Determinants of human adaptation and gain of pandemic potential

Kanta Subbarao
WHO Collaborating Centre for Reference and Research on Influenza & Department of Microbiology and Immunology, University of Melbourne, Peter Doherty Institute for Infection and Immunity

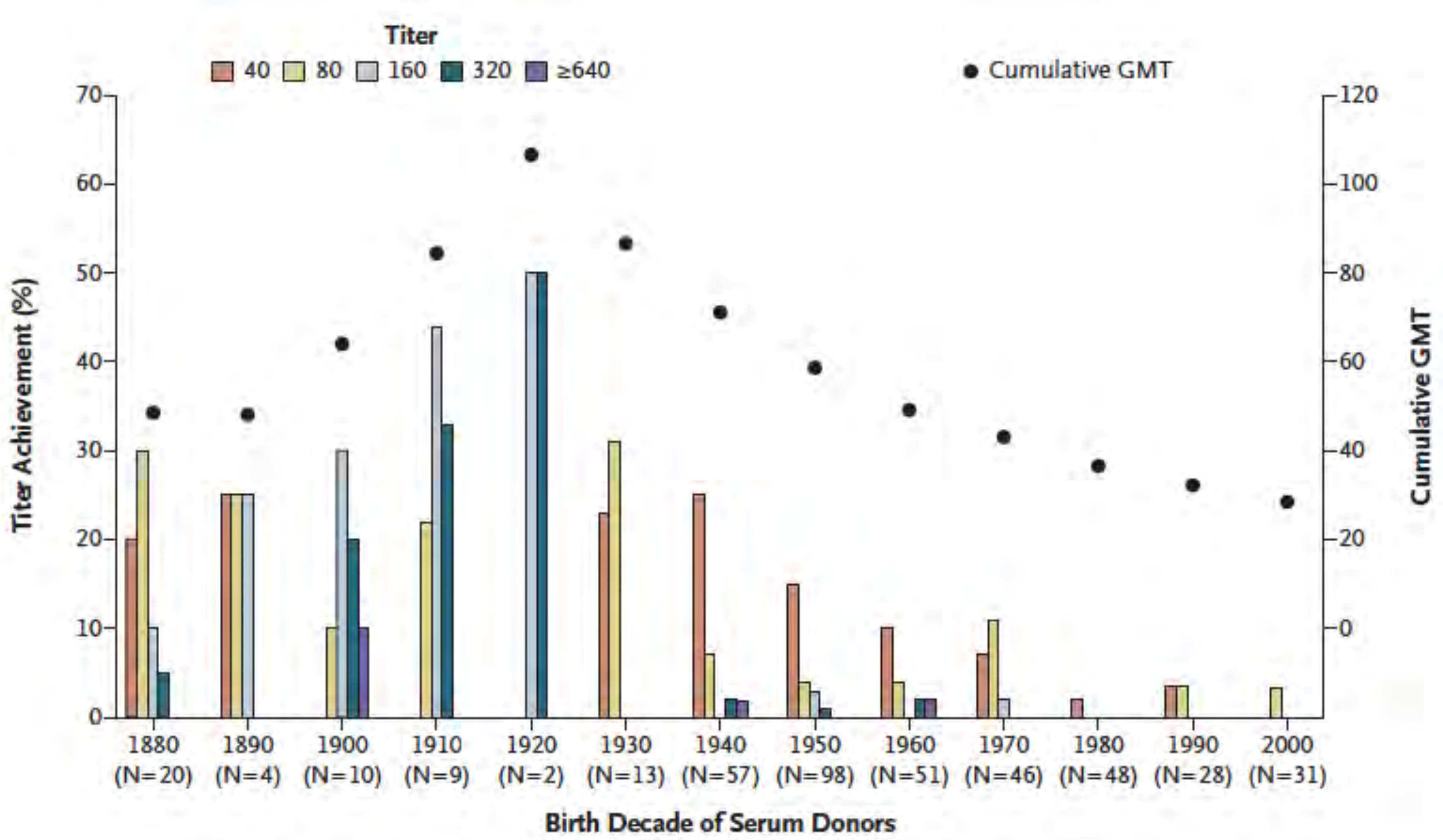
Doherty Institute

WHO Collaborating Centre for Reference and Research on Influenza
VIDRL

Features of a Pandemic Influenza Virus

- Isolation from humans of a novel subtype of influenza, to which the general population has little or no immunity.
- Demonstrated ability of the virus to replicate and cause disease in humans.
- Efficient spread from person-to-person, expressed as sustained chains of transmission causing community-wide outbreaks.

Serosusceptibility to a novel influenza virus

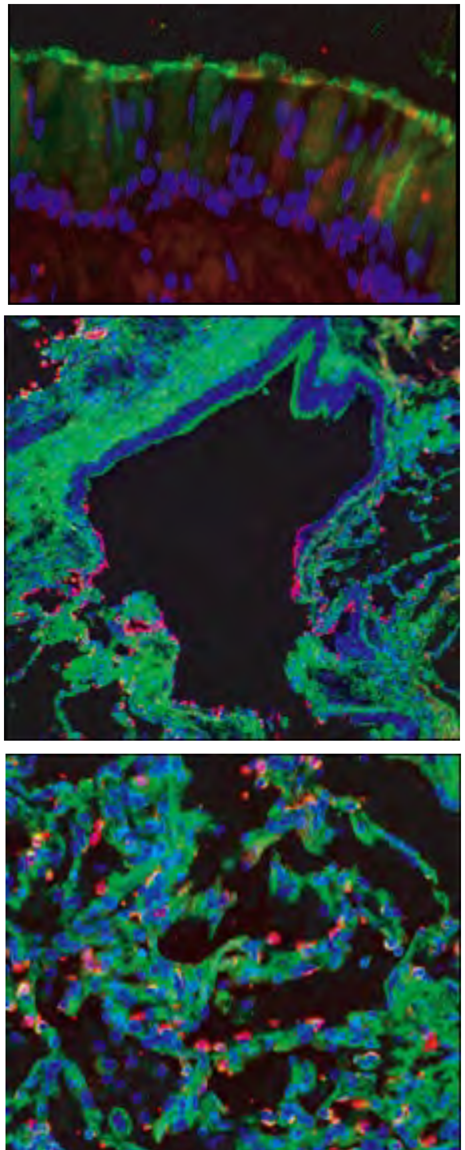


Hancock et al *NEJM* 2009

Sialic Acid Receptor Distribution and Specificity

Reactivity of human respiratory tract tissues with lectins specific for sialic acid linkages

Green α2,6Gal
Red α2,3Gal



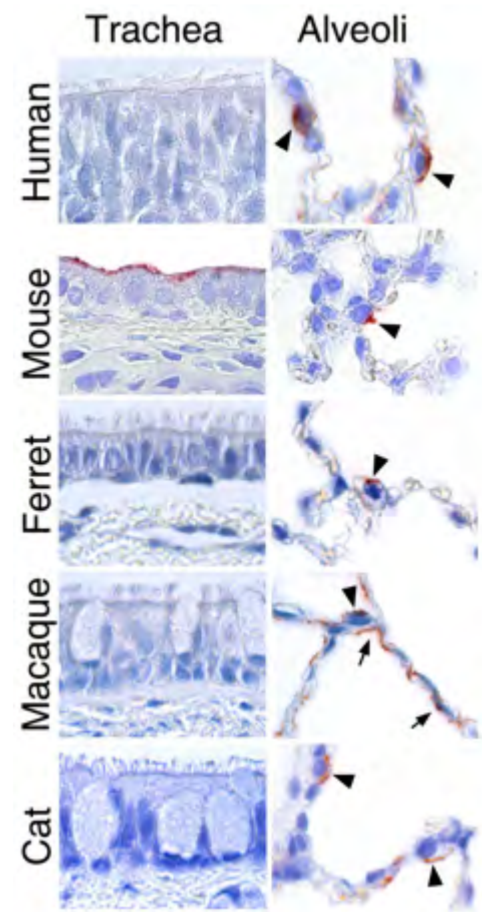
Trachea

Bronchiole

Alveoli

Shinya et al Nature 2006

Staining for H5 Ag



Van Riel et al Science 2006

Ability to replicate and cause disease in humans

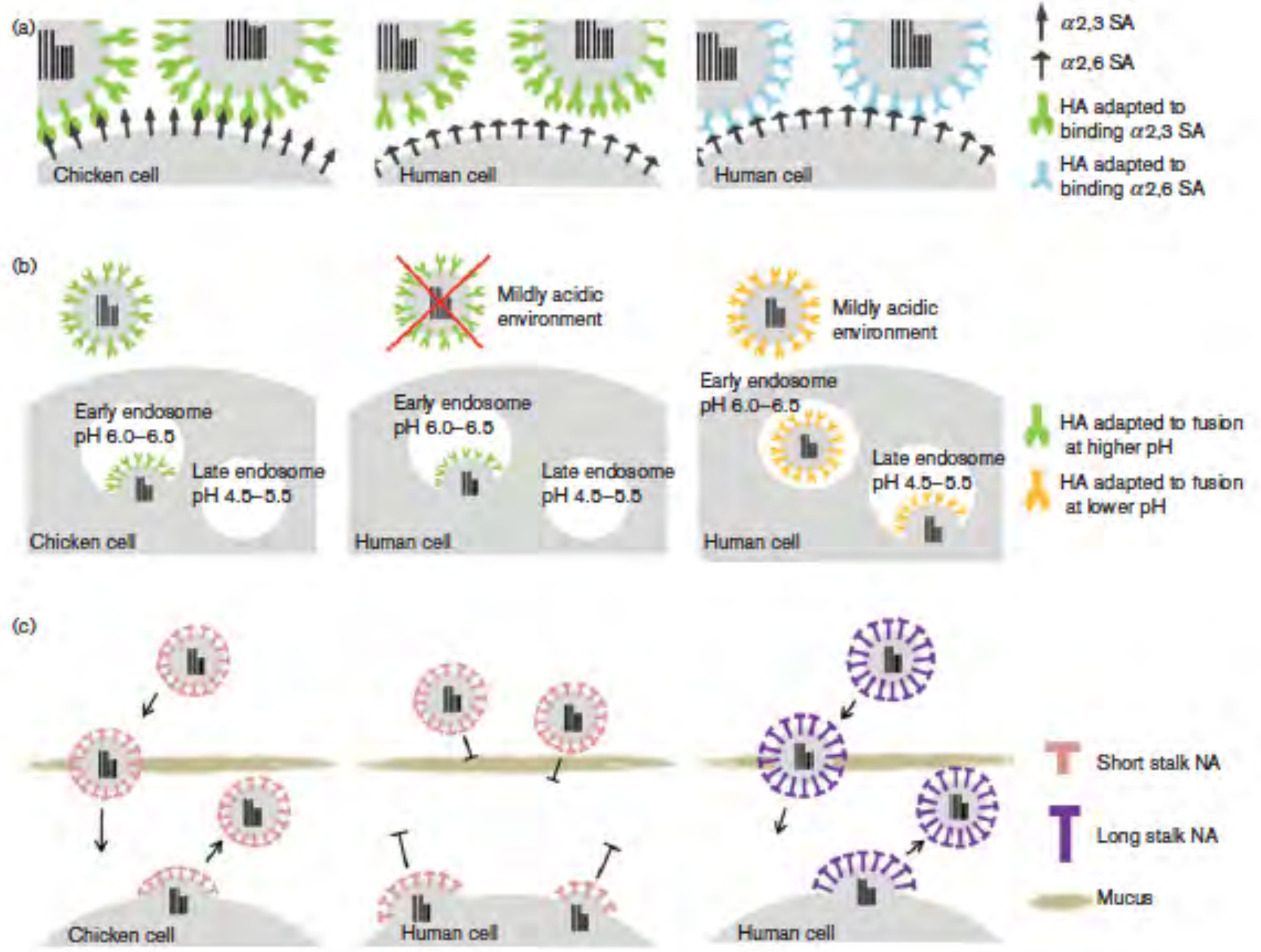
HA

- Receptor specificity
  - Long chain α2,6
  - Q226L and G228S in H3, E190D and D225G in H1
  - Change in receptor specificity alone is not enough

NA

- Shortened NA stalk-reduced release
- HA-NA balance

Characteristics of avian IAV HA and NA that restrict transmission in humans



Cauldwell et al *J Gen Virol* 2014

Ability to replicate and cause disease in humans

HA

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NA

- Shortened NA stalk- reduced release
- HA-NA balance

PB2

- PB2 627 or 701
  - via promoter binding
  - via nuclear transport involving importin α
- G590S+Q591R (H1N1pdm09) or T271A

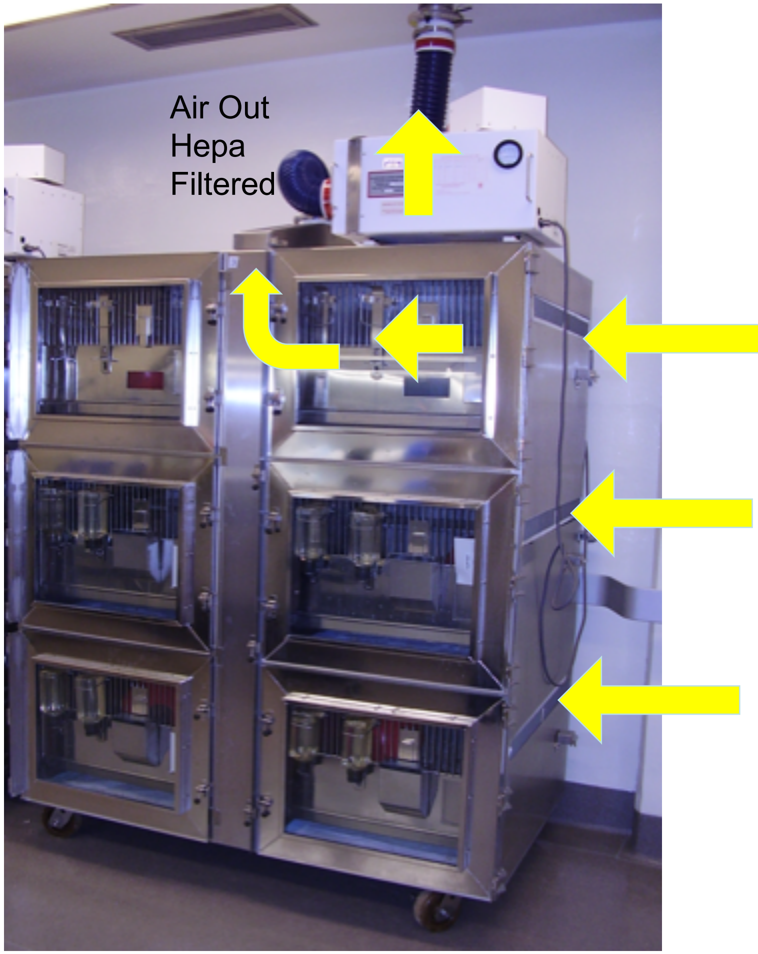
PB1-F2 N66S

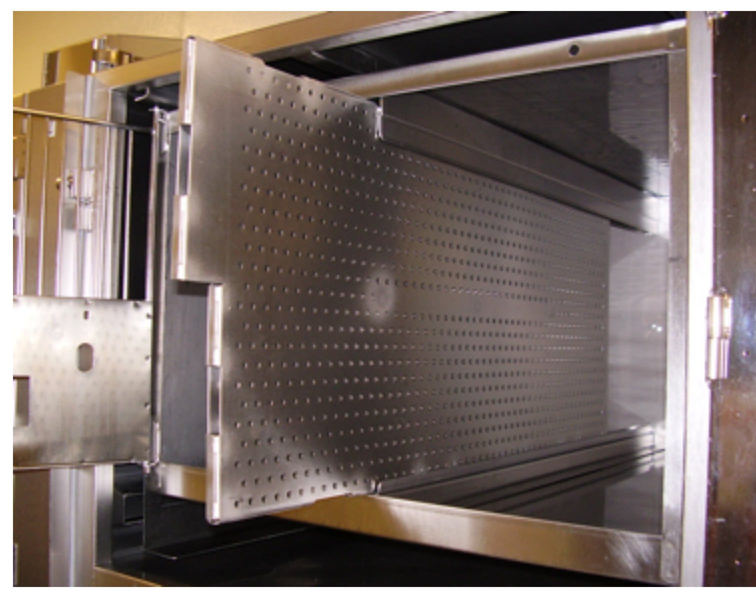
PA-X truncation

NS

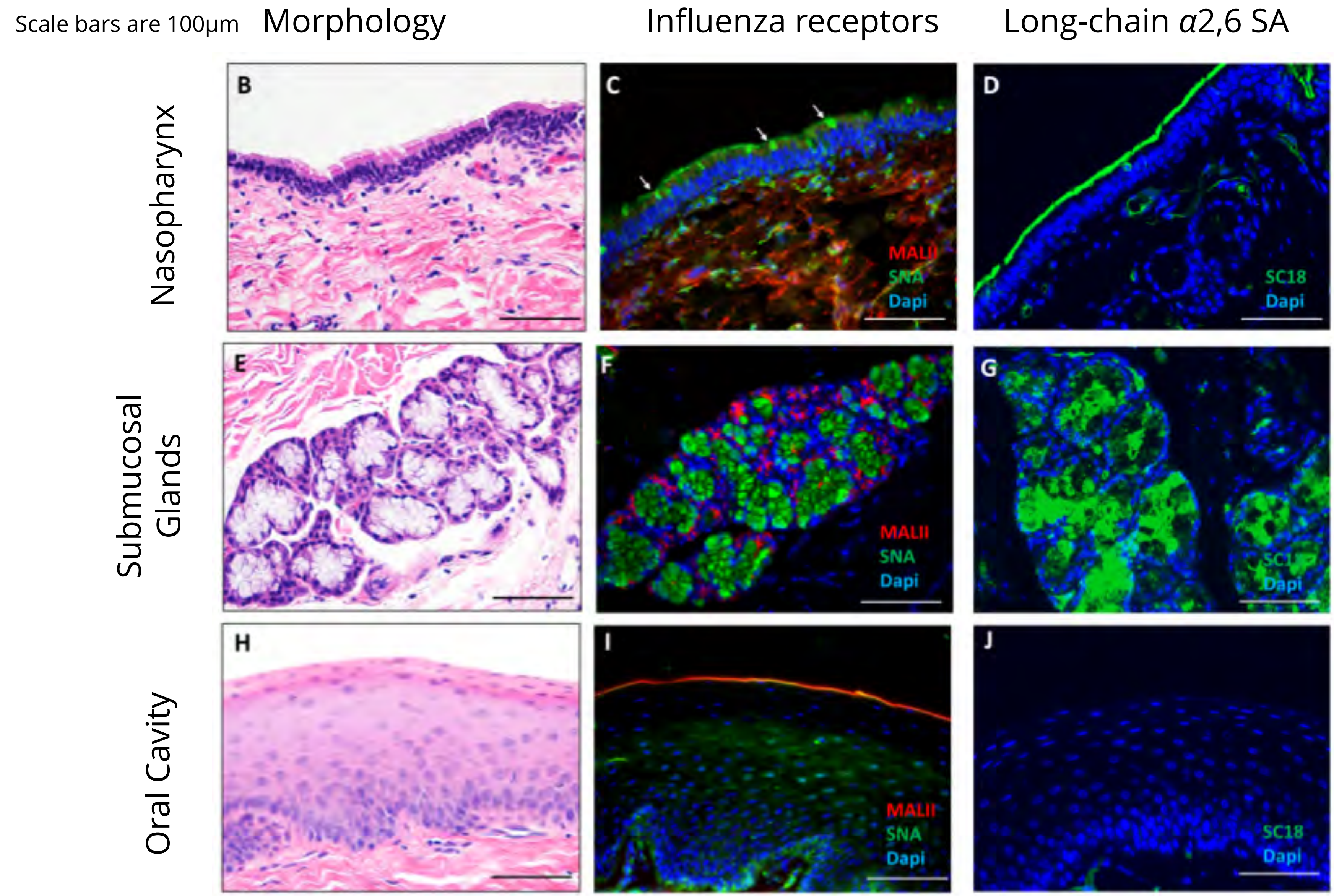
- IFN sensitivity,
- C terminal PDZ binding
- CPSF binding

NIH airborne transmission ferret cage setup





The soft palate is an important site of adaptation and selection of transmissible influenza viruses



Lectins: MALII: α2,3; SNA: α2,6

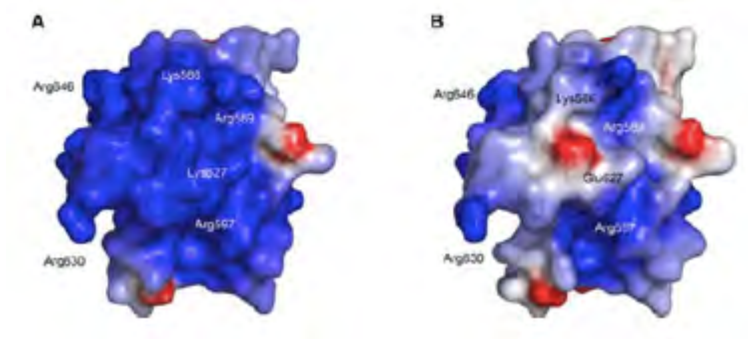
IHC: SC18: long chain α2,6

Lakdawala *Nature 2015*

PB2 627 mediated host range restriction

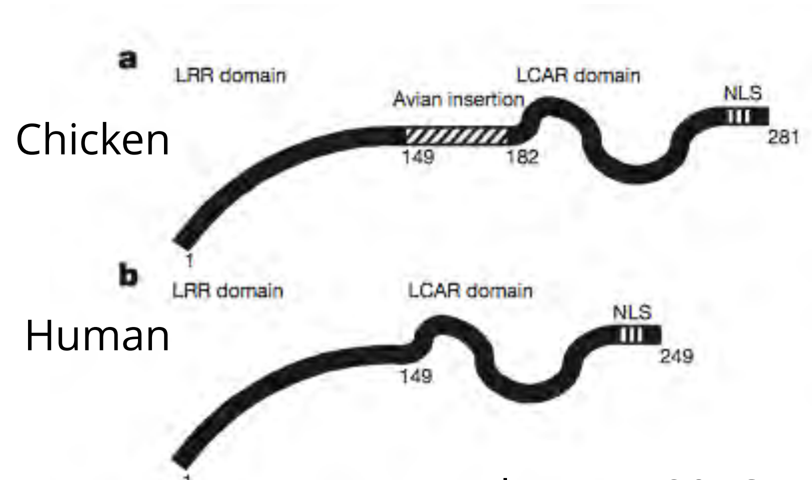
- Interaction with NP or by affecting promoter binding
- Crystal structure of a 538-676 domain shows a unique fold with 627 in the centre of a solvent exposed surface.

627 Lysine 627 Glutamic acid



Tarendeau et al *PPath* 2008

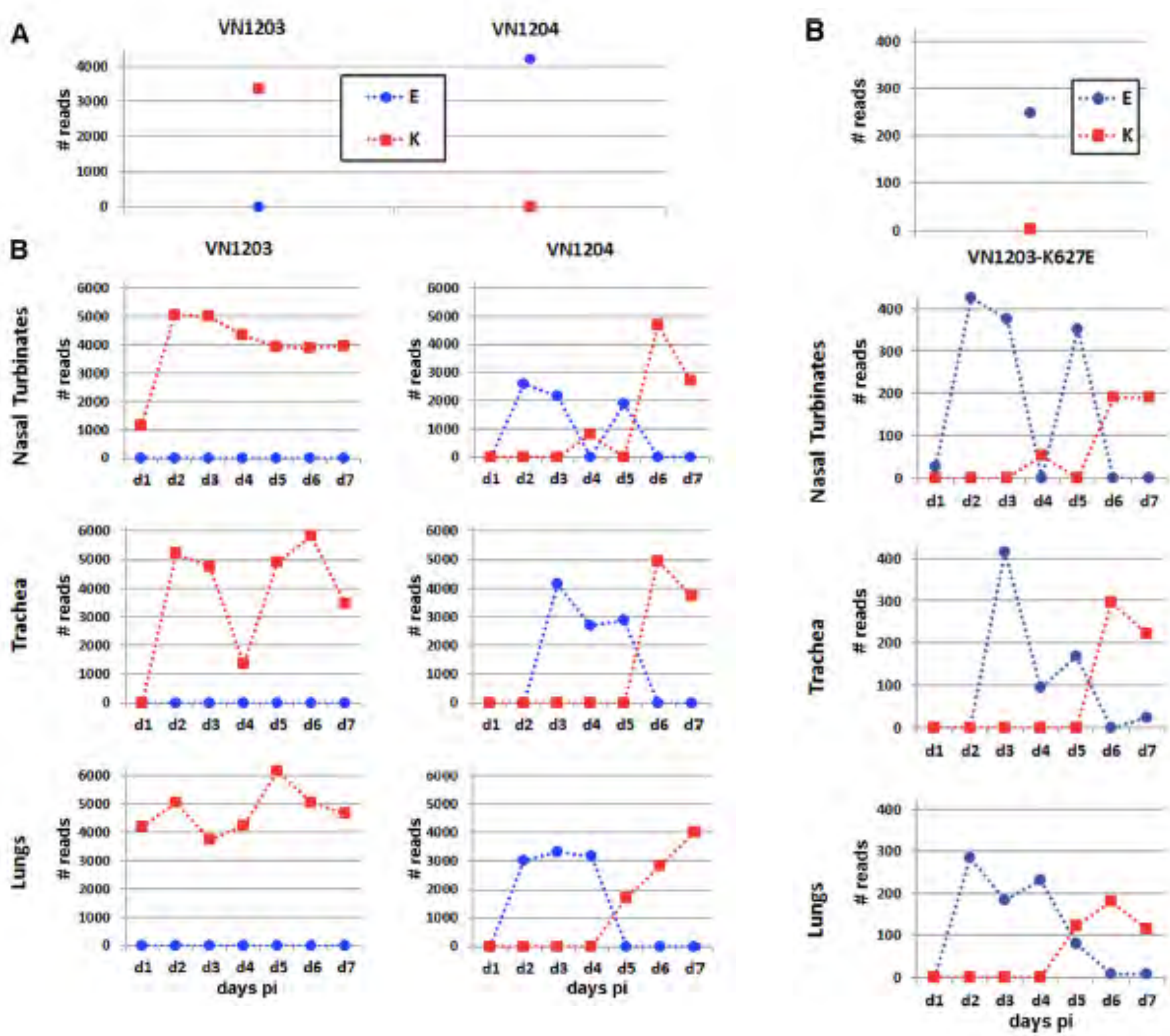
- A species-specific difference in host protein ANP32



Long et al *Nature* 2016

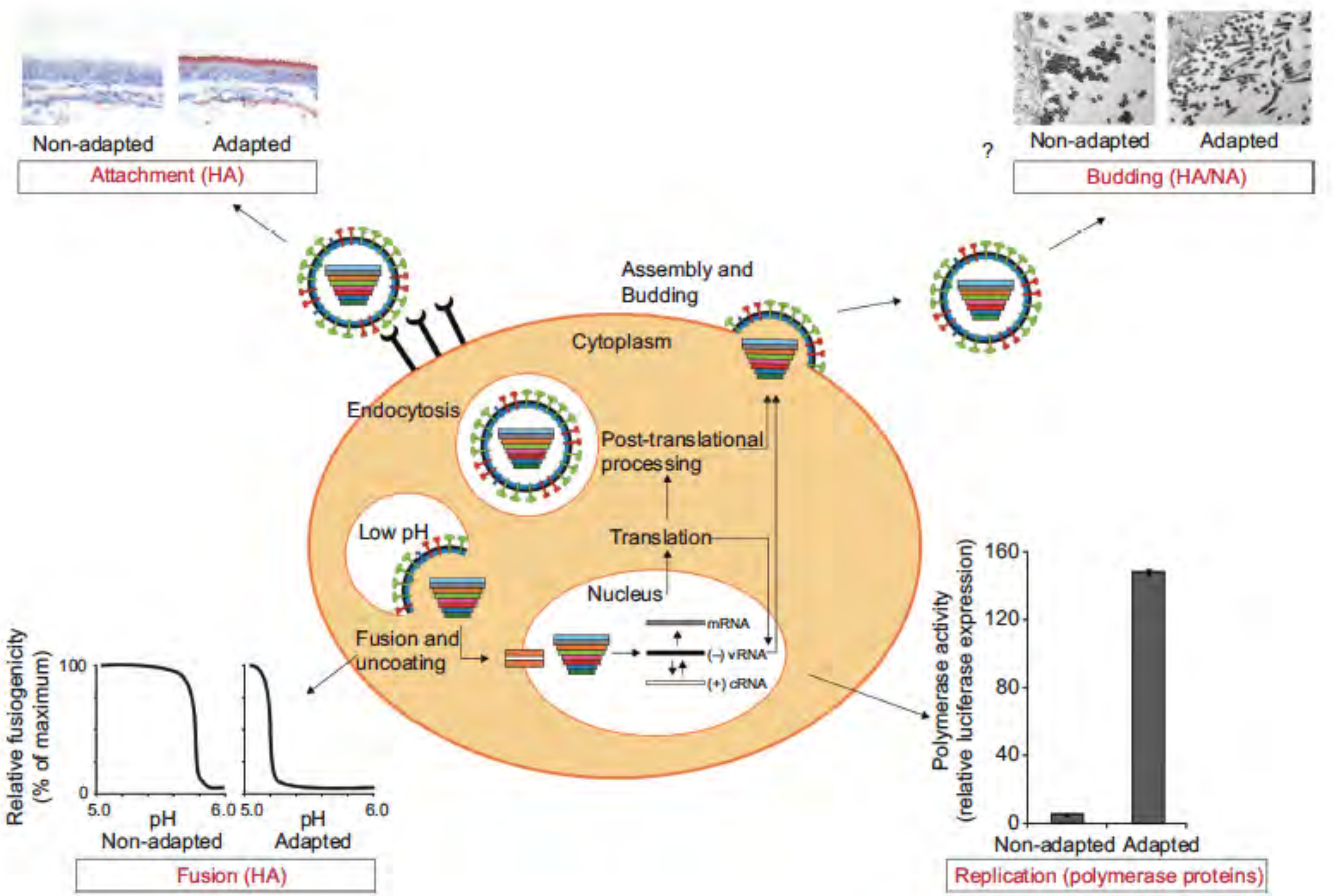
Mammalian adaptation of H5N1 viruses at PB2 627

VN/1203/2004 mutant K627E



Min et al *J Virol* 2013

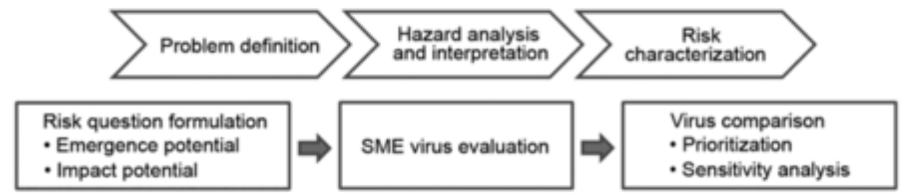
Traits associated with airborne transmissibility in mammals

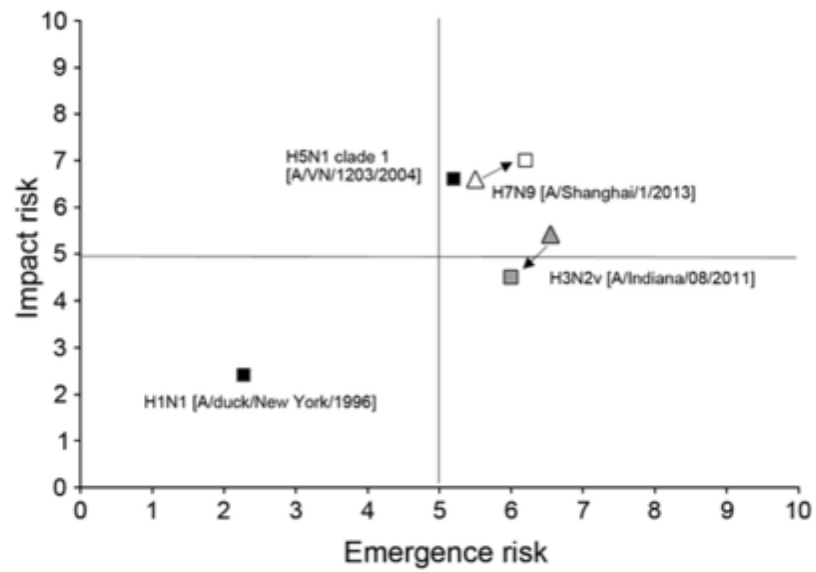


Schrauwen and Fouchier *Emerging Microbes and Infections* 2014

CDC's Influenza Risk Assessment Tool (IRAT)

Category	Risk Element
Virus properties	Genomic variation
	Receptor binding properties
	Transmissibility in animal models
	Antiviral susceptibility
Host properties	Population immunity
	Disease severity
	Antigenic relationship to vaccines
Ecology and Epidemiology	Human infections
	Infections in animals
	Global distribution in animals



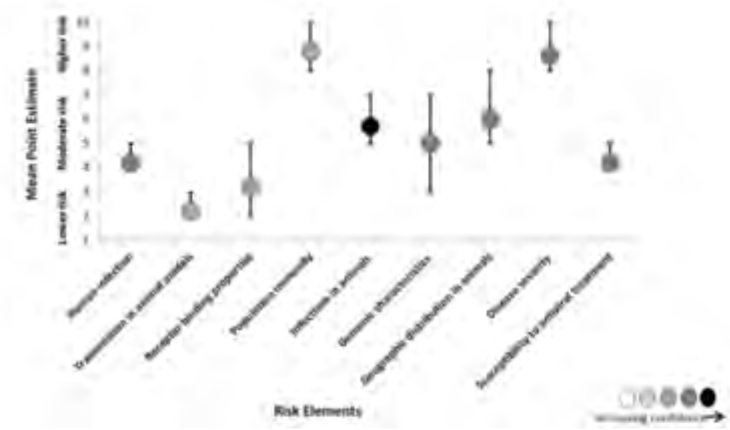


Trock et al *EIDJ* 2015

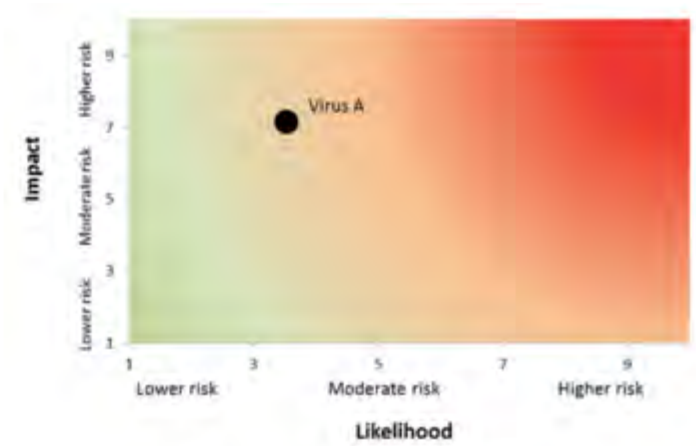
Tool for Influenza Pandemic Risk Assessment (TIPRA)

Question	Rank	Risk element	Weight
Likelihood of sustained human to human transmission of the virus	1	Receptor binding properties	0.408
	2	Transmission in animal models	0.242
	3	Human infection	0.158
	4	Genomic characteristics	0.103
	5	Infection in animals	0.061
	6	Geographic distribution in animals	0.028
Impact to the human population of sustained human to human transmission of the virus	1	Disease severity	0.408
	2	Population immunity	0.242
	3	Antiviral susceptibility	0.158
	4	Genomic characteristics	0.103
	5	Receptor binding properties	0.061
	6	Human infection	0.028

Technical experts provide point estimates & confidence scores- converted to Confidence Coefficient scores



Calculate weighted risk scores



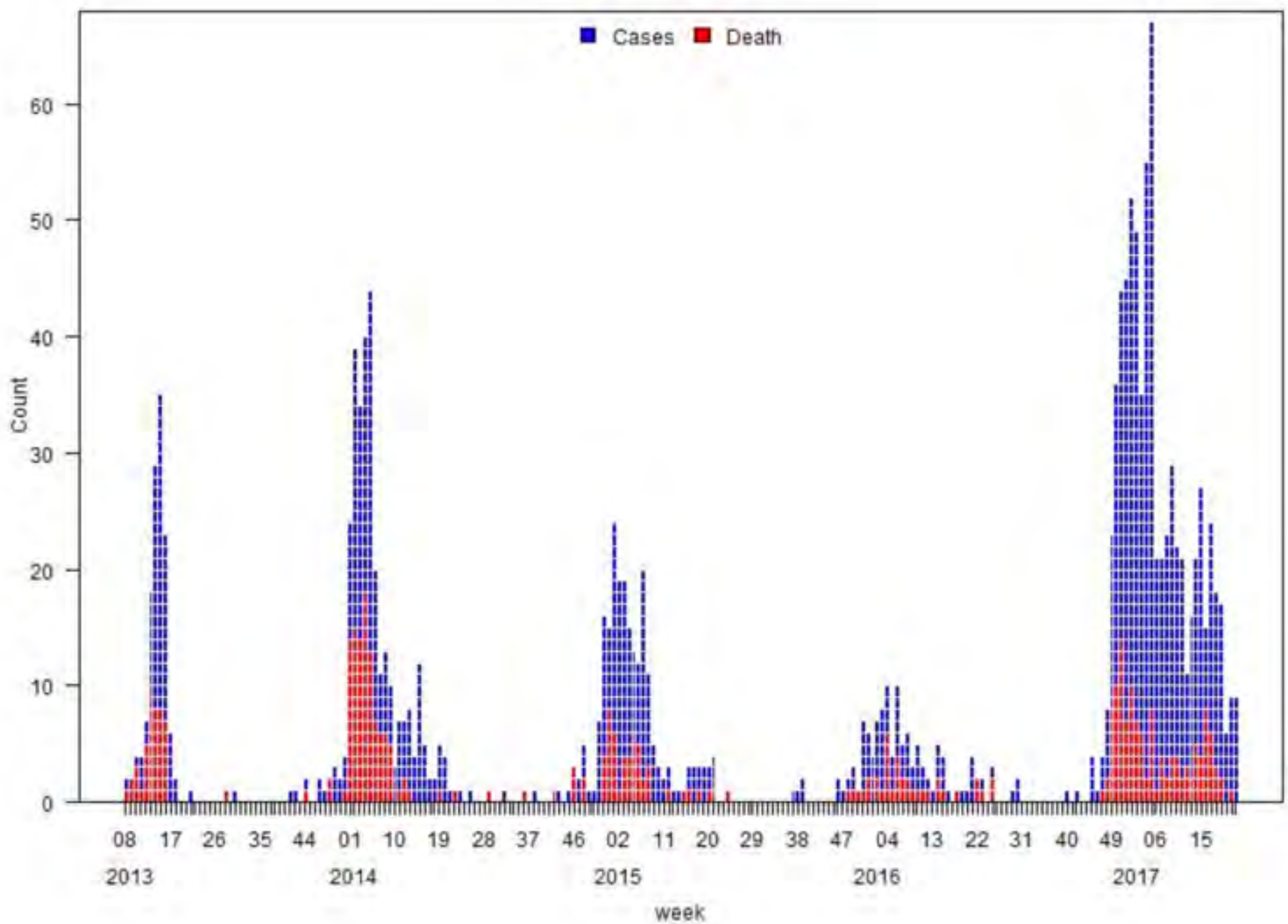
http://www.who.int/influenza/publications/TIPRA_manual_v1/en/

Direct Infection of Humans with Avian Influenza Viruses

Year	Country	Subtype/pathotype	Cases	Fatalities
1959	US	H7N7 HPAI	1	0
1978-79	US	H7N7 LPAI	?	0
1996	England	H7N7 LPAI	1	0
1997	Hong Kong	H5N1 HPAI	18	6
1999	China	H9N2 LPAI	5	0
1999, 2003	Hong Kong	H9N2 LPAI	3	0
2002-03	US	H7N2 LPAI	2	0
2003	Hong Kong	H5N1 HPAI	5	2
2003	Netherlands	H7N7 HPAI	89	1
2004	Canada	H7N3 HPAI	2	0
2004	Egypt	H10N7 LPAI	2	0
2003-2017	16 countries	H5N1 HPAI	859	453
2013-2017	China	H7N9 LPAI	1533	592
2014	Taiwan	H6N1 LPAI	1	0
2014	China	H10N8 LPAI	3	2
2014-2017	China	H5N6 HPAI	16	6

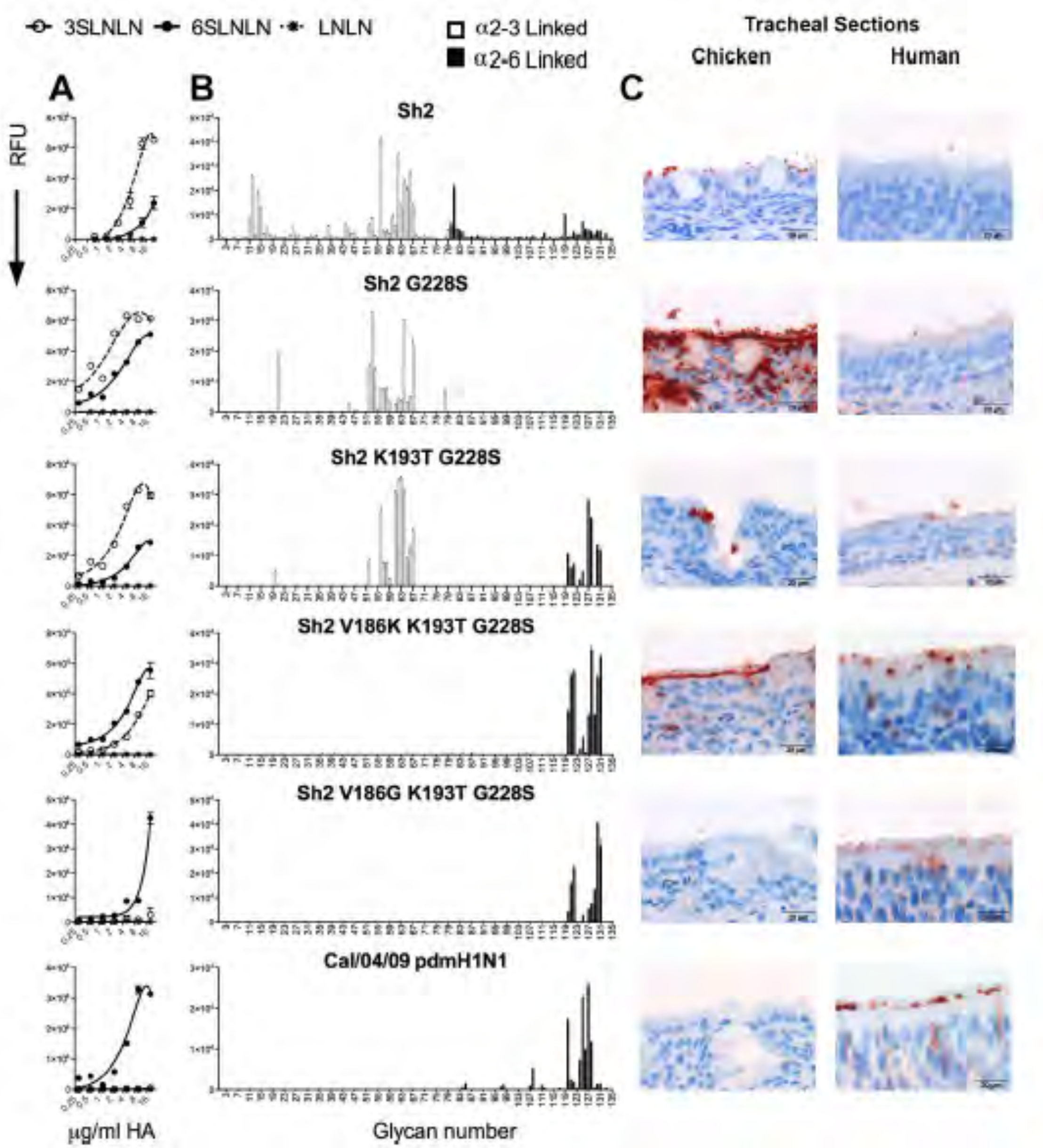
Sources: Perdue & Swayne (2005) Avian Dis 49:317 and EID Weekly Updates (2004) 2(18), 2, WHO

Avian influenza A (H7N9) cases in humans (2013- 2017)



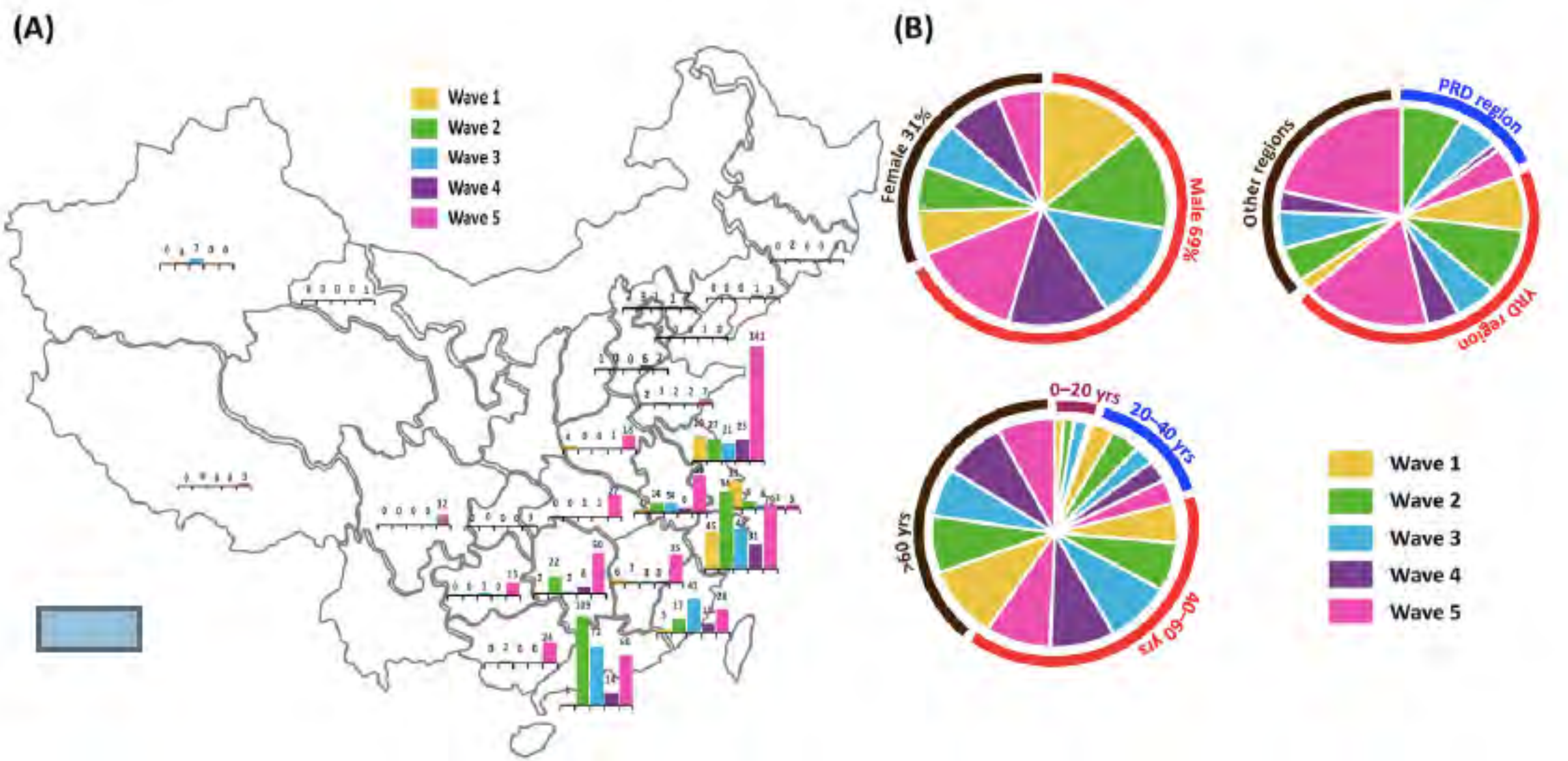
(https://wwwnc.cdc.gov/travel/notices/watch/avian-flu-h7n9)

3 amino acids that can change the receptor binding of H7N9 viruses



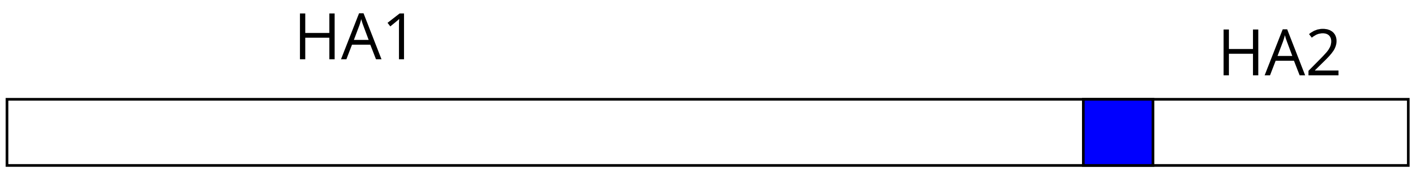
De Vries *PPath* 2017

H7N9 virus distribution over 5 waves in China



Su *Trends in Microbiol* 2017

The Influenza H5 Haemagglutinin Gene



Avirulent ....RETR*GLF

Highly pathogenic H5 and H7 ....RKKR*GLF

1997 HK human isolates ...RERRRKKR*GLF

2003/7 human H5 isolates ...RERRRKKR*GLF

The presence of multiple basic amino acids adjacent to the HA cleavage site increases the tissue range of the virus in birds

Is the HA multibasic site a critical virulence motif for humans?

- In mice and ferrets, the presence of the H5 HA MBS was associated with
  - lethality
  - significantly higher virus titers in the respiratory tract,
  - virus dissemination to extrapulmonary organs
  - lymphopenia
  - significantly elevated levels of pro-inflammatory cytokines and chemokines
  - inflammation in the lungs
- In AGMs,
  - neither H5N1 nor ΔH5N1 virus was lethal and neither caused clinical symptoms.
  - The H5 HA MBS was associated with mild enhancement of replication and delayed virus clearance.

Thus, the contribution of H5 HA MBS to the virulence of the HPAI H5N1 virus varies among mammalian hosts and is most significant in mice and ferrets and less remarkable in nonhuman primates.

Suguitan et al *J Virol* 2012

Molecular characteristics of a human HPAI H7N9 isolate*

Strain	HA			NA	M2	PB2			PA
	321–331†	G186V‡	Q226L‡	R292K§	S31N¶	E627K#	K526R**	K702R††	V100A††
A/Guangdong/ 17SF006/2017 (H7N9)‡‡	PEVPKRKRTAR/ GL	V	Q	K	N	K	K	K	V
A/Guangdong/ 17SF003/2016 (H7N9)	PEVPKRKRTAR/ GL	V	Q	K	N	E	K	R	V
A/Taiwan/1/2017 (H7N9)	PEVPKRKRTAR/ GL	V	Q	K	N	K	R	K	A
A/Environment/ Guangdong/ C16283222/2016 (H7N9)	PEVPKGKRTAR/ GL	V	Q	R	N	E	R	K	A
A/Fujian/3/2016 (H7N9)	PEIPKG—-R/GL	V	L	R	N	K	R	K	A
A/Zhejiang/3/2017 (H7N9)	PEIPKG—-R/GL	V	L	R	N	K	K	K	V
A/Netherlands/ 219/2003 (H7N7)	PEIPKRRRR/GL	G	Q	R	S	K	R	K	A

*HA, hemagglutinin; NA, neuraminidase; NAI, neuraminidase inhibitor; M2, matrix protein 2; PA, polymerase acidic; PB2, polymerase basic 2.
†Cleavage site.
‡Receptor binding site.
§Associated with NAI drug resistance.
¶Associated with amantadine resistance.
#Associated with increased virulence in mice.
**Associated with enhanced viral replication in mammalian hosts.
††Species-associated signature position.
‡‡Virus from patient in this report.

*A/Guangdong/17SF006/2017

Ke et al *EIDJ* 2017

An HPAI H7N9 is more virulent than the LPAI H7N9 virus in mammalian models

Virulence in animal models:

- Mice: Both HPAI and LPAI are lethal for mice; titers of HPAI>LPAI in RT; HPAI spread to the brain
- Ferrets: Both HPAI and LPAI replicated to high titers in the resp tract; HPAI cause more severe lung lesions than LPAI; both spread to the brain
- Cynomolgus macaques: Both HPAI and LPAI caused no/mild clinical signs; replicated similarly

Transmissibility in ferrets: No difference in transmissibility but some HPAI experimentally-infected and recipient animals died.

GD/3, isolated from a fatal human case treated with oseltamivir
+ recombinant GD/3 with and without NAI resistance mutation

Imai et al *Cell Host & Microbe* 2017

Caveats in pandemic risk assessment

- Difficulty in applying genetic data for risk assessment due to context (Herfst mSphere 2018) - used 3 phenotypic assays for H5N6 viruses that were associated with transmissibility of H5N1 viruses by the airborne route in ferrets:
  - receptor binding using resialylated RBCs,
  - HA acid stability
  - polymerase activity
- Difficult to get quantitative data from ferret transmission studies
- Virulence and transmissibility may not be linked - in animal models or in people (PISA)

Indicators to describe influenza severity

INDICATOR	DESCRIBES	INFLUENCED BY	INFORMED BY
Transmissibility 	How many people in a population get sick from influenza on a weekly basis	• Ease of movement of virus between individuals (virus shedding, viral replication, and viral binding) • Immunity and vaccination status • Age, contact patterns and health-seeking behaviour • Climatic factors	Routine surveillance parameters
Seriousness of disease 	How severely sick individual people get when infected with the influenza virus	• Virus factors • Host factors • Context (e.g. access to health care and availability of ventilators)	Hospital-based surveillance parameters
Impact 	How the influenza epidemic or pandemic affects the health-care system (and society)	• Public health interventions • Health-care use • Public concern	• Hospital-based surveillance • Vital statistics (e.g. death records) • School and work absenteeism

Pandemic Influenza Severity Assessment (PISA): A WHO guide to assess the severity of influenza epidemics and pandemics. Geneva: World Health Organization; 2017.

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