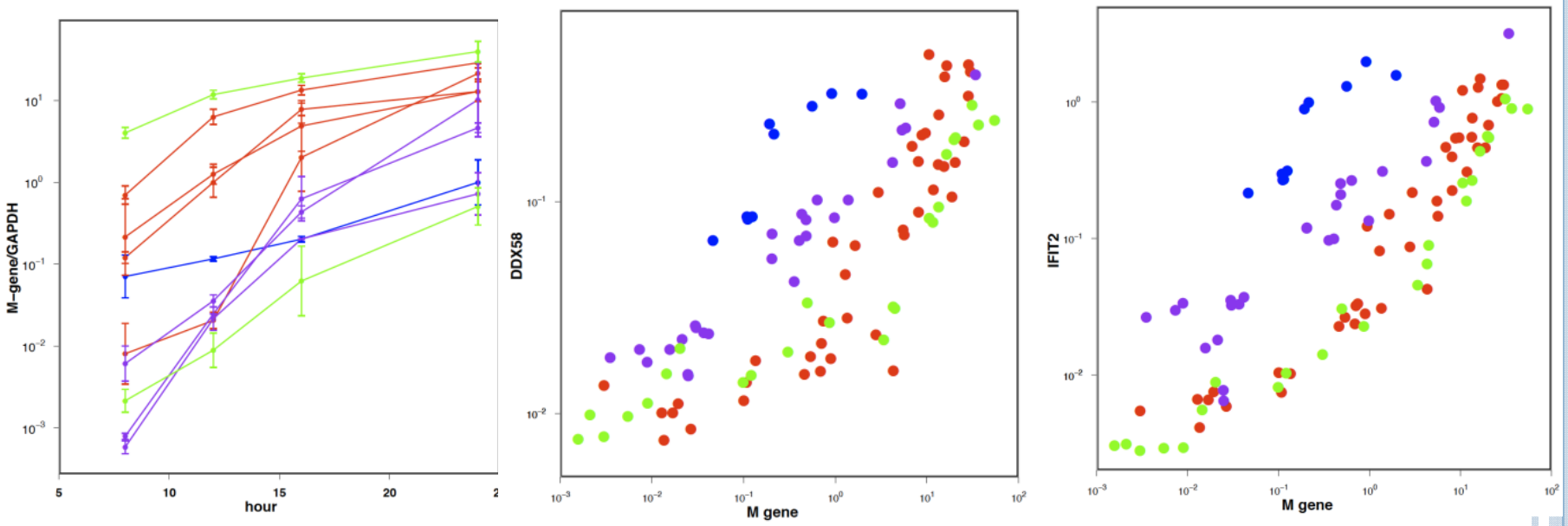
A

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



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

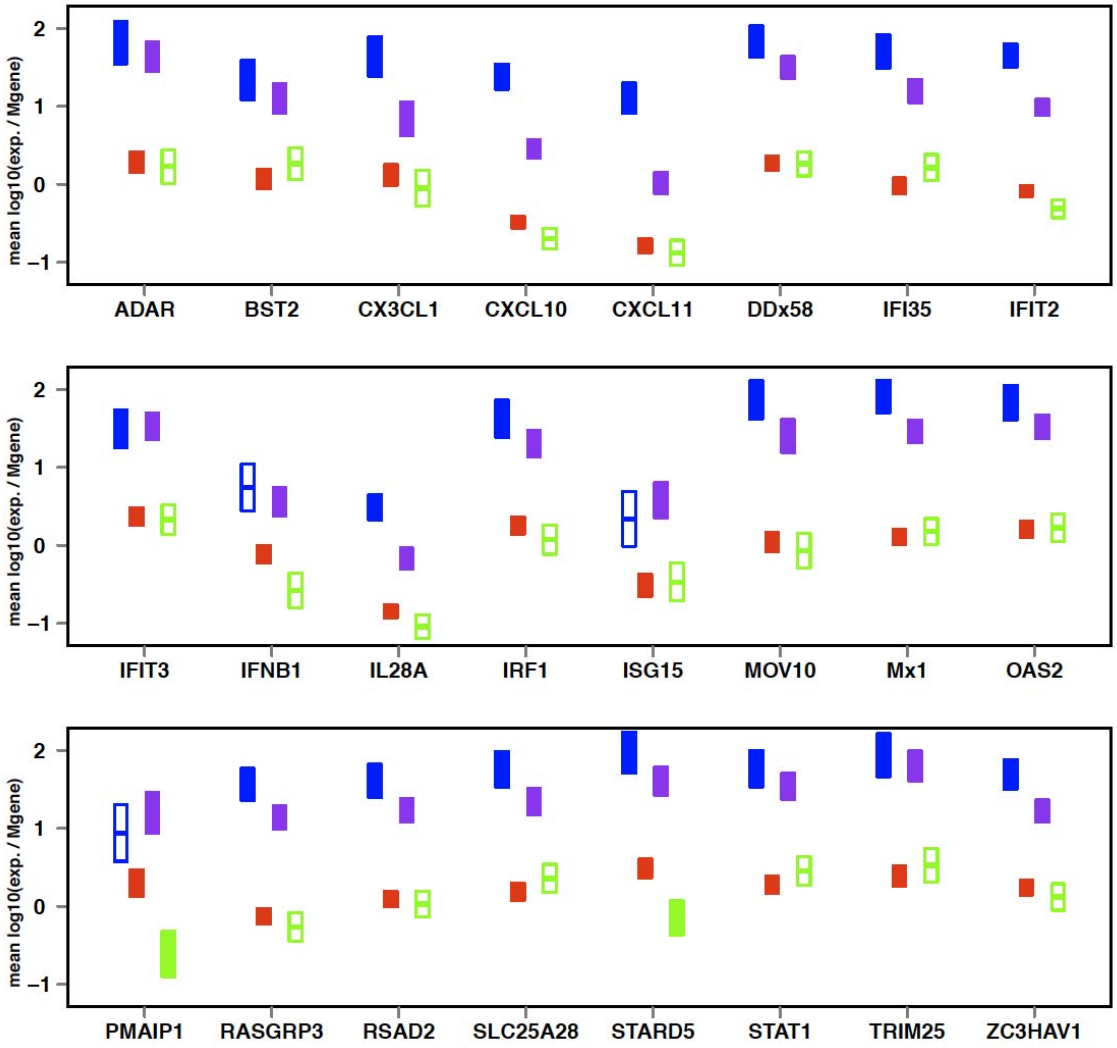
Brisbane California Italy North Carolina

HOST RESPONSE AS A FUNCTION OF VIRUS II

Brisbane California

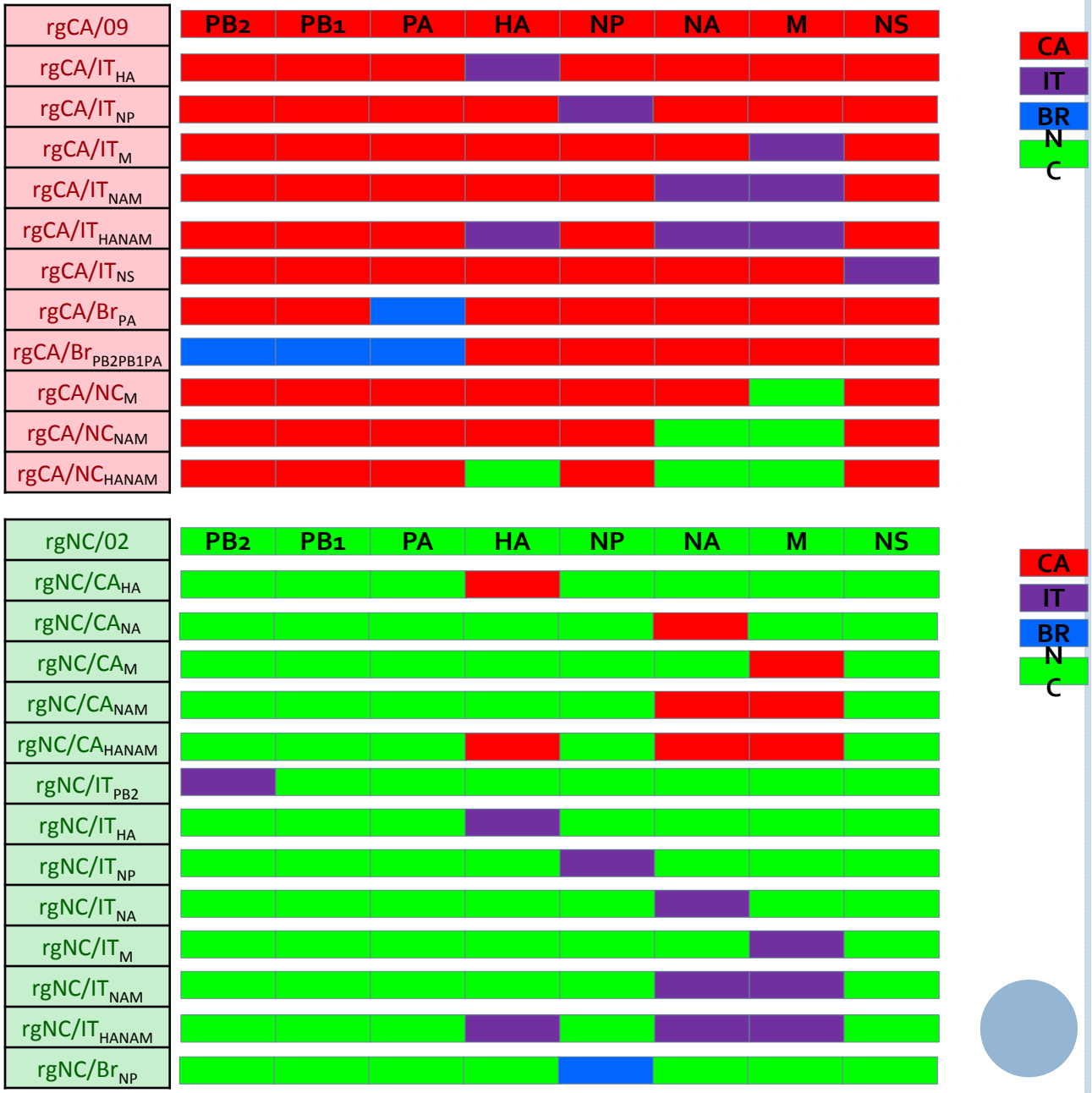
Italy North Carolina

$$\frac{expression - expression_{mock}}{\frac{\max(expression)}{Mgene}}$$

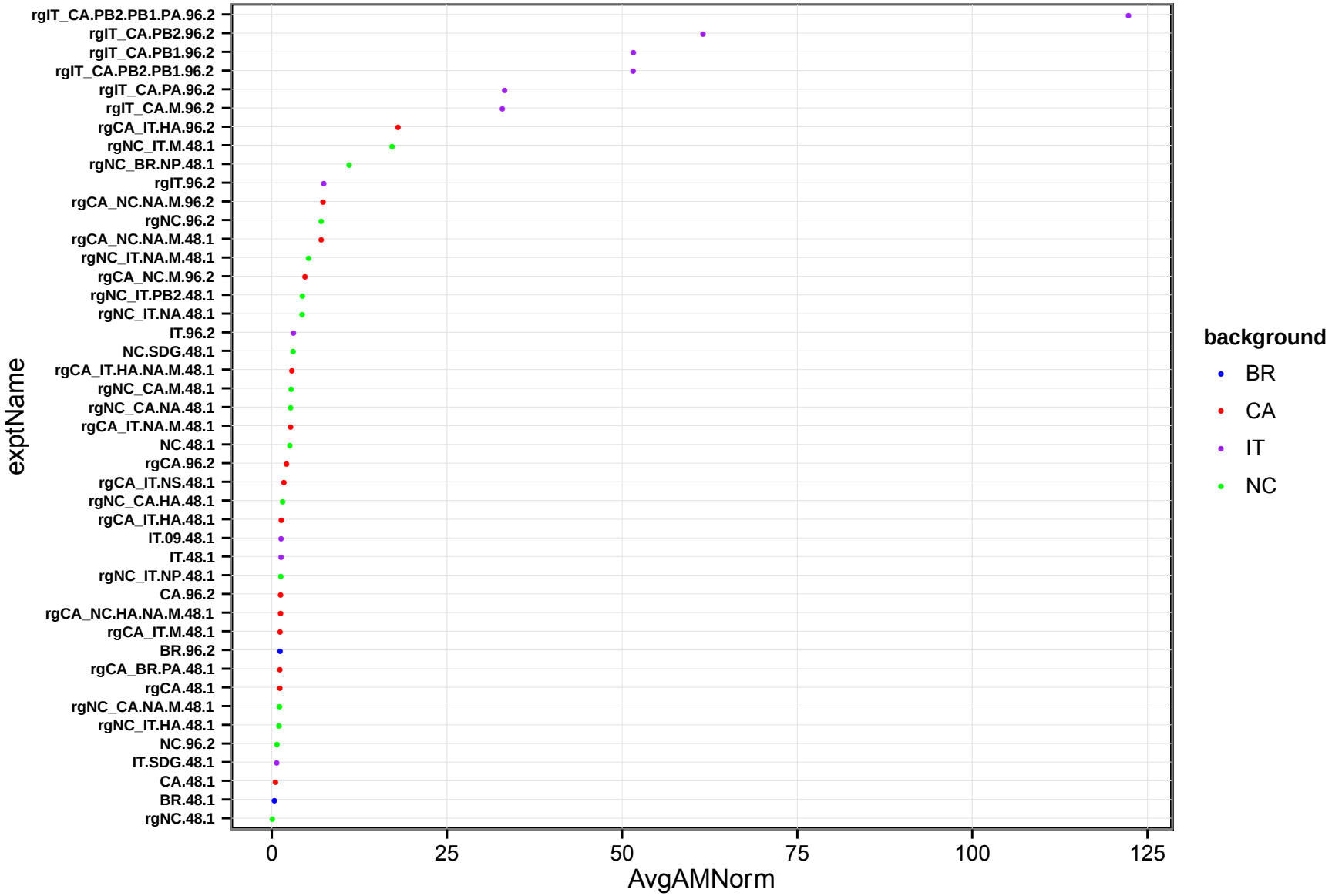


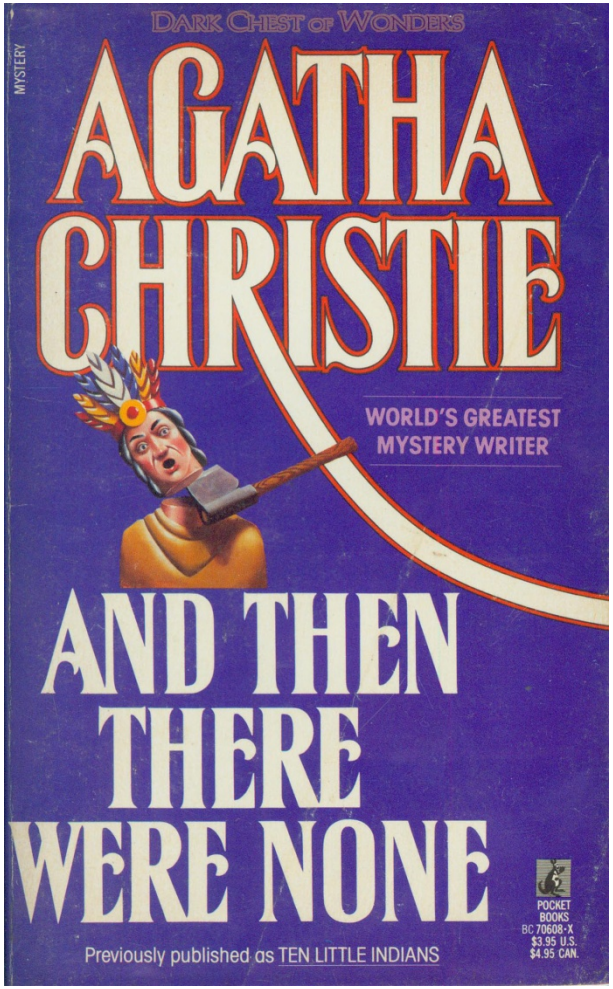
SWAPS

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



Average amplitude across all genes normalized to M-gene

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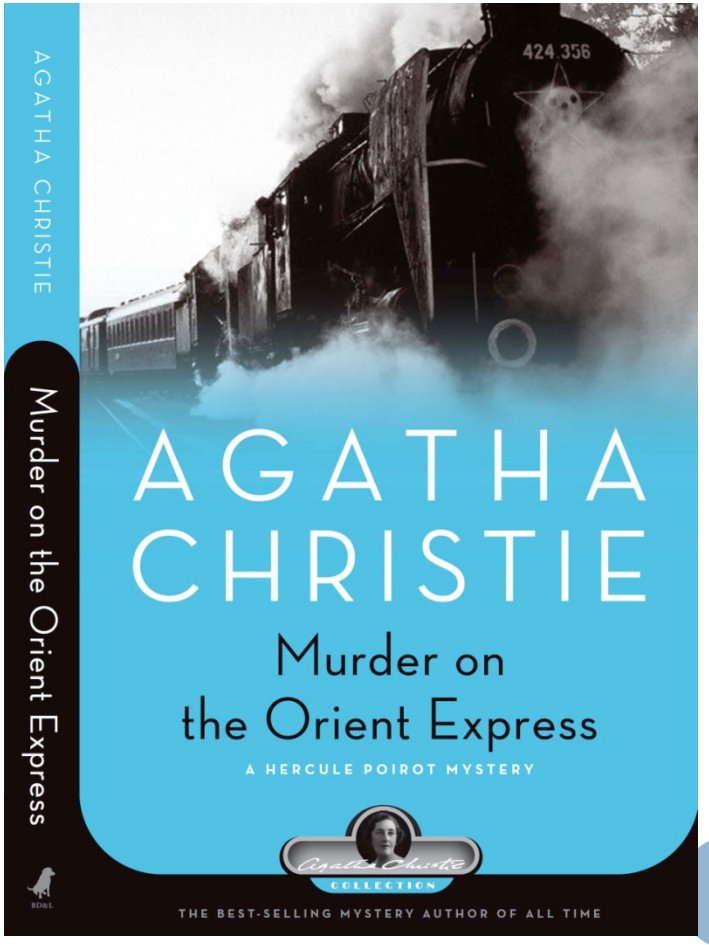
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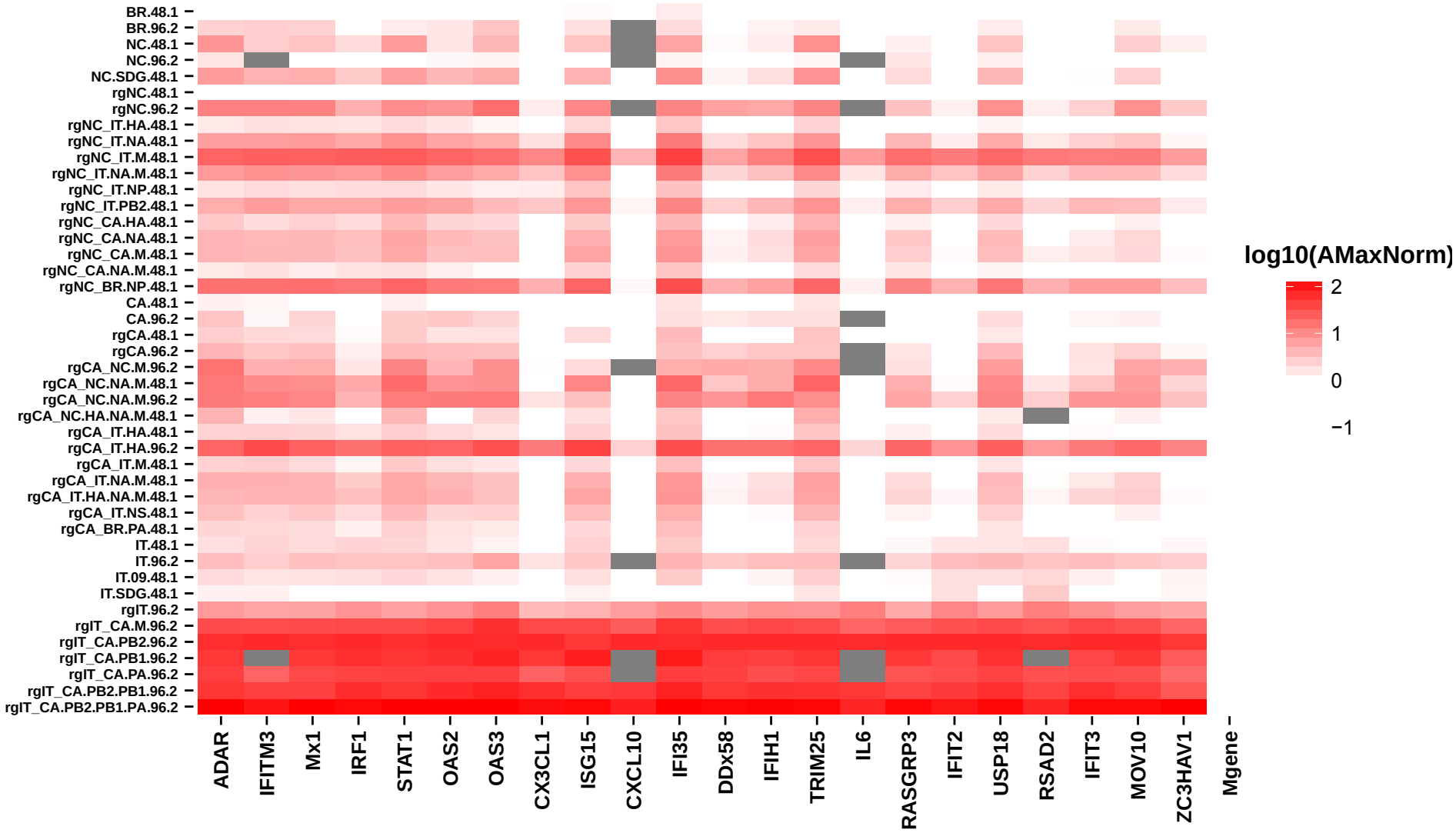
Amplitude (“A”) normalized to M-gene



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 ismediated by contriubtions of the P-gene complex, with potential roles for NP and NS

ODE MODEL OF INFLUENZA INFECTION

$$\frac{dU}{dt} = \lambda D - \frac{b}{1+s_1X}UV \qquad \text{uninfected cells}$$

$$\frac{dE}{dt} = \frac{b}{1+s_1X}UV - \frac{g}{1+s_3X}E \qquad \text{latent infected cells}$$

$$\frac{dI}{dt} = \frac{g}{1+s_3X}E - dI \qquad \text{productively infected cells}$$

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

$$\frac{dV}{dt} = \frac{p}{1+s_2X}I - cV - \gamma\frac{b}{1+s_1X}VU \qquad \text{free virus}$$

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

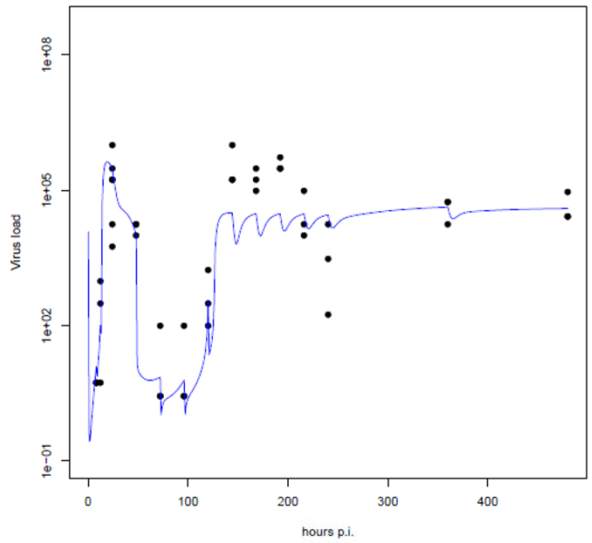
AICc VALUES OF 8 DIFFERENT MODELS

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

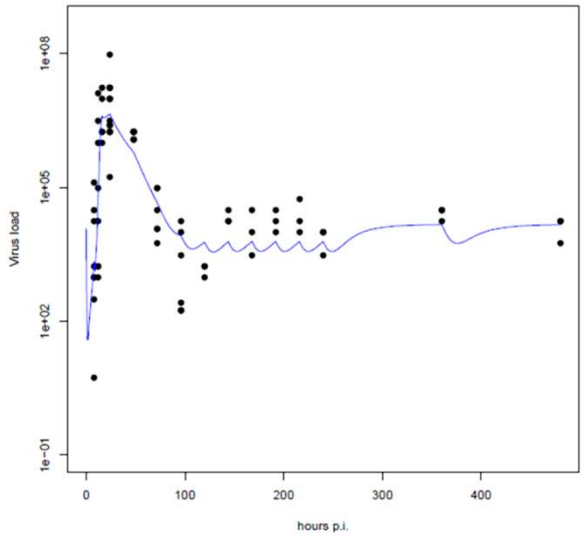
Model	BB	CA	IT	NC
1	54.5	54.7	33.1	28.2
2	48.8	-22.6	0.8	28.5
3	52.8	24.8	17.0	30.3
4	59.9	33.2	38.3	33.6
5	53.2	32.1	24.6	31.7
6	-11.6	-17.6	-11.1	33.2
7	54.5	-17.7	6.1	29.3
8	56.1	-17.3	6.2	34.3

Fits for Model 6—IR reduces virus production and cells regrow

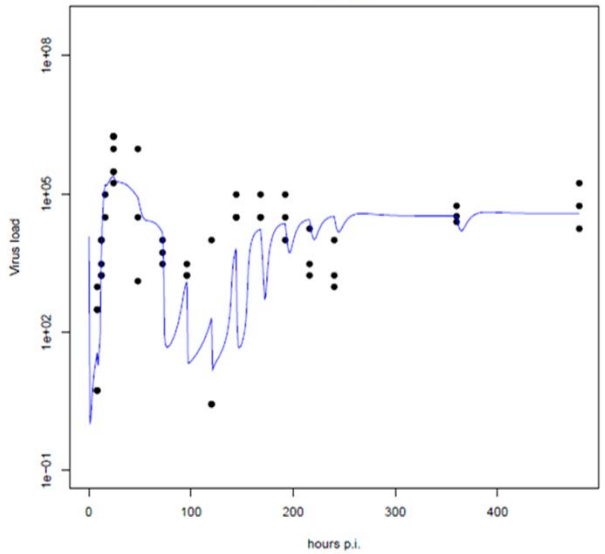
BR



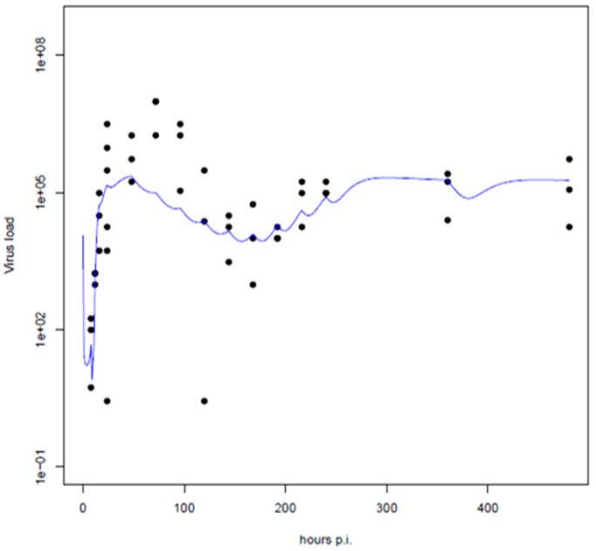
CA



IT

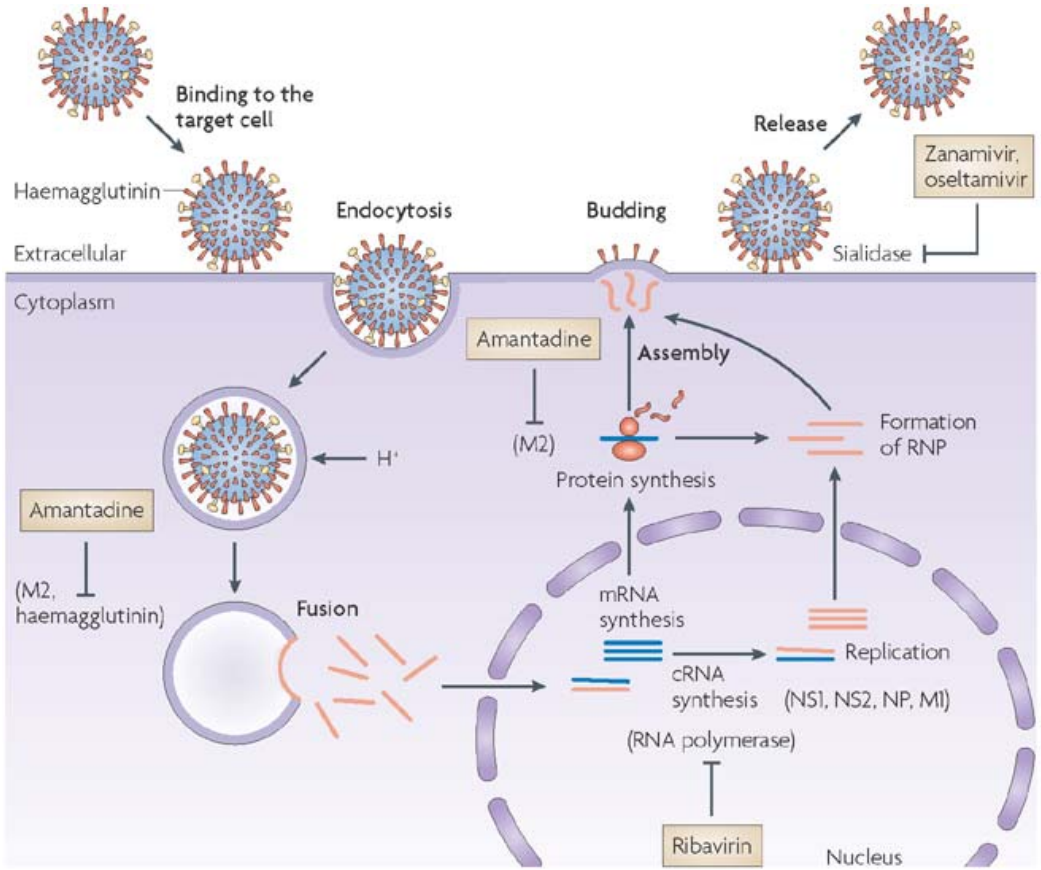


NC



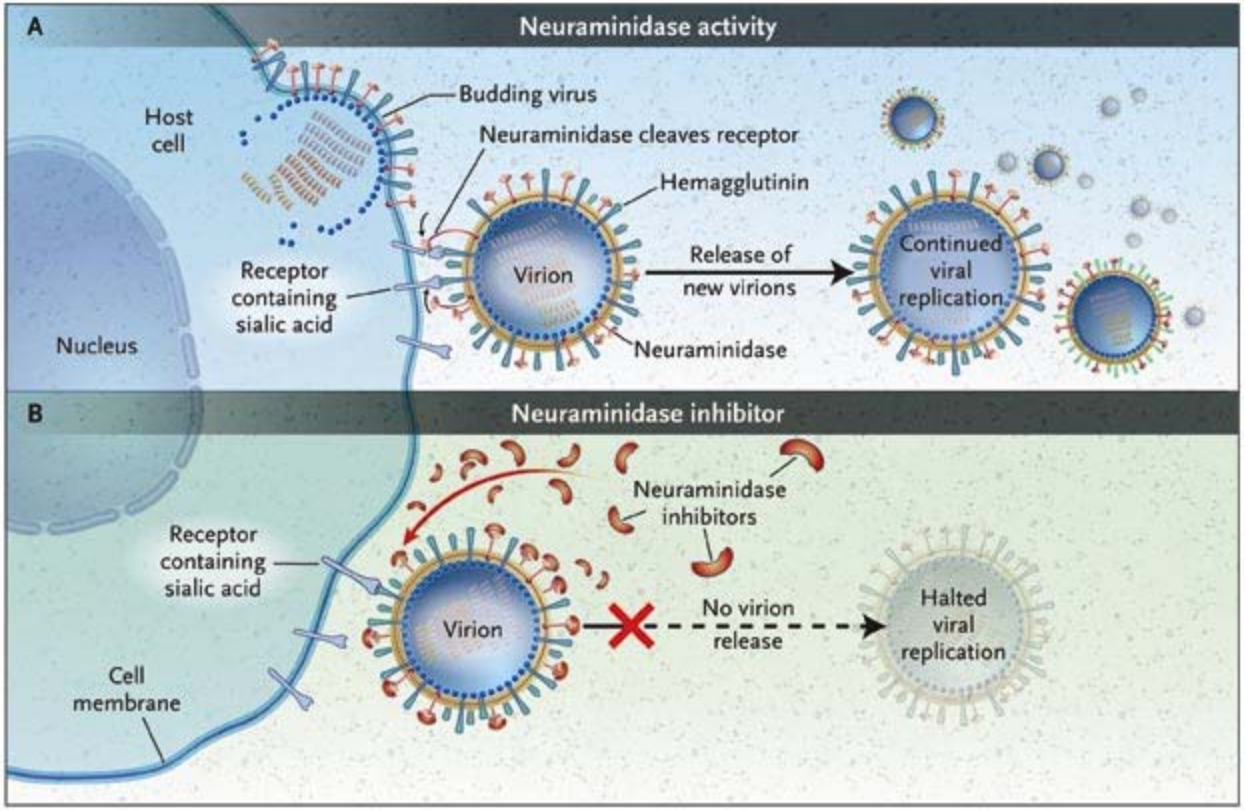
Drug Targets for Influenza Treatment

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



Nature Reviews | Drug Discovery

Neuraminidase Inhibitors prevent Viral Spread



Neuraminidase inhibitor resistance has emerged in Seasonal H1N1 influenza, Amantaidne resistance in H3N2

	Isolates tested (n)	Resistant Viruses, Number (%)	Isolates tested (n)	Resistant Viruses, Number (%)	
		Oseltamivir	Zanamivir		Adamantanes
Seasonal Influenza A (H1N1)	825	820 (99.4%)	0 (0)	832	4 (0.5%)
Influenza A (H3N2)	132	0 (0)	0 (0)	141	141 (100%)
Influenza B	403	0 (0)	0 (0)	N/A*	N/A*
Novel Influenza A (H1N1)	68	0 (0)	0 (0)	96	96 (100%)

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?