Importance of 1918 virus reconstruction on current assessments to pandemic risk

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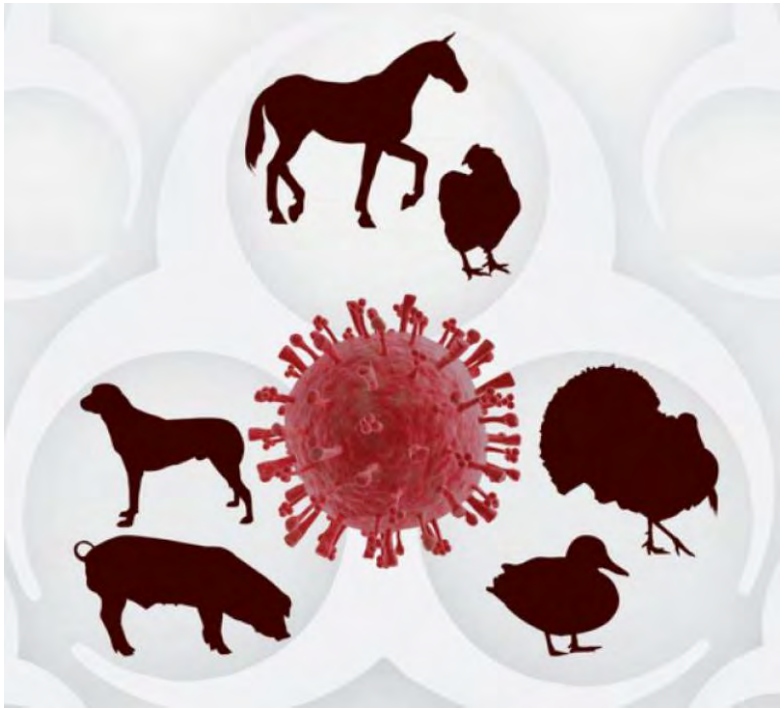
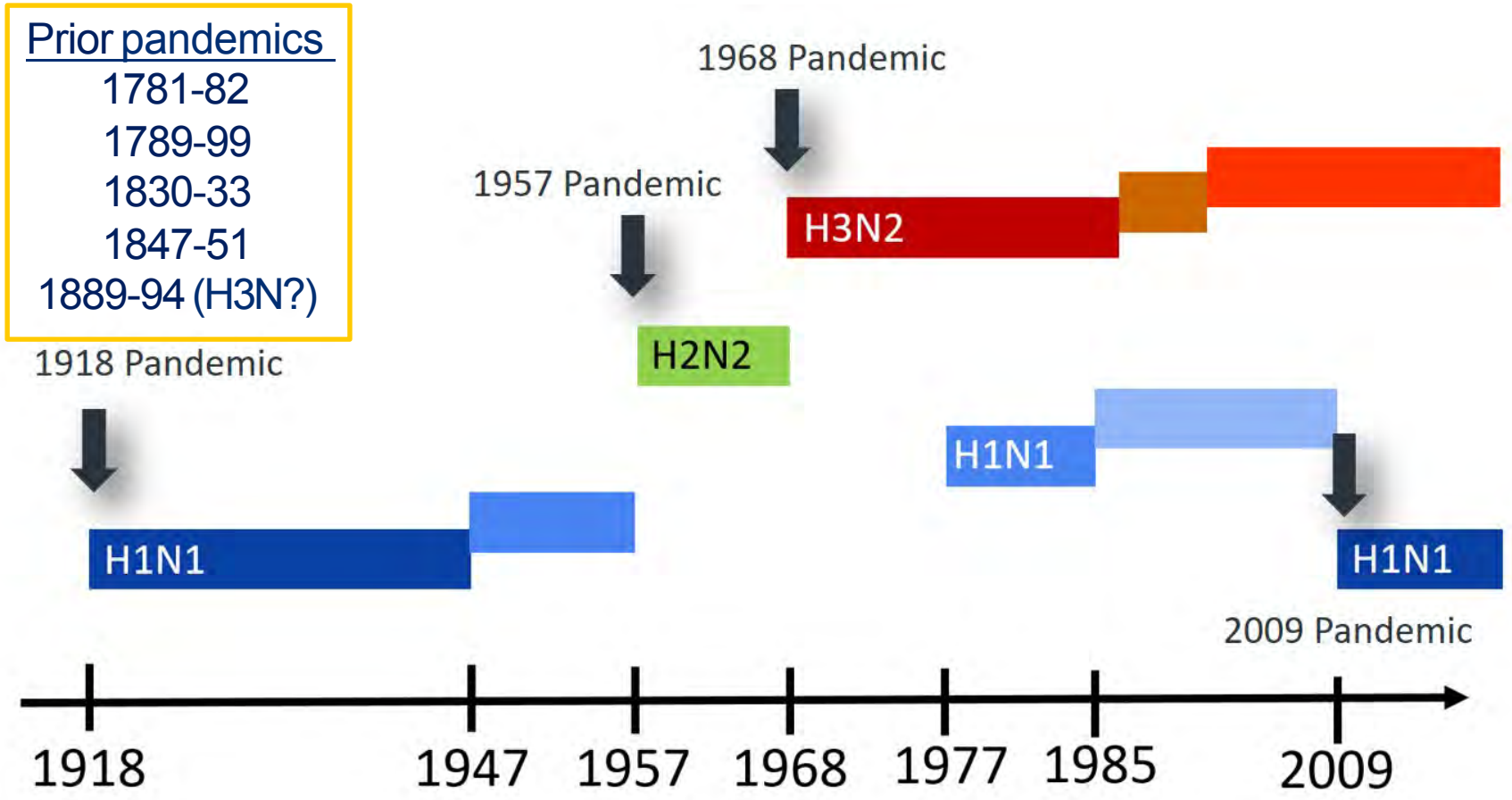
1918

2018



Belser and Tumpey, Science 2018; Image US National Archives and Records Admin; Animal Influenza, 2016 (Swayne)

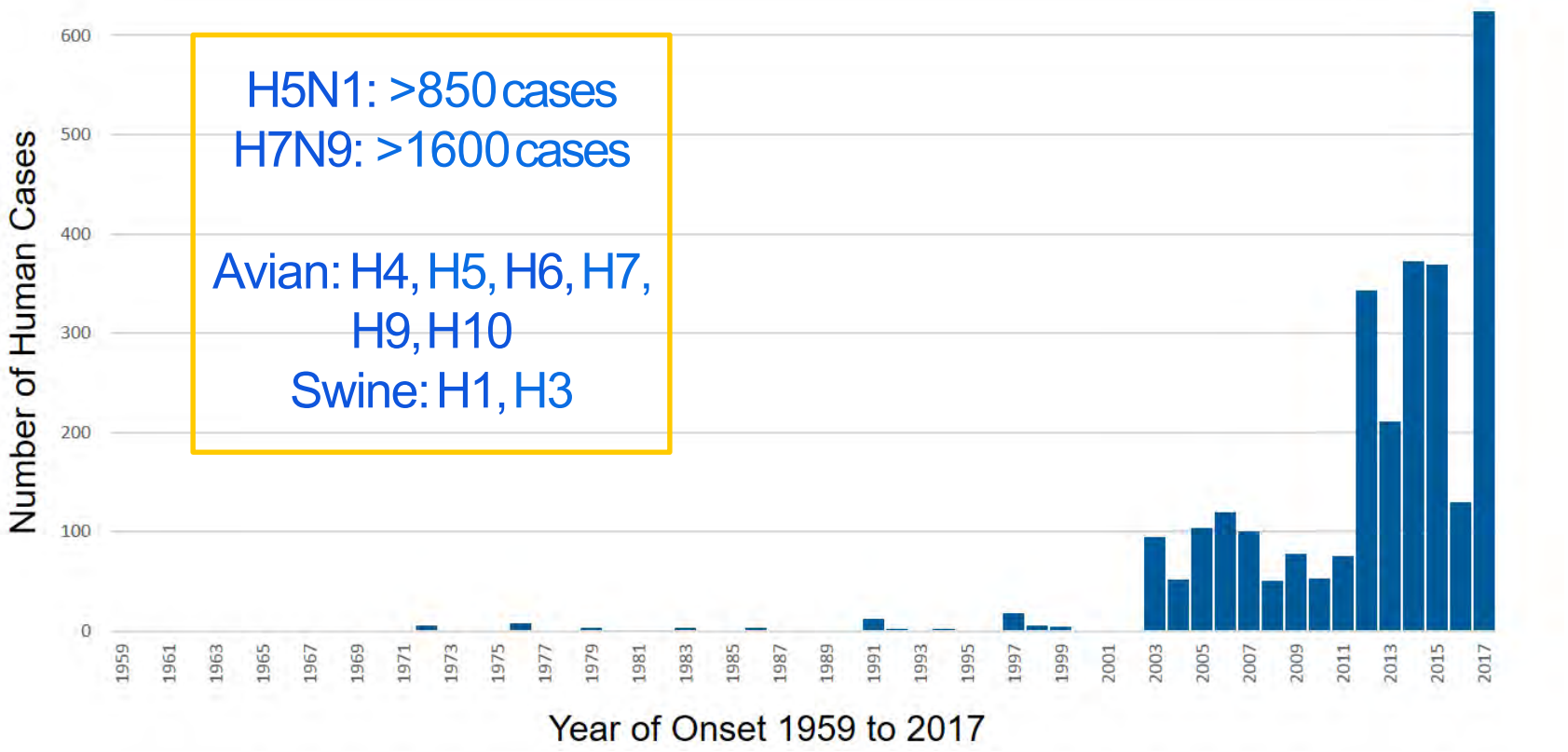
Influenza A viruses circulating in humans since 1918



Taubenberger et al, Antiviral Ther 2007; Image D Jernigan/B Jester

CDC

Reported human cases of novel influenza A infection, 1959-2017



Diversity of viruses jumping the species barrier to humans

Subtypes of novel influenza A viruses and clinical syndromes associated with human infection

	LPAI viruses	HPAI viruses	Variant viruses*
Conjunctivitis	H7N2, H7N3, H7N7, H10N7	H7N3, H7N7	H1N1v, H3N2v
Upper respiratory tract illness	H6N1, H7N2, H7N3, H7N9, H9N2, H10N7	H5N1, H5N6, H7N7	H1N1v, H1N2v, H3N2v
Lower respiratory tract disease, pneumonia	H7N2, H7N9, H9N2, H10N8	H5N1, H5N6, H7N7, H7N9	H1N1v, H3N2v
Respiratory failure, acute respiratory distress syndrome	H7N9, H10N8	H5N1, H5N6, H7N7, H7N9	H1N1v, H3N2v
Multiorgan failure	H7N9, H10N8	H5N1, H5N6, H7N7, H7N9	..
Encephalopathy or encephalitis	H7N9	H5N1	..
Fatal outcomes†	H7N9, H9N2, H10N8	H5N1, H5N6, H7N7, H7N9	H1N1v, H3N2v

Diversity of clinical syndromes highlights the challenge in studying the human risk posed by these viruses

Uyeki et al, The Lancet, 2017

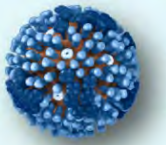
CDC

Pandemic preparedness and risk assessment

- Assess potential pandemic risk of zoonotic influenza viruses
  - Advance preparedness
  - Response timing and capacity
  - Employs both current and historical data

CDC Influenza Risk Assessment Tool **(IRAT)**

WHO Tool for Influenza Pandemic Risk Assessment **(TIPRA)**



Virus

1. **Genomic variation**
2. **Receptor binding**
3. **Transmission in Laboratory animals**
4. **Antivirals and Treatment Options**

Population

5. **Existing Population Immunity**
6. **Disease Severity and Pathogenesis**
7. **Antigenic Relationship to Vaccine Candidates**

Ecology

8. **Global Geographic Distribution**
9. **Infection in Animals, Human Risk of Infection**
10. **Human Infections and Transmission**

Jernigan and Cox, Textbook of Influenza 2013

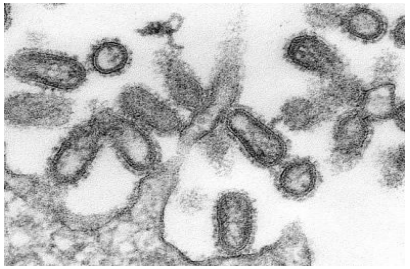
CDC

Importance of 1918 virus reconstruction

- Identify properties that were responsible for the extraordinary virulence of the 1918 influenza virus
- Identify genetic determinants responsible for the transmissibility of this pandemic virus

- Apply this knowledge to current assessments of pandemic risk

- Archaevirology: the systematic study of pastviruses by the recovery and examination of remaining material evidence



Tumpey and Belser, Ann Rev Microbiol 2009; 1918 EM by Cynthia Goldsmith

CDC

Rescuing The 1918 Influenza Virus

1951

Johan Hultin at permafrost gravesite, Brevig Mission AK

1951

Hultin unable to grow live 1918 virus in lab

1997

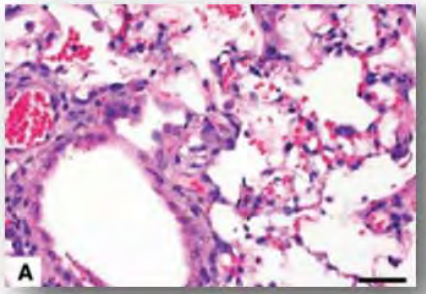
Hultin returns to gravesite for frozen lung tissue

1997

Taubenberger at AFIP begins sequencing the 1918 virus genes

2004

Tumpey at CDC rescues 1918 virus in high containment lab



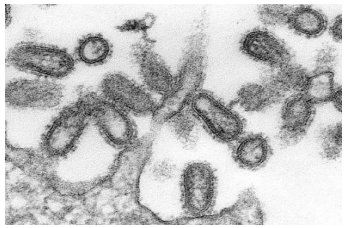
2005

CDC shows 1918 virus causes severe pneumonia in mice and identifies the genes responsible for high virulence

Taubenberger et al, Science 1997 and Antiviral Ther 2007; Tumpey et al, Science 2005; image D Jernigan/B Jester

Initial assessments of the reconstructed 1918 virus

- High-titer replication in vitro
- Lethal in mice, ferrets, macaques



- Efficient spread throughout respiratory tract
  - Nasal turbinates, trachea, lung in ferrets
  - Systemic spread not pronounced

- Severe pulmonary inflammation in both mouse and ferret lungs
  - Necrotizing bronchitis, moderate to severe alveolitis with edema

Tumpey et al, Science 2005; Tumpey et al, Science 2007; Pearce et al J Virol 2012; Kobasa et al, Nature 2007

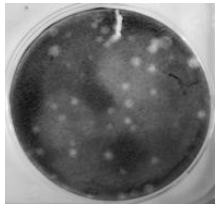
CDC

Molecular determinants of 1918 virus virulence

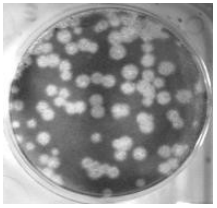
Single-gene reassortant viruses in BALB/c mice

Gene segment	1918/Tx91 (7:1) recombinant viruses	Virulence	1918/Tx91 (1:7) recombinant viruses	Virulence
PB2	Same as 1918	High	Same as Tx/91	Low
PB1	Reduced replication	Reduced	Elevated replication	Low
PA	Same as 1918	High	Same as Tx/91	Low
HA	Reduced replication	Low	Elevated replication	High
NA	Reduced replication	Reduced	Elevated replication	Low
NP	Same as 1918	High	Same as Tx/91	Low
M	Same as 1918	High	Same as Tx/91	Low
NS	Same as 1918	High	Same as Tx/91	Low

Tx/91



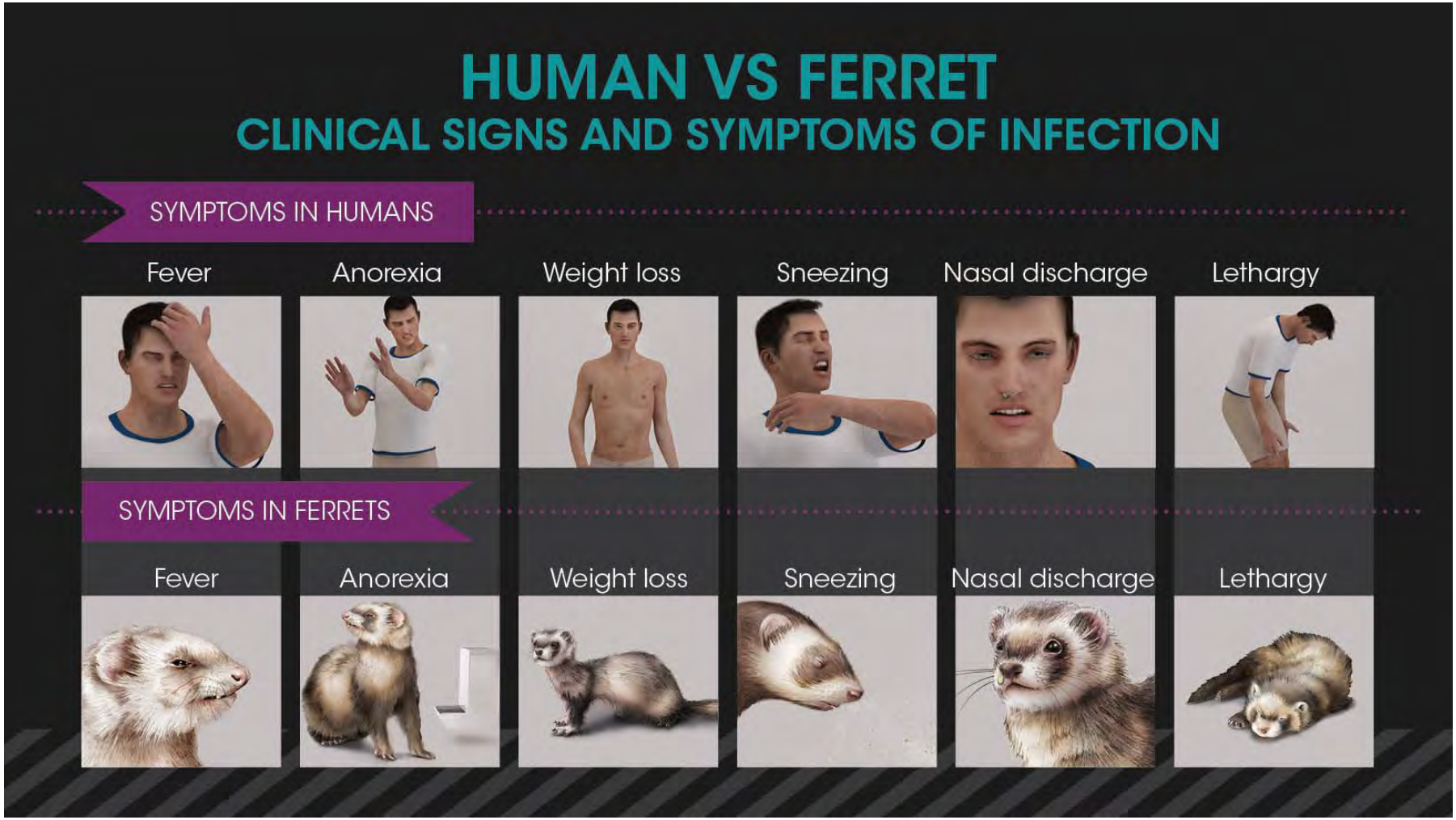
+1918 PB1



1918 PB1 increases replication efficiency of Tx/91 H1N1 virus in NHBE cells

Tumpey et al, Science 2005; Pappas et al, PNAS 2008

CDC

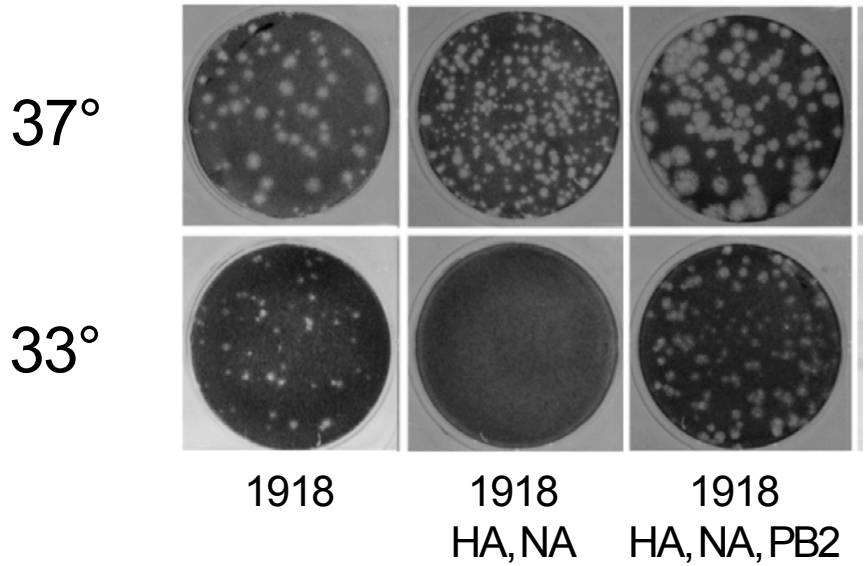


Close physiologic links between ferret and human respiratory tissues
Similar binding patterns of influenza viruses to sialic acid receptors in both species

Belser et al, 2016 MMBR; image by Alissa Eckert

Molecular determinants of 1918 virus virulence

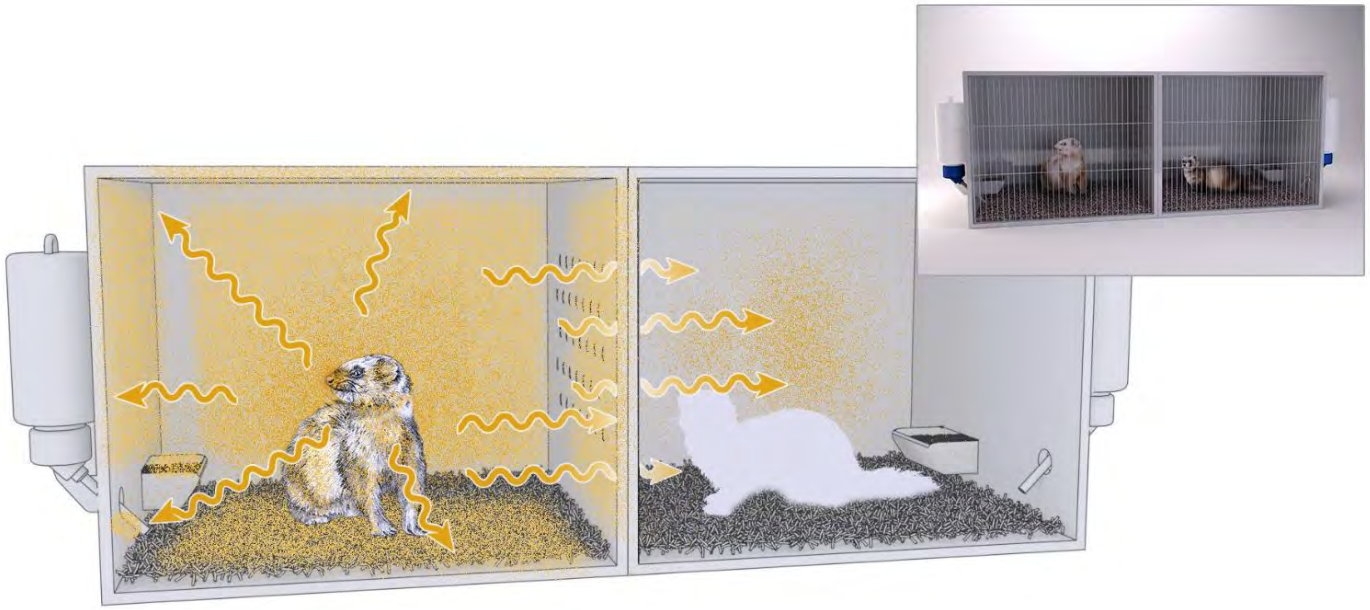
Gene segments from 1918 virus	Morbidity (weight loss)	Peak NW titer Log_{10} EID_{50}/ml
None (avian H1N1)	6%	6.3
HA, NA	9.1%	6.5
HA, NA, PA	17.5%	8.4
HA, NA, PB1	12.4%	7.1
HA, NA, PB2	12.9%	8.1



Contribution of 1918 surface glycoproteins and polymerase genes contribute to virulence in ferrets and replication at URT temperatures

van Hoeven et al, PNAS 2009; ferrets (through 9 days p.i.) and MDCK cells (48hrs p.i.)

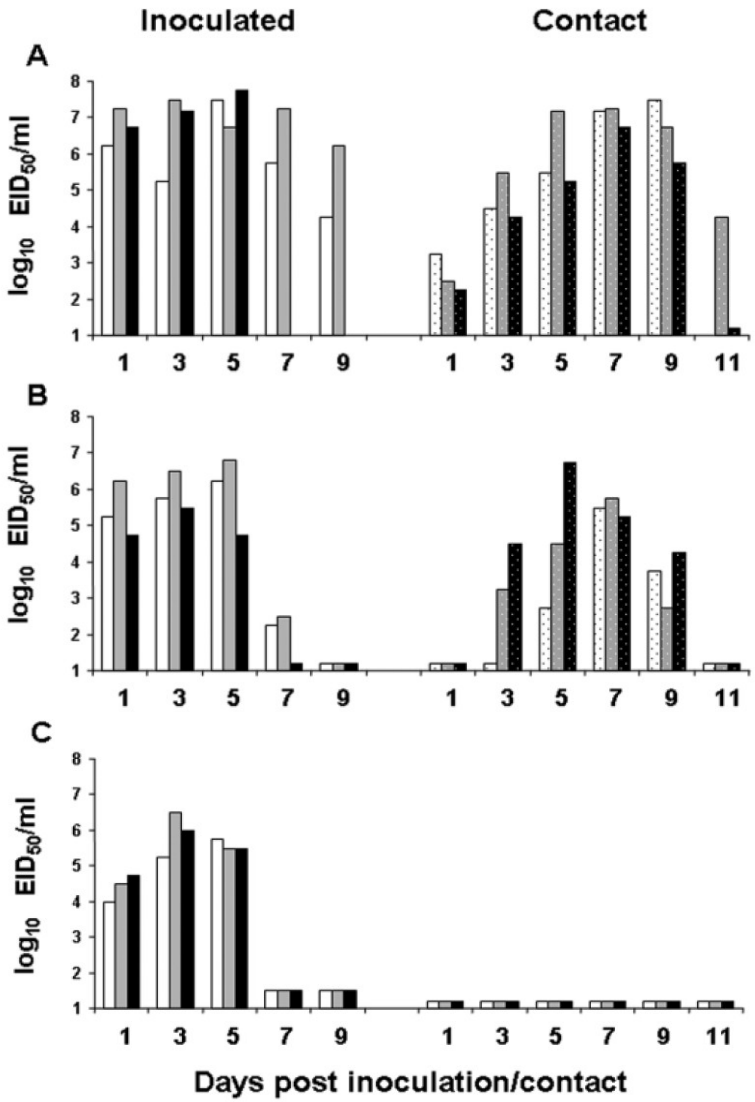
Transmission: respiratory droplet model



- Naive ferrets (white silhouette) are placed adjacent to inoculated ferrets
- Areas of potential exposure to influenza virus are depicted in yellow
- Arrows indicate dispersion of respiratory droplets expelled from the inoculated ferret
- **Respiratory droplet model represents the most stringent transmission setup**

Belser et al. 2016 MMBR.

1918 virus transmissibility in ferrets



Reconstructed 1918 virus (SC/18)

- Highly transmissible by airborne route
- Lethality in one contact ferret

Seasonal H1N1 virus (Tx/91)

- Transmissible by airborne route
- Delay in shedding among contacts

Avian H1N1 virus (Dk/Alb)

- Not transmissible by airborne route
- No seroconversion

Tumpey et al. 2007 Science.

Receptor binding and 1918 virus transmission

		aa position (H3 numbering)		Receptor binding		Respiratory droplet
Virus	**Description**	**190 aa**	**225 aa**	**α2-6**	**α2-3**	**Transmission**
Tx/91	Seasonal H1N1	D	D	+	⊘	Efficient, 3/3
SC/18	Reconstructed 1918 virus	D	D	+	⊘	Efficient, 3/3
NY/18	Natural variant 1918 virus	D.	G	+	+	Inefficient, 2/3 (1 sero only)
AV/18	"avianized" 1918 virus	E.	G	⊘	+	None, 0/3
Dk/NY	Avian H1N1	E	G	⊘	+	None, 0/3
Dk/NY-DD	Avian H1N1 with DDmut	D	D	+	⊘	None, 0/3

α2-6 receptor binding necessary but not sufficient for virus transmissibility

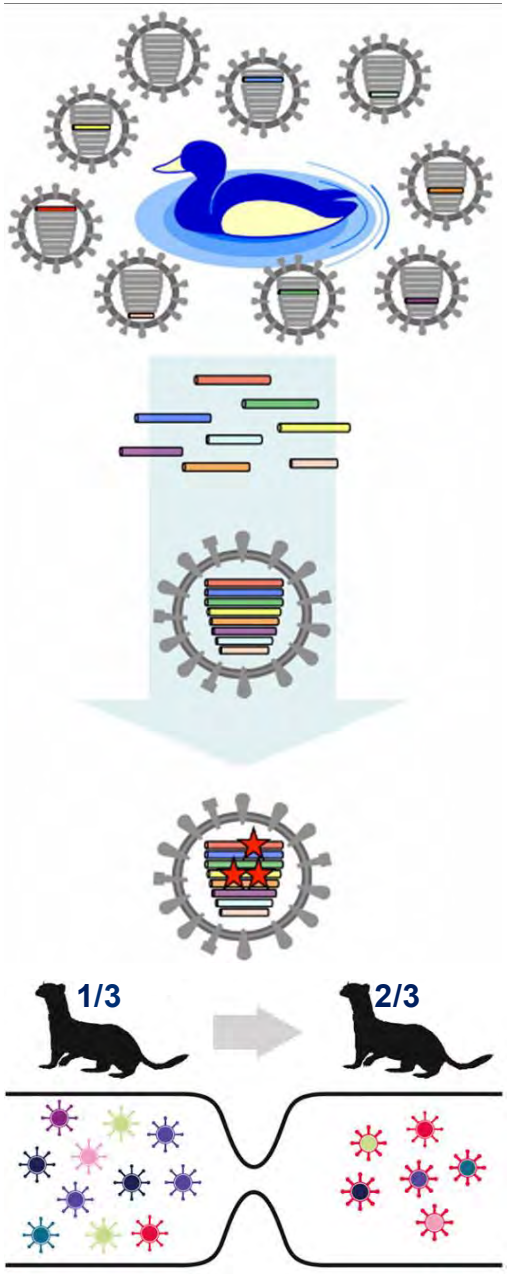
Tumpey et al. Science, 2007; van Hoeven et al, PNAS 2009.

Molecular determinants of 1918 virus transmission

On background of avian H1N1 virus	Respiratory droplet	
Gene segments from 1918 virus	**Transmission**	**Conclusion**
All (fully reconstructed 1918 virus)	Efficient, 3/3	
None (avian H1N1 virus)	None, 0/3	
HA	None, 0/3	1918 surface glycoproteins not sufficient
HA, NA	None, 0/3	
HA, NA, pol complex (PB1, PA, PB2)	Efficient, 3/3	Role for internals
HA, NA, PA	None, 0/3	Role for PB2 specifically
HA, NA, PB1	None, 0/3	
HA, NA, PB2	Efficient, 3/3	
HA, PB2	Efficient, 3/3	
Everything but PB2	None, 0/3	PB2 necessary but not sufficient
PB2	Inefficient, 1/3	
All, PB2 K627E mutation	Inefficient, 1/5	Role for 627K

van Hoeven et al, PNAS 2009; unpublished data; Subbarao et al. J Virol 1993.

CDC



1918-like viruses

Likelihood and impact of "1918-like" viruses emerging from the contemporary avian reservoir

- Viral segments that encoded proteins with high homology to 1918 viral proteins
- Generated "1918-like" avian influenza virus
- Higher pathogenicity in mice and ferrets than authentic avian virus
- Transmissibility (1/3, then 2/3) following aa substitutions (HA, PB2, PA), then serial
- 0/3 to 1/3: "loose" bottleneck with high within-host HA diversity
- 1/3 to 2/3: strongly selective bottleneck after additional HA mutations emerged

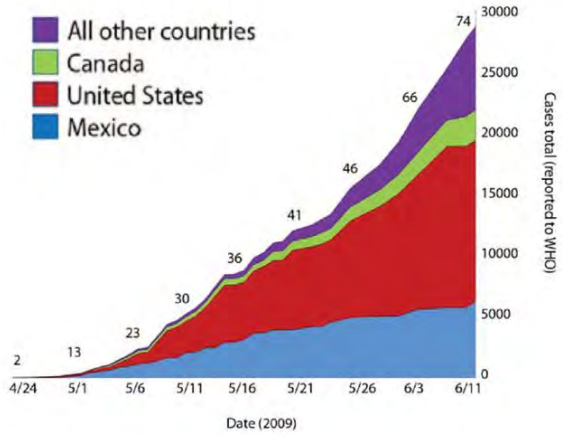
Watanabe et al, Cell Host & Microbe, 2014; Moncla et al, Cell Host & Microbe 2016.

Risk assessment post-1918 virus reconstruction

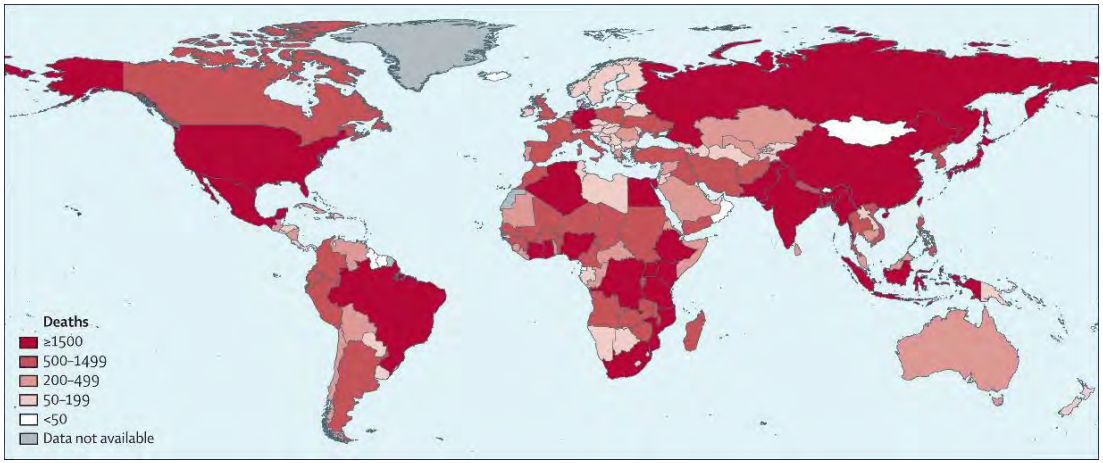
- 2009 H1N1 pandemic
  - Importance of receptor binding preference
- Variant H1 and H3 viruses from swine reservoirs
  - Role of HA and NA on mammalian pathogenicity and transmissibility
- Novel H5 and H7 viruses from avian reservoirs
  - Contribution of PB1-F2

Belser and Tumpey. Microbe, 2010.

Pandemic H1N1 91 years later



Escalation of confirmed 2009 H1N1 human cases from first detection to declaration of a pandemic



Global distribution of estimated deaths associated with 2009 H1N1 during the first year of virus circulation

- 18,500 laboratory-confirmed deaths (April 2009-August 2010)
- Global mortality estimates >200,000 respiratory, >83,000 cardiovascular
- >80% in persons <65 years old, ~50% in southeast Asia and Africa

- Symptomatic case-fatality ratio estimates:
  - 1918: 1-2.1%
  - 2009: 0.05%

Belser and Tumpey. Microbe, 2010; Dawood et al. Lancet Inf Dis, 2012; Nishiura. Expt Rev Respir Med, 2010

Receptor binding specificity: 2009 vs 1918

- 2009 H1N1 does not possess many known markers of mammalian virulence/transmissibility
  - No 627K/701D in PB2, truncated PB1-F2

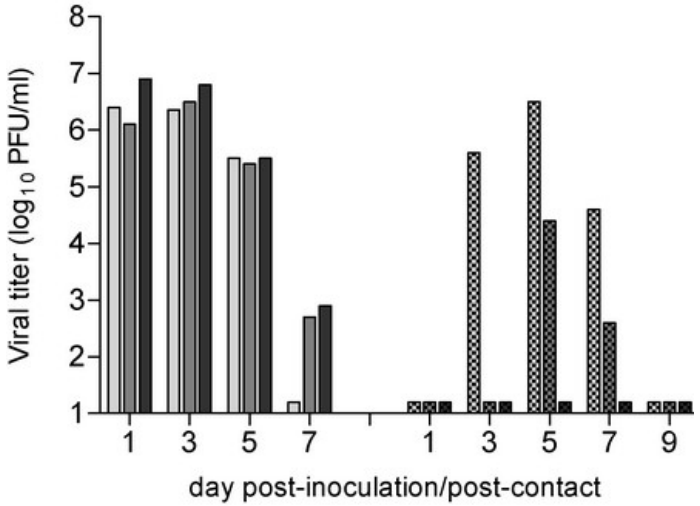
- D225G (H3 numbering) present in >1% of 2009 H1N1 viruses
  - Associated with severe pneumonia, ICU admittance, and death
  - Present during 1918 pandemic

Virus	225 aa	α2-6	α2-3
1918	D	T	⊘
1918 D225G	G	T	T
2009	D	T	⊘
2009 D225G	G	+	+

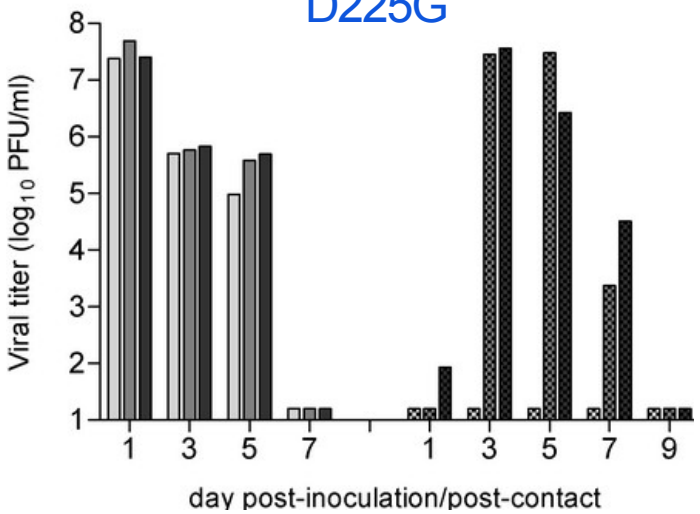
Zhang et al. J Virol, 2013; Reid et al, PNAS, 1999; Chutinimitkul et al, J Virology 2010; Belser et al. PLoS ONE, 2011.

Role of HA 225 in H1N1 pandemic influenza

A/California/4/2009

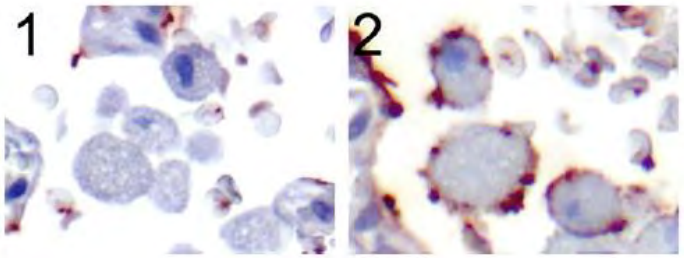


B D222G A/California/4/2009 D225G



- D225G mutation did not augment transmissibility of 2009 H1N1 virus
- Comparable mouse and ferret pathogenicity

2009 H1N1 D225G



11% 53%

Increased binding to human type II pneumocytes and alveolar macrophages

- Enhanced replication in respiratory epithelial cells

Belser et al. PLoS ONE, 2011; Chutinimitkul et al, J Virology 2010.

CDC

HA glycosylation patterns of 1918 and seasonal flu

- Presence of glycans in close proximity to receptor binding domain can modulate binding affinity and specificity
  - 1918 and 2009 H1N1: 1 glycosylation site on HA globular head
  - Seasonal (pre-2009) H1N1: 4-5 glycosylation sites

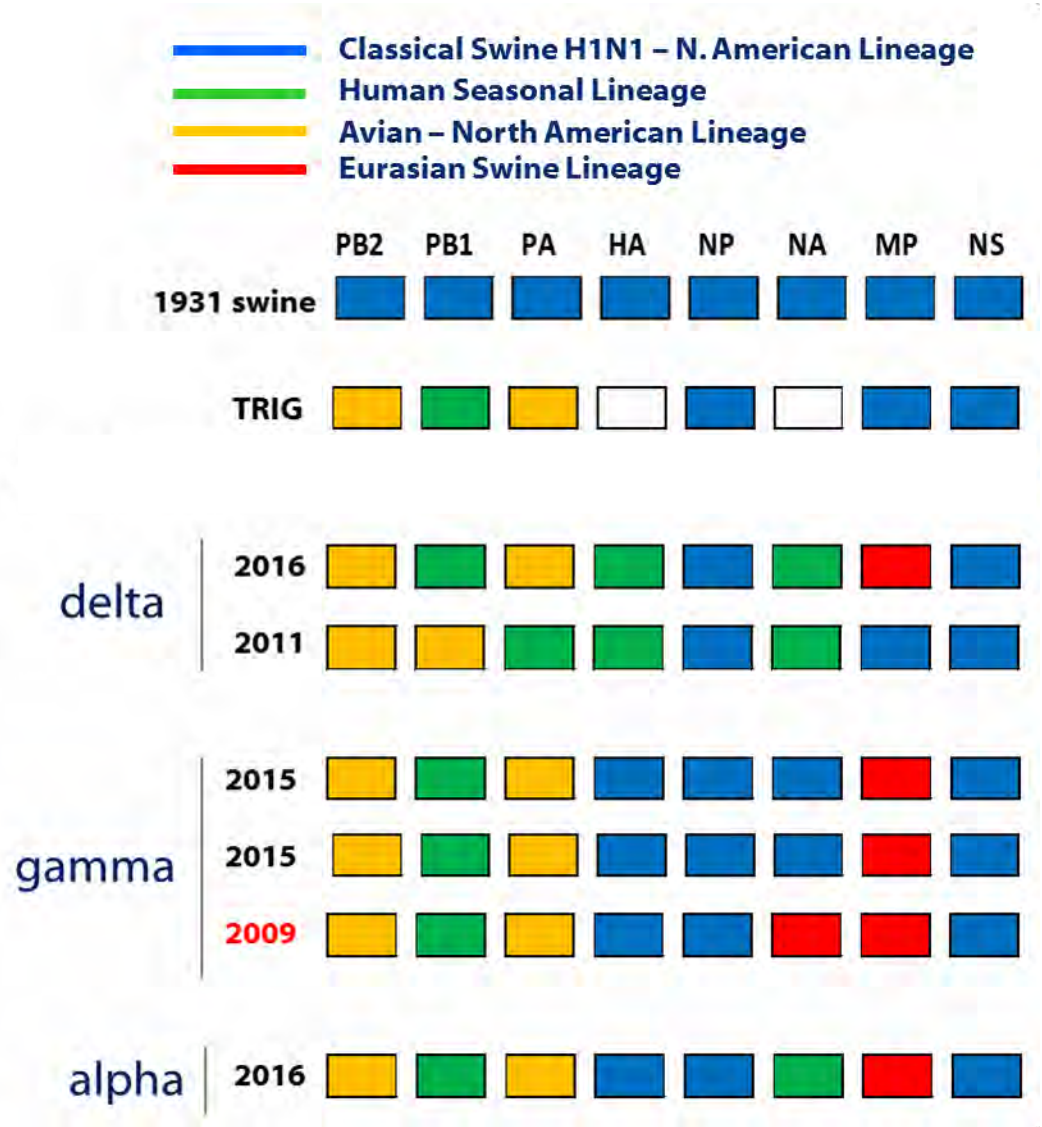
- Modulation of glycans (G) can influence pathogenicity and binding specificity in mice

Virus HA	Weight loss	LD_{50}	Receptor binding
1918	24.8%	$10^{2.2}$	+2G reduced binding to α2-6 SA
1918 + 2G (142, 172)	20%	$10^{4.6}$	
2006 seasonal H1N1	6.2%	$>10^{6}$	-2G increased binding to α2-3 SA
H1N1 – 2G (142, 177)	25%	$10^{3.5}$	

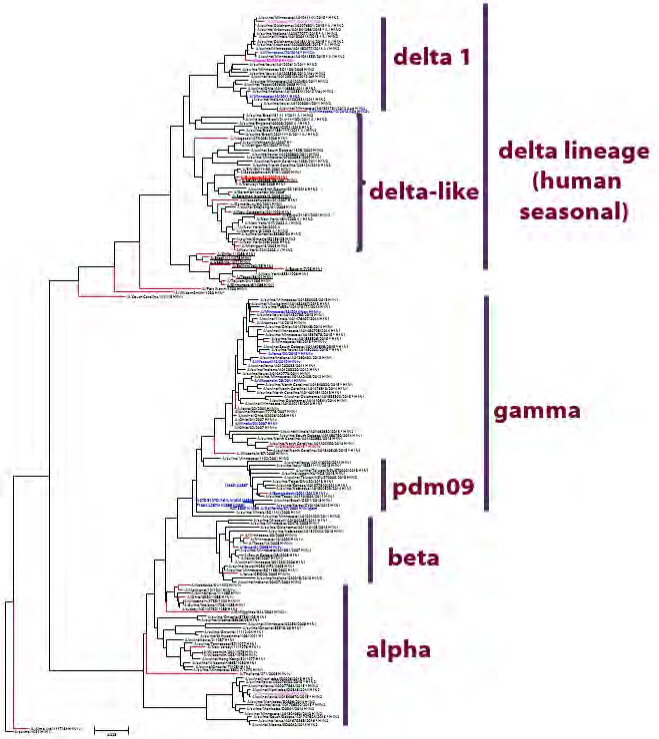
Sun et al. J Virology 2013. Mutant viruses have wt/mut HA and NA on PR8 backbone, 10^5 PFU dose; H1 numbering

CDC

Diversity of North American H1 viruses



Evolution of North American H1 HA



Continuous evolution resulted the generation of genetically distinct clades, leading to many human infections

Pulit-Penaloza et al, manuscript under review.

Importance of HA and NA lineage on virus transmissibility from swine

				Ferret peak morbidity	Log_{10} PFU/ml	Respiratory droplet
Subtype	**year**	**HA**	**NA**	**Weight loss**	**Lung titer d3**	**Trans**
H1N1	2000	swine	swine	5%	3.2	0/2
H1N1	2002	swine	swine	9%	3.05	0/2
H1N2	1999	swine	human	5%	5.3	1/2
H1N1	2005	human	human	5%	4.95	2/2
H1N2	2008	human	human	5%	5.45	2/2

All viruses isolated from swine bearing triple reassortant internal gene cassette

Surface glycoprotein lineage can play a role in virus transmissibility

Barman et al, PLoS Pathogens 2012.

CDC

Virus transmissibility of novel influenza A viruses following exposure to swine

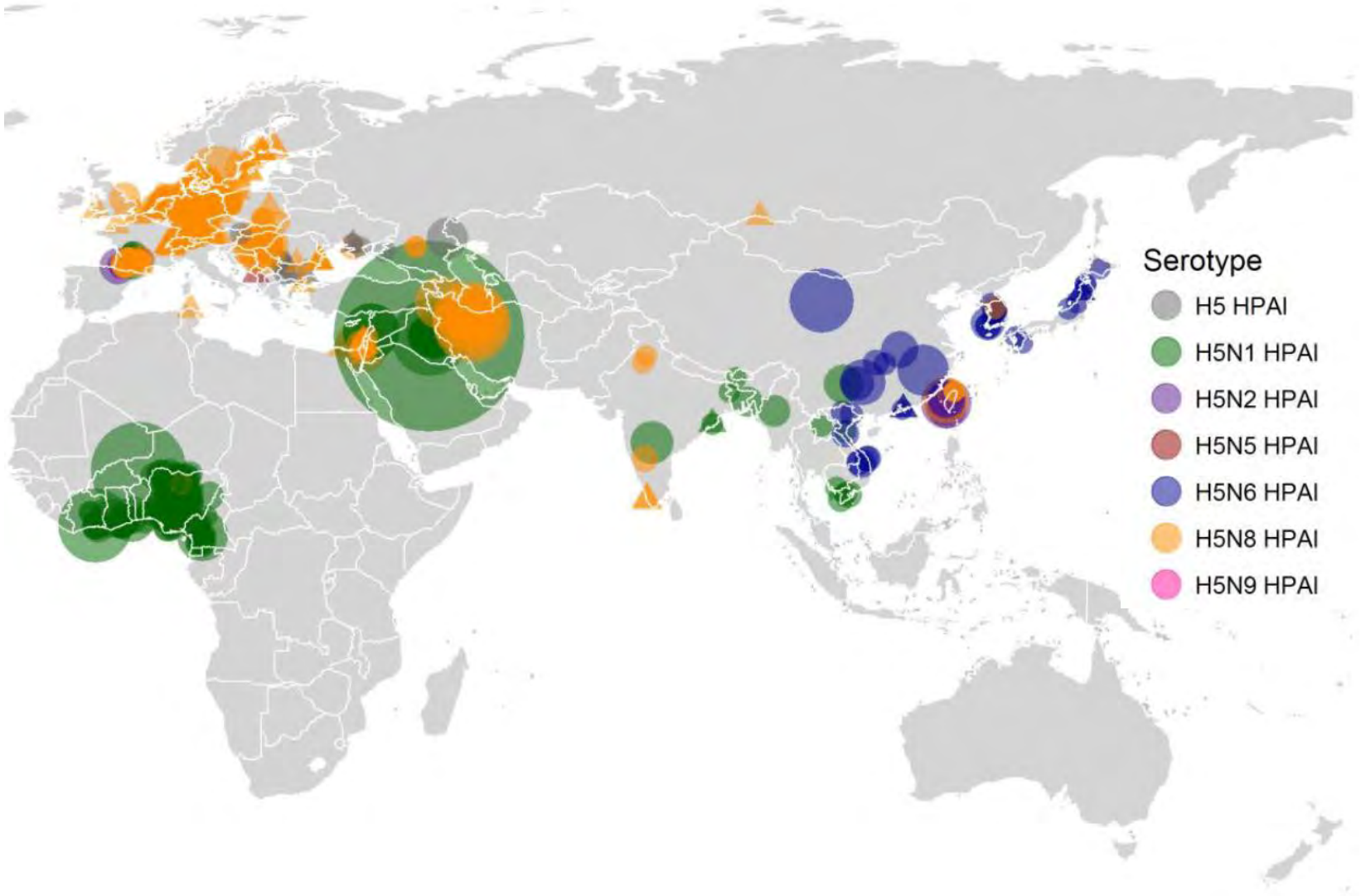
				Ferret peak morbidity	Log_{10} EID_{50} or PFU/ml	Respiratory droplet
Subtype	**Year**	**HA**	**NA**	**Weight loss**	**Lung titer d3**	**Trans**
H1N1 pandemic	1918	swine	swine	11.7%	6.0	3/3
H1N1 seasonal	2008	human	human	4.9%	<1 (0/3)	3/3
H1N1 pandemic	2009	swine	Eur swine	9.9%	4.4	3/3
H1N1v	2015	swine	swine	12.5%	3.2 (1/3)	1/3
H1N1v	2015	swine	swine	5.1%	4.3	2/3
H1N2v	2016	swine	human	14.4%	3.8	3/3
H1N2v	2016	human	human	14.9%	4.1	1/3
H1N2v	2011	human	human	6.0%	3.7	2/3

All viruses associated with human infection
Virus transmissibility in ferrets independent of H1 virus lineage

Pulit-Penaloza et al, manuscript under review; manuscript in preparation.

CDC

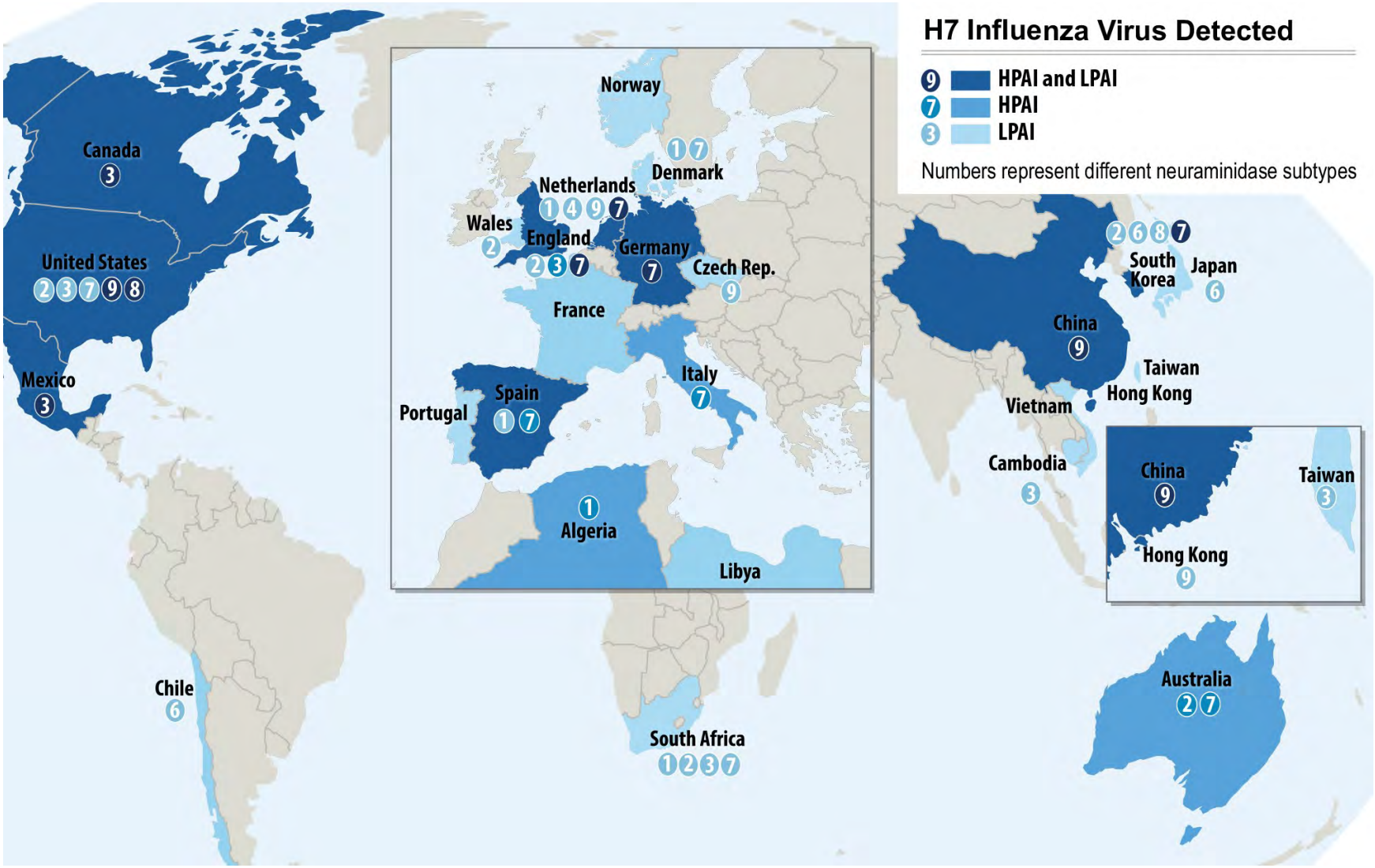
Detection of H5 subtype influenza viruses in avian species



D Jernigan/B Jester

CDC

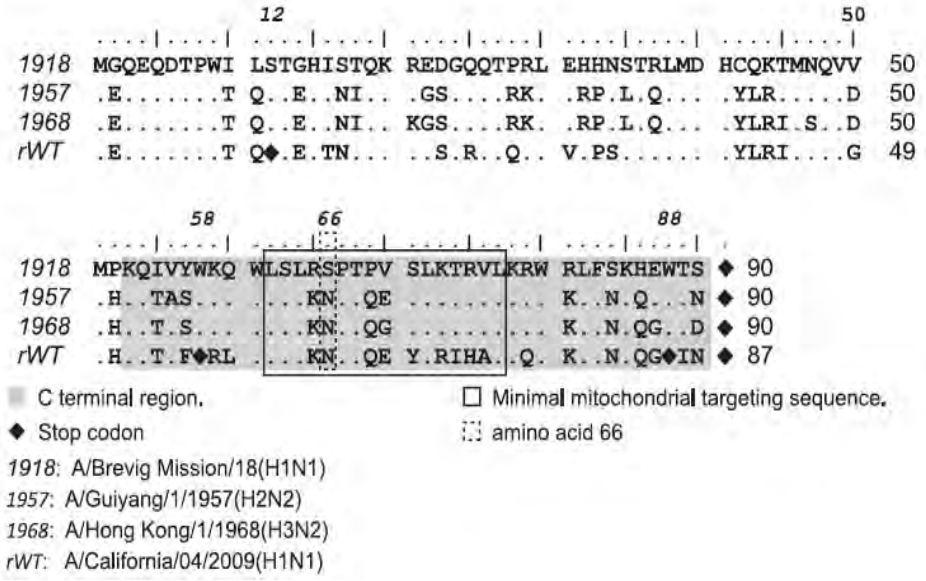
Detection of H7 subtype influenza viruses in avian species



Belser et al, Lancet Inf Dis 2018. Cartographic perfection by Ryan Lash.

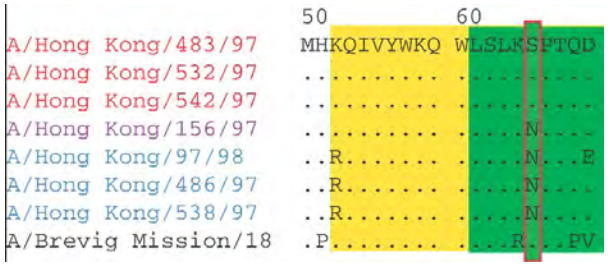
1918, HPAI H5N1, and PB1-F2

- Similarities following infection with 1918 and H5N1 viruses:
  - Increased cellularity in lungs (macrophages and neutrophils)
  - Susceptibility of lung macrophages and dendritic cells to infection
  - High levels of proinflammatory cytokines elicited
- PB1-F2 has known roles in influenza virus virulence
  - Full length vstruncated
  - Presence of virulence-associated residues



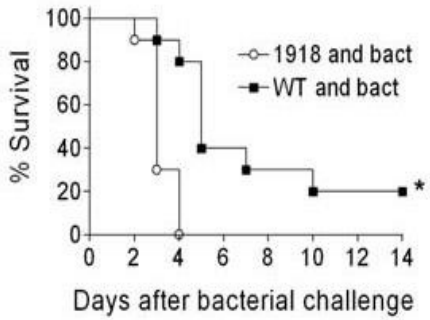
Perrone et al. PLoS Pathogens, 2008; Hai et al. J Virol 2010; Kamal et al. IJMS 2017

1918, HPAI H5N1, and PB1-F2

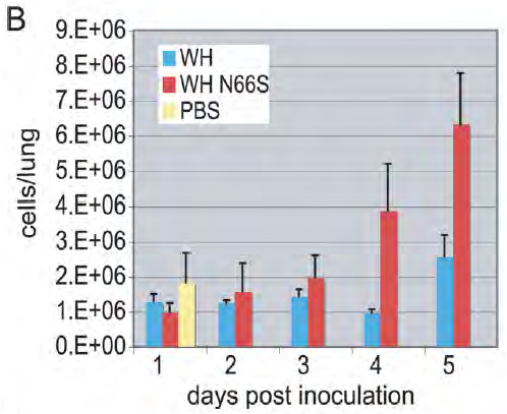


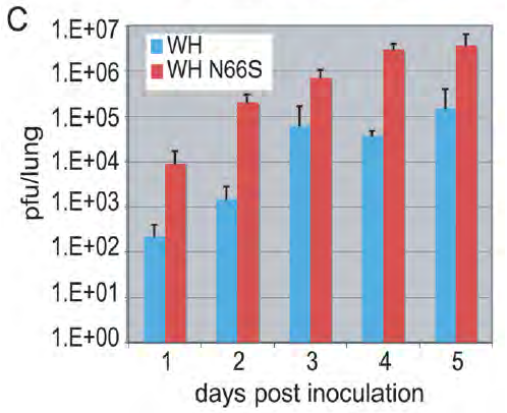
Both 1918 and H5N1 possess single aa substitution in PB1-F2

Virus	aa 66	MLD_{50}	Lung titer d3
1918 WT	S	$10^{2.5}$	$>10^{7}$ pfu/ml
1918 mut	N	$10^{5.25}$	$<10^{6}$ pfu/ml



Expression of 1918 PB1-F2 enhances pathogenesis of secondary bacterial pneumonia in mice





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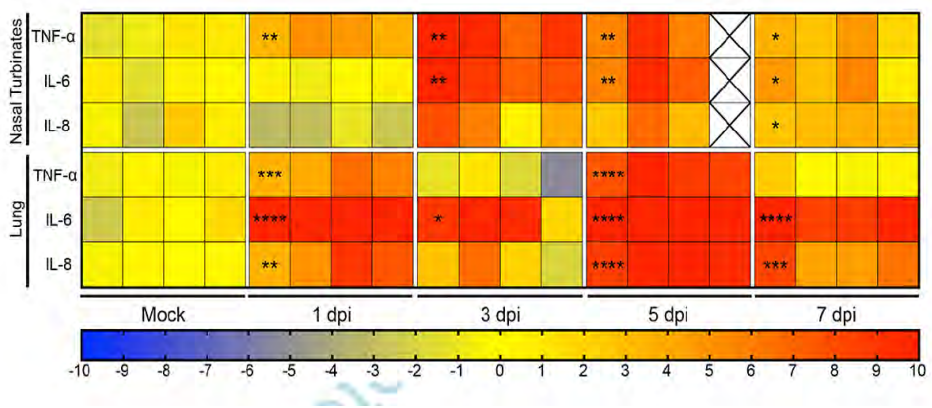
Conenello et al. PLoS Pathogens, 2007; McAuley et al. Cell Host & Microbe, 2007.

Summary and conclusions

- Looking to the past:
  - Archaevirology elucidated the composition of the virus responsible for the 1918 pandemic
  - Role of surface glycoproteins and polymerase genes in both enhanced virulence and transmissibility of the virus
- Looking towards the future:
  - Additional context and understanding of sequence
  - Side-by-side comparisons of 1918 and current pandemic threats
- Research is still ongoing



de Wit et al, J Inf Dis 2018

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