Avian influenza A (H5N1): Where did it come from & where is it going!!



H5N1: First detected in Hong Kong 1997

Expect the unexpected !!

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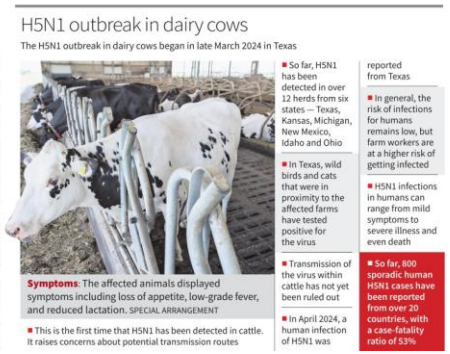
HKU Med
LKS Faculty of Medicine
HKU-Pasteur Research Pole
香港大學-巴斯德研究中心

Influenza A H5N1 detected in dairy cows in 6 states in the U.S.

The exact extent of the spread of H5N1 in cows has not yet been ascertained since cattle are not routinely tested for avian influenza and the symptoms have been relatively mild

Bani Jolly
Vinod Scaria

Avian influenza (bird flu) is a highly contagious viral infection that primarily affects birds. An emerging new lineage 2.3.4.4b of avian influenza has been spreading across the globe since late 2020, carried by migratory birds following specific routes. This panzootic has a significant effect on the avian population with disastrous consequences to the ecology and significant economic loss affecting poultry across the world. In rare instances, the virus can infect mammals from birds causing spillovers, and in recent years, several such instances of spillovers

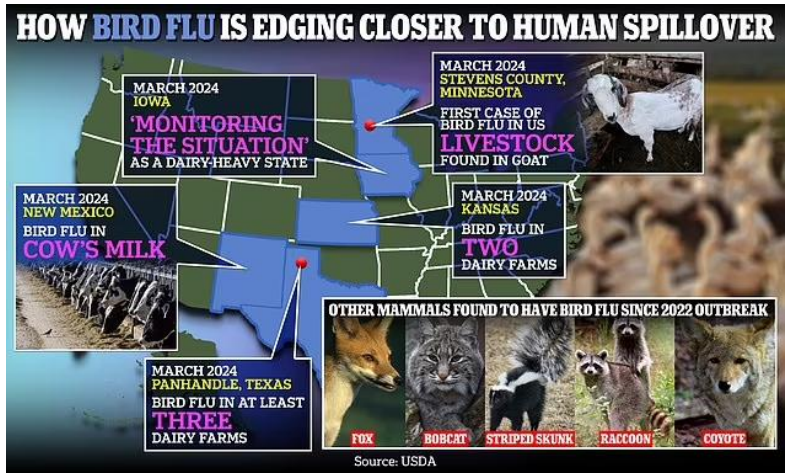


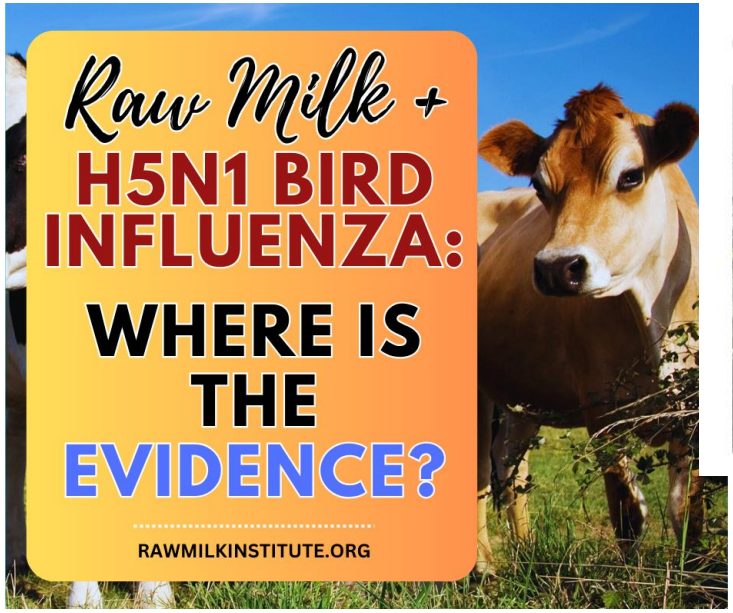
ly one resulting in death.

Researchers sequenced the H5N1 virus from both the infected Texas cows and the human case and found that both viruses belonged to clade 2.3.4.4b of H5N1, with the human strain having one minor mutation potentially linked to adaptation in mammals. However, this change has not led to increased transmissibility among humans, and the overall public health risk remains low according to the CDC. Since late 2021, H5N1 clade 2.3.4.4b has circulated in wild birds in the U.S.

Globally, 2023 also saw potential spillover events with H5N1 linked to the deaths of seals in Russia and infections in marine







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Animal apocalypse: Deadly bird flu infects hundreds of species pole-to-pole

Sharon Guynup
6 Aug 2024 Global Planetary Boundaries

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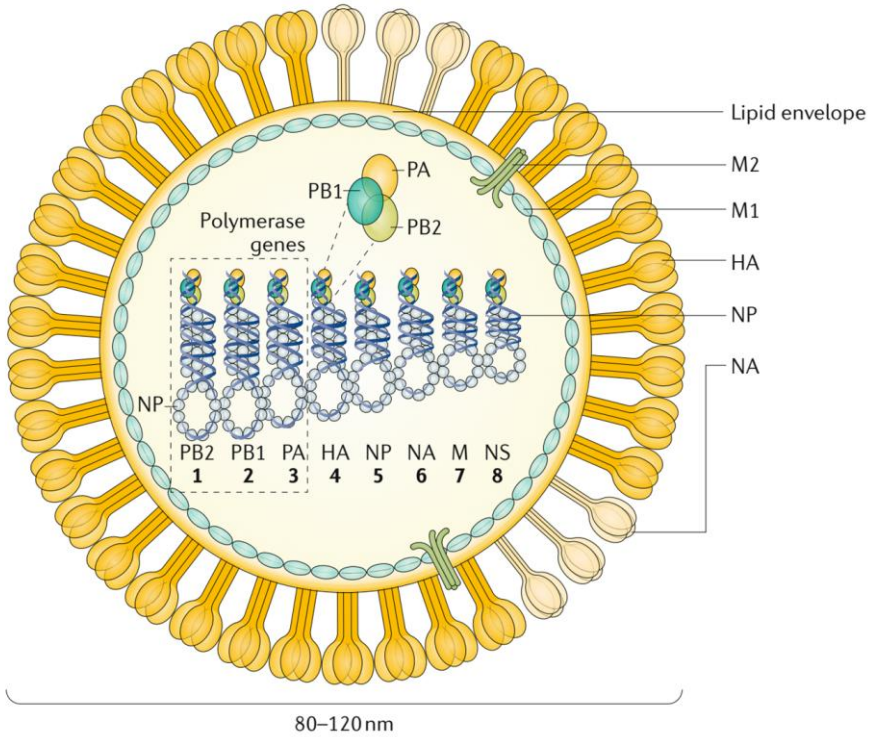


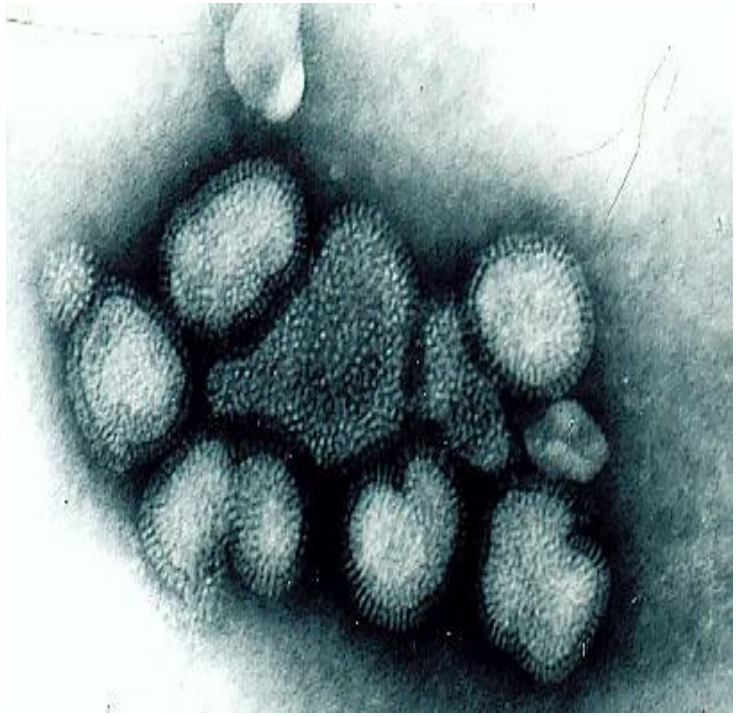
Outline

- Pandemic influenza emergence and impact
- Avian influenza in humans – pre 1997
- H5N1 avian influenza outbreak in Hong Kong in 1997 and responses
- Wider recognition of H5N1 influenza threat
- H5N1 influenza takes wing !
- Recent spread to Americas, marine mammals and dairy cows

Influenza viruses

- Types A (human, animal), B (human, Victoria, Yamagata now extinct), C (human, animal); D (from cattle, pigs, sheep, goats)
- Pandemics only from type A
- segmented genome; *ss RNA (single stranded, negative sense)*





Influenza A subtypes

Haemagglutinin H1-19 ***(17,18 in bats)***

Neuraminidase N1-11 ***(10,11 in bats)***

Influenza nomenclature:
type / (host) / where isolated / lab number / year of isolation [HN subtype]

- A / chicken / New York / 14009 / 1993 [H5N2]
- **A/California/4/2009 [H1N1]** - a human virus does not need species name specified

Influenza A

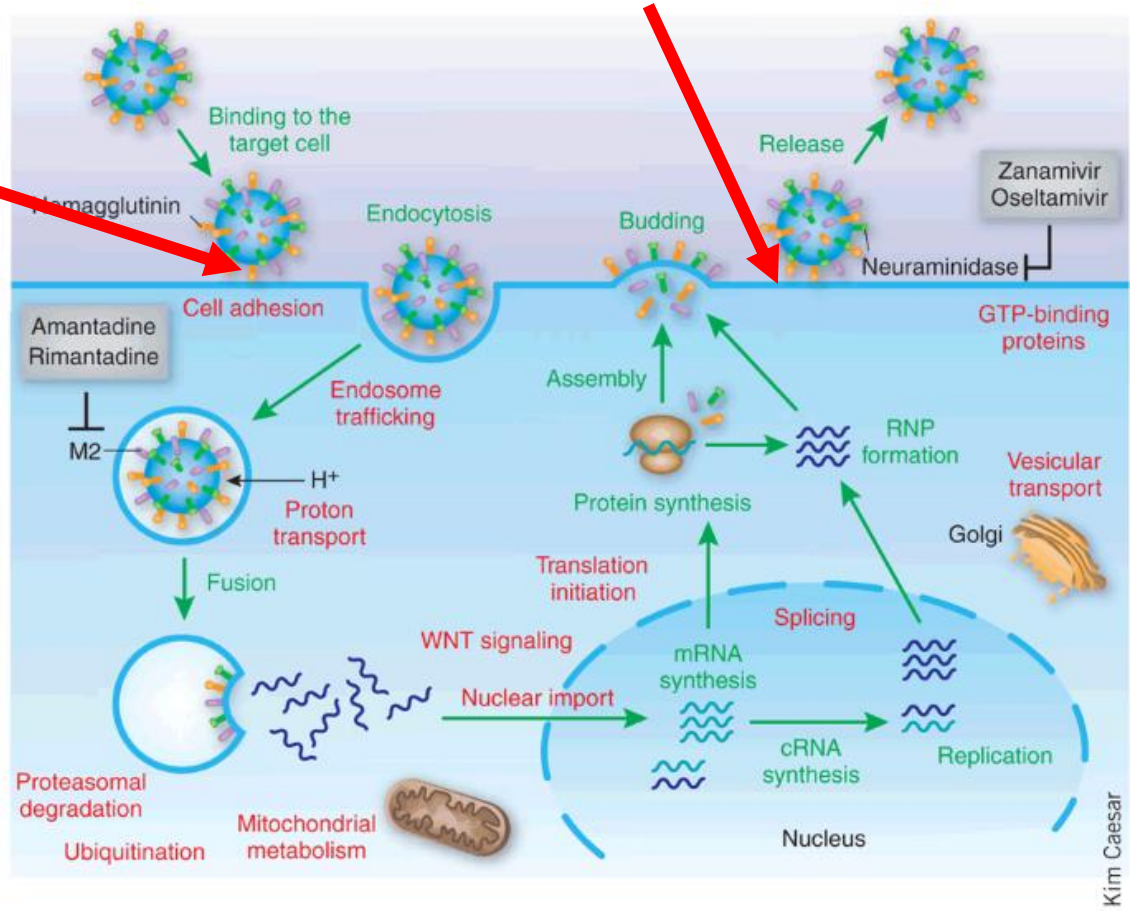
Haemagglutinin

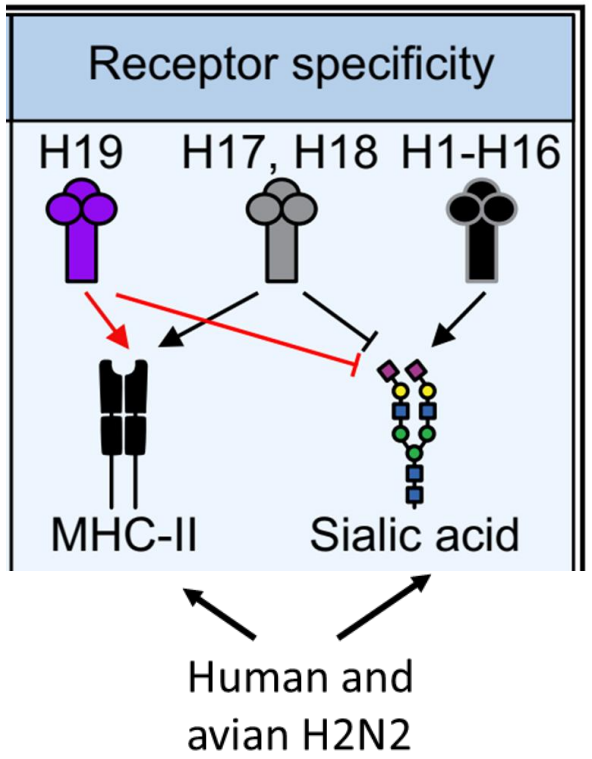
binds to cell receptor in host cell – sialic acid (sialyl-oligosaccharides).

Neuraminidase (sialidase):
Cleaves terminal sialic acid from various glycoconjugates.
Releases the virus from infected cell.
Allows virus to spread through the mucin of the respiratory tract.



H17 and H18:
Yellow-shouldered bat found in Guatemala. Photo credit: CDC/OID/NCEZID – Amy T. Gilbert.
Use MHC-II as receptor, NOT sialic acids





Fereidouni S, et al. Emerg Microbes Infect. 2023 12(2):2225645.
Karakus U et al Cell Host Microbe 2024; 32: 1089
Karakus et al Nat Microbiol 2024; 9:2626

New World Bats Harbor Diverse Influenza A Viruses

Tong et al PNAS 2012
Tong et al PLoS Path 2013
Wu et al Trends in Microbiol 2014



Artibeus planirostris
New world fruit bats

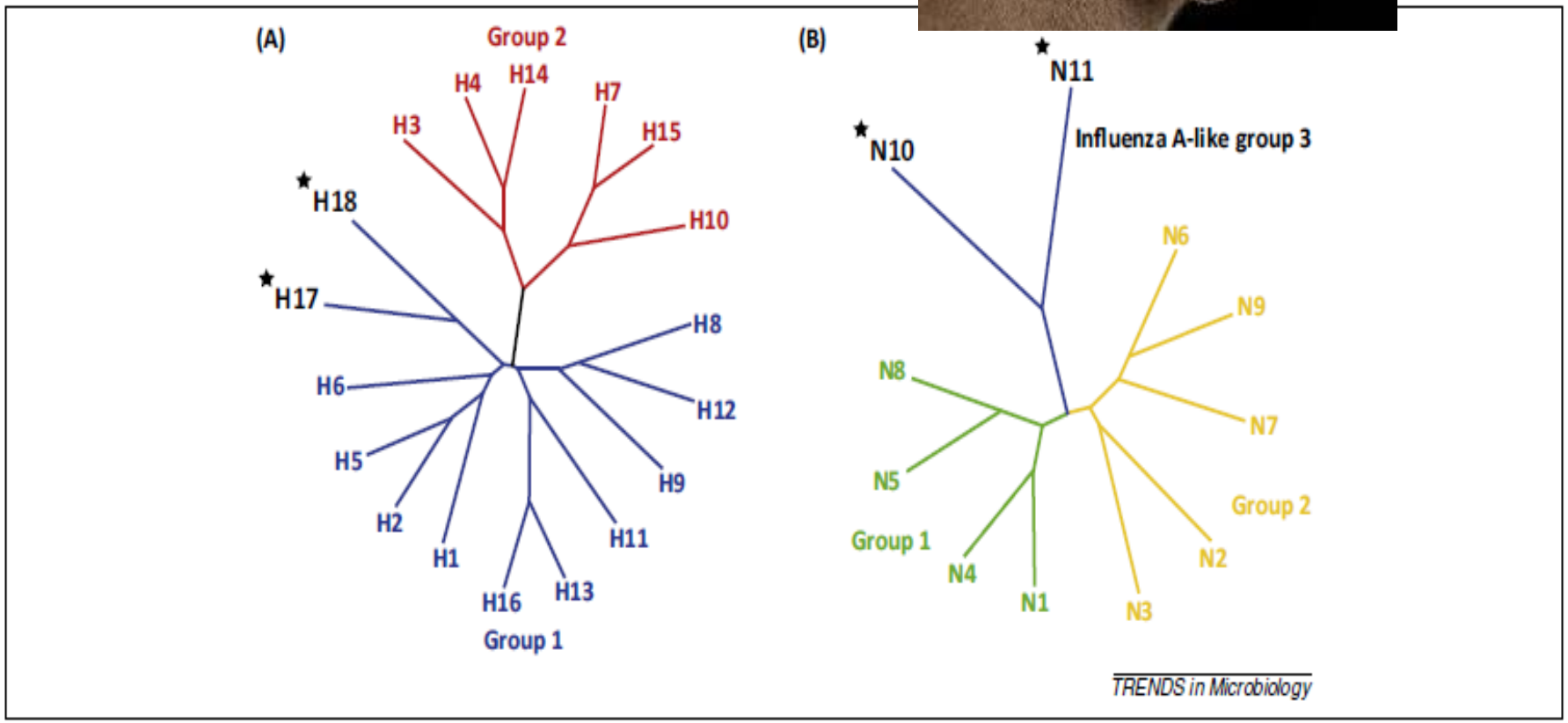


Figure 1. Phylogenetic trees of the hemagglutinin (HA) and neuraminidase (NA) genes of all known influenza A virus subtypes including H17/18 and NA-like N10/11. **(A)** The categorized HA molecules can be grouped into two groups, group 1 and group 2. The bat-derived H17 and H18 (each marked with a star) belong to group 1, displaying a typical sequence feature of group 1 HA molecules. **(B)** All the known NAs could also be grouped into two groups: group 1 and group 2. The bat-derived N10 and N11 (each marked with a star) do not belong to either group. They are NA homologs or could be called NA-like molecules. Here we propose that they be categorized as influenza A-like group 3.

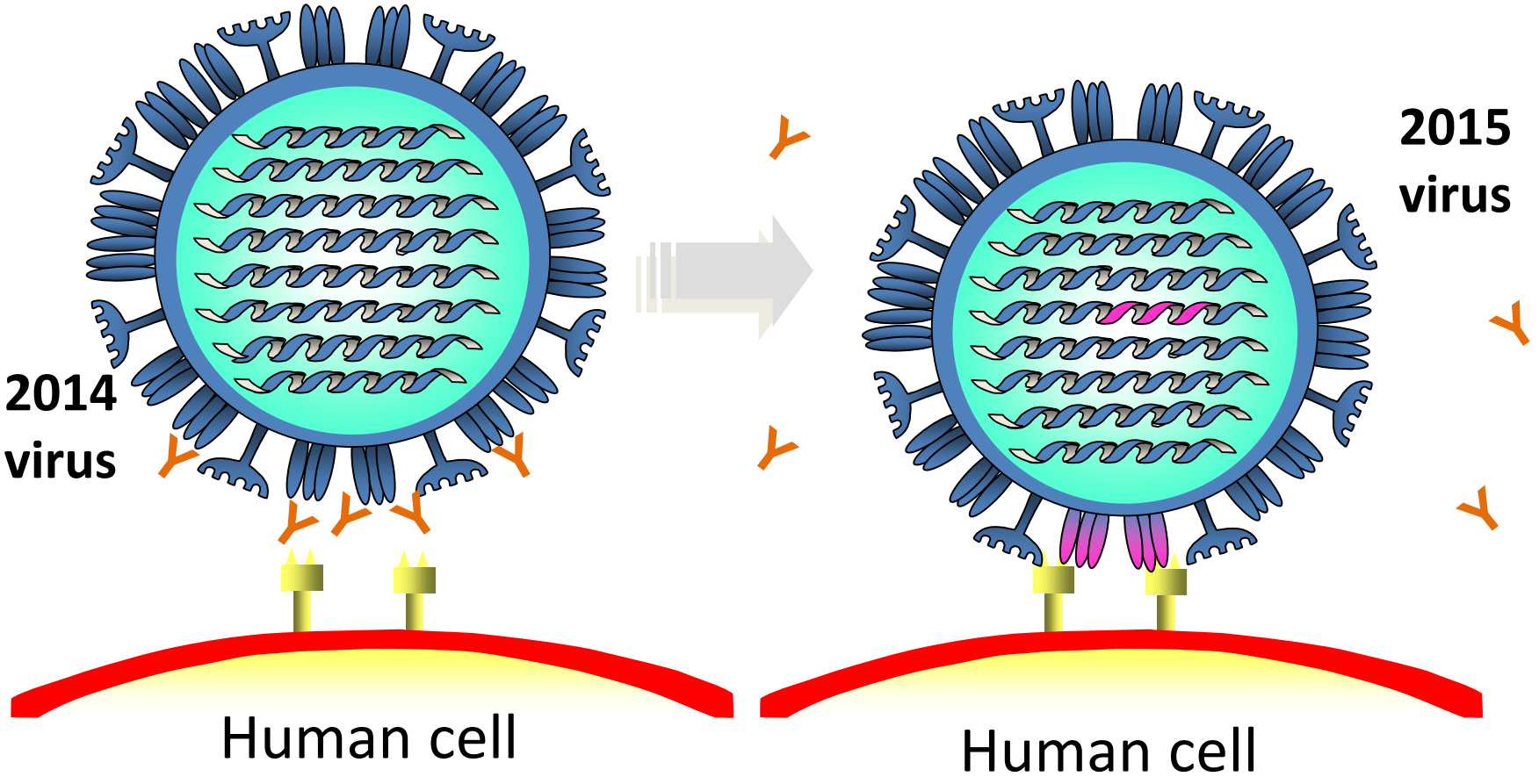
- Chimeric virus containing six bat virus gene segments with human HA and NA can replicate in human cells (including primary human airway cells).
- Poor replication in avian cells and chicken embryos.
- No natural reassortment with human viruses.

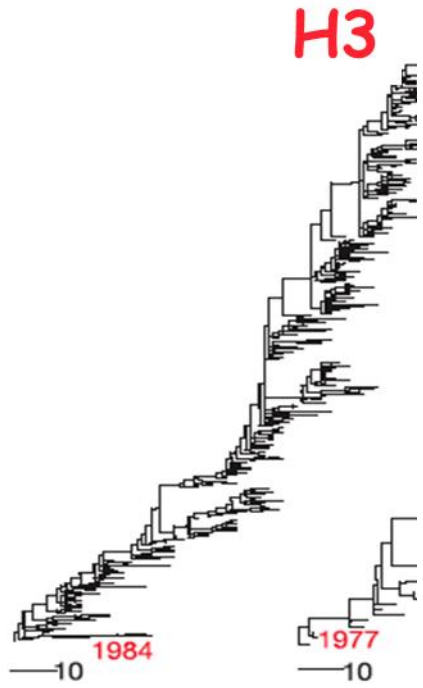
Juozapaitis M et al. Nat Commun. 2014 Jul 23;5:4448.

Antigenic Drift

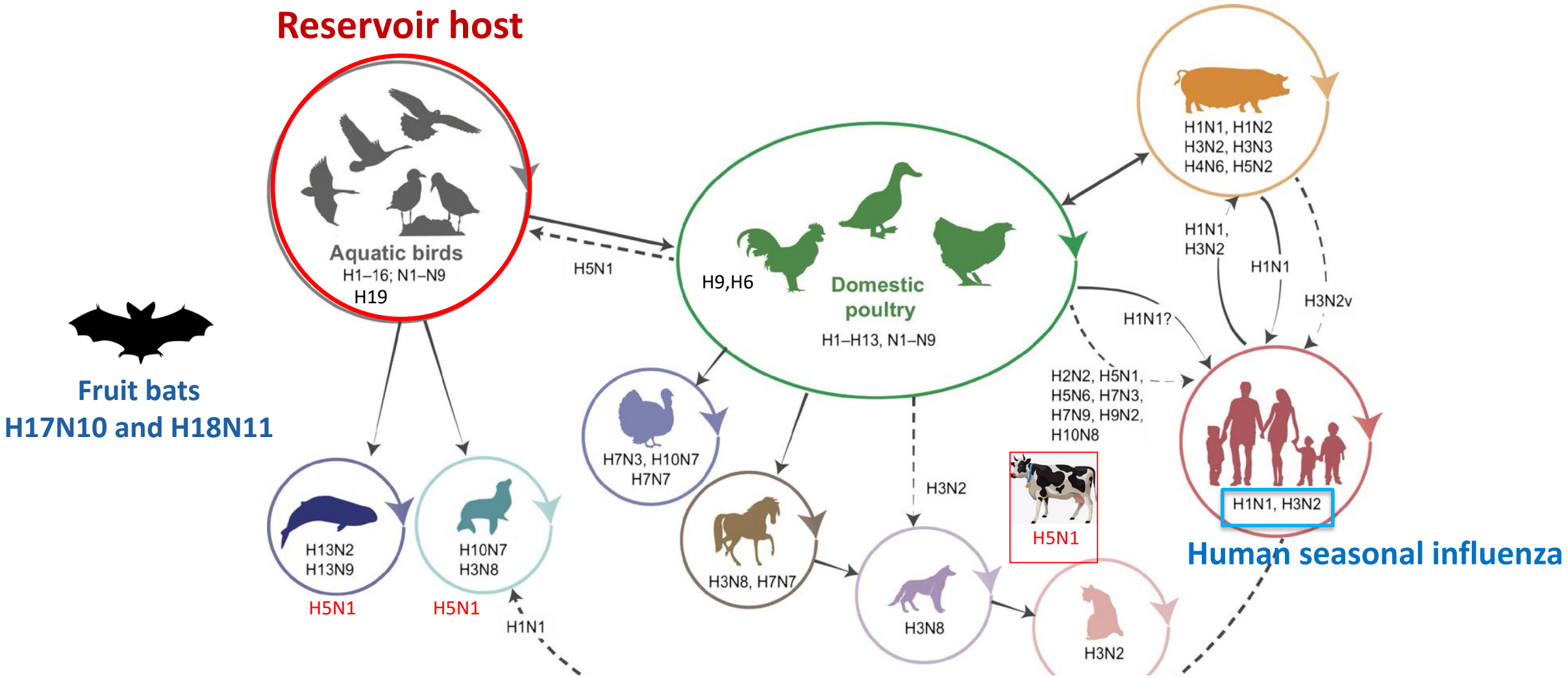
Mutation and minor antigenic change - “antigenic drift”

RNA polymerase lacks “proof reading” ability. More prone to mutation





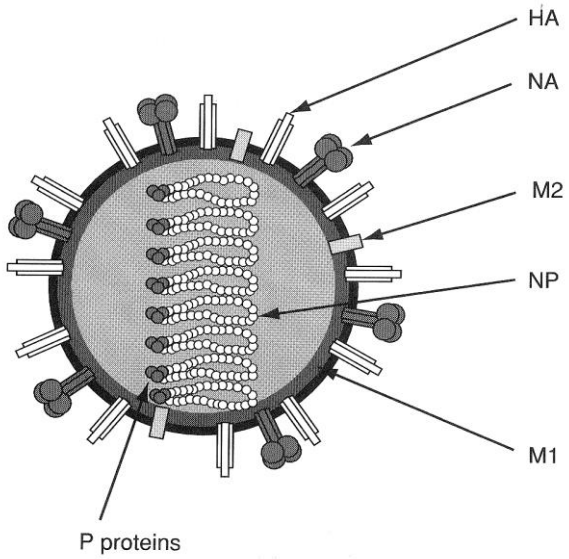
Influenza A viruses infect a wide range of hosts



Each of the animal species have their own "species-adapted" influenza A viruses!

Joseph et al. Influenza Other Respir Viruses 2017

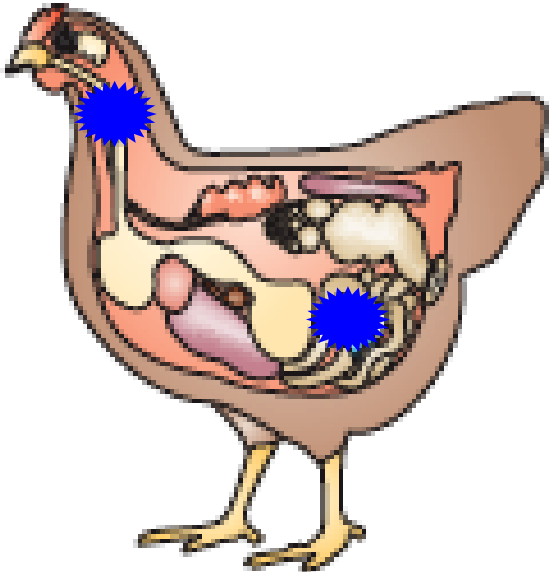
Highly pathogenic avian influenza (HPAI) viruses in chicken

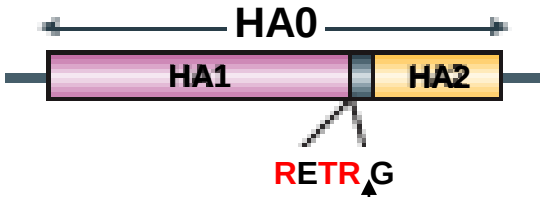


Haemagglutinin
HA0 →
cleavage by host cell proteases → HA1 and HA2

Reviewed in Horimoto & Kawaoka Nature Reviews Microbiol 2005; 3; 591-600

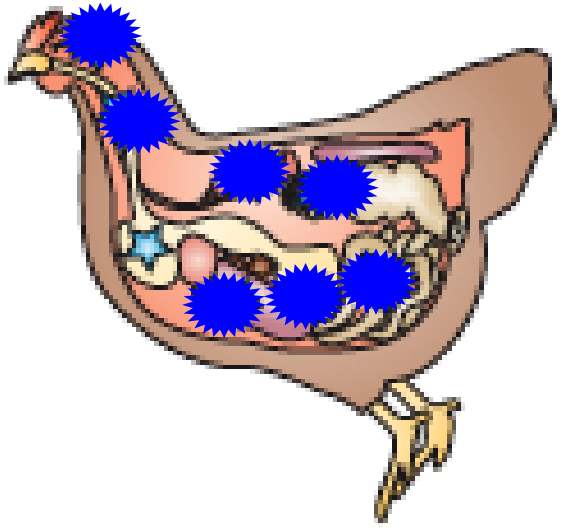
Low pathogenic

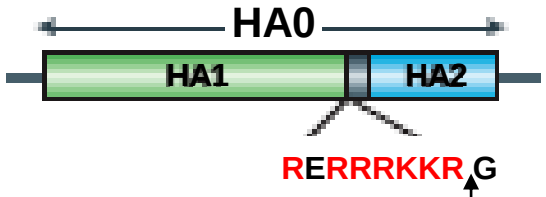




Cleaved by extra-cellular Trypsin like proteases found in respiratory and intestinal tracts.
Infection restricted to resp and GI tracts → mild symptoms

High pathogenic





Acquisition of a multiple basic amino acid motif at the cleavage site permits cleavage by intracellular Furin-like proteases which are found in many organs
Infection disseminated → brain, spleen, liver, pancreas etc→ rapidly lethal
Naturally occurs only with H5 or H7 subtypes

Aquatic poultry e.g. mallard ducks typically do not get severe disease, but some H5N1 HPAI viruses can cause severe disease



Healthy chicken





HPAI H5N1 caused rapid and high mortality in chicken farm in Kam Tim, 2022



Acknowledgement Les Sims
AFCD

Influenza Pandemics in History

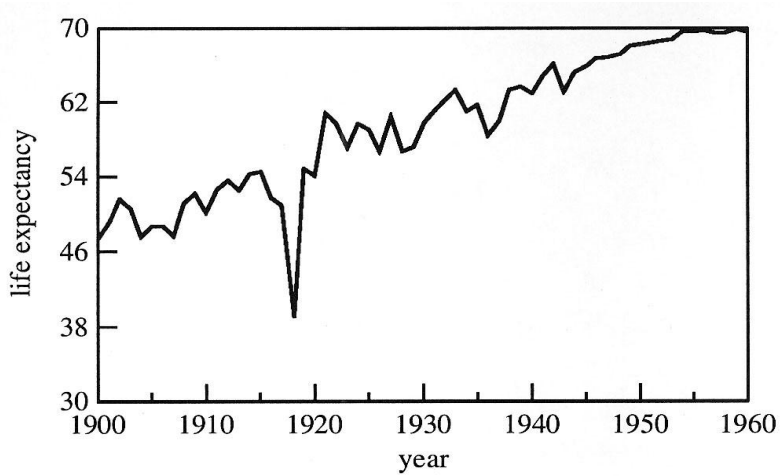
1918 pandemic

- 28% of the global population (then 500 million) affected in 1 year.
- Mortality rates: > 2.5% (cf. 0.1% in other pandemics)
- Estimated global mortality: ~ 50 million



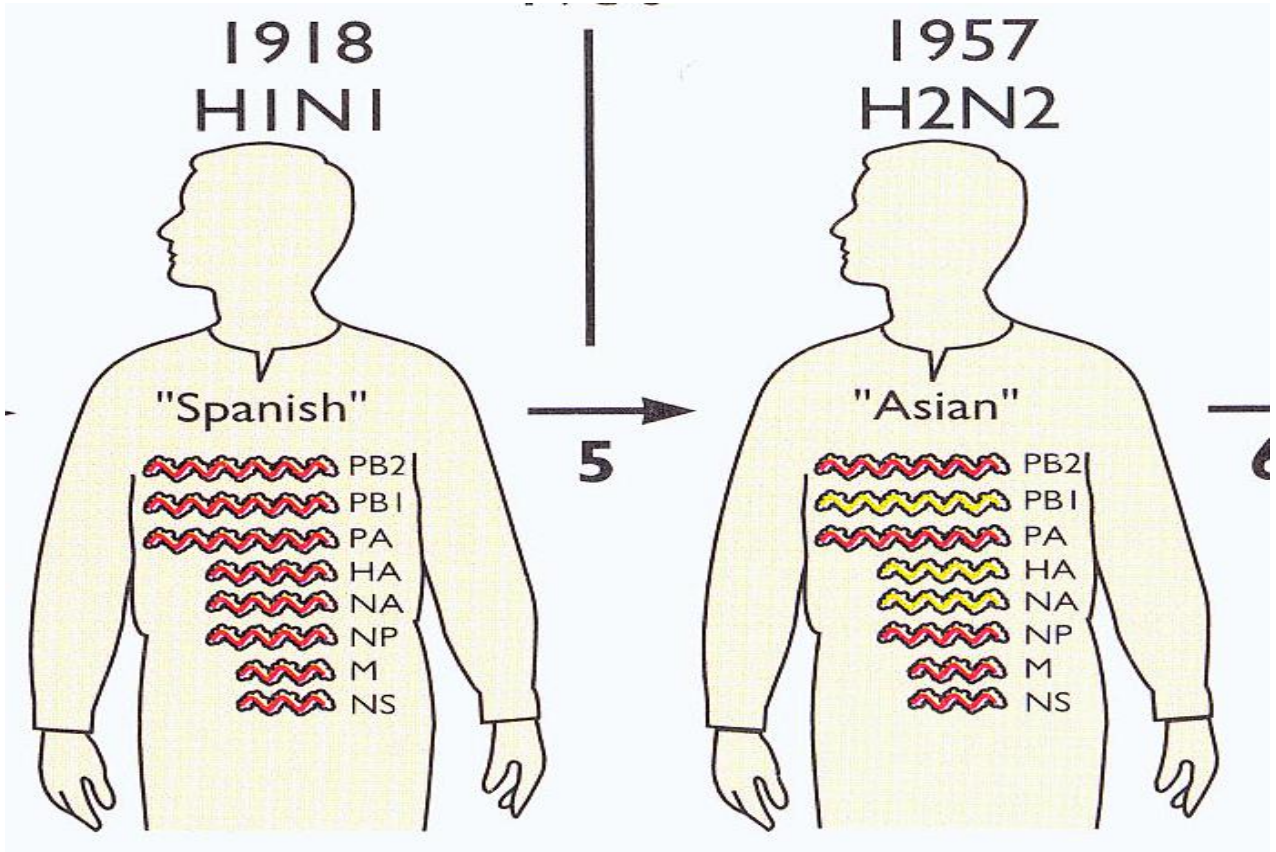
- In 1957 'Asian' influenza caused more than 1-4 million deaths
- In 1968 'Hong Kong' influenza caused more than 1-4 million deaths
- 2009 H1N1 "swine flu" pandemic estimated 151-575 thousand deaths

For comparison, **seasonal influenza** causes approx. 291-645 thousand deaths annually.



Life expectancy at birth: USA 1900-1960

The origin of pandemic influenza viruses



How did H1 and N1 change so extensively to H2 and N2?

Expedition to Great Barrier Reef to sample migratory birds for influenza, 1967

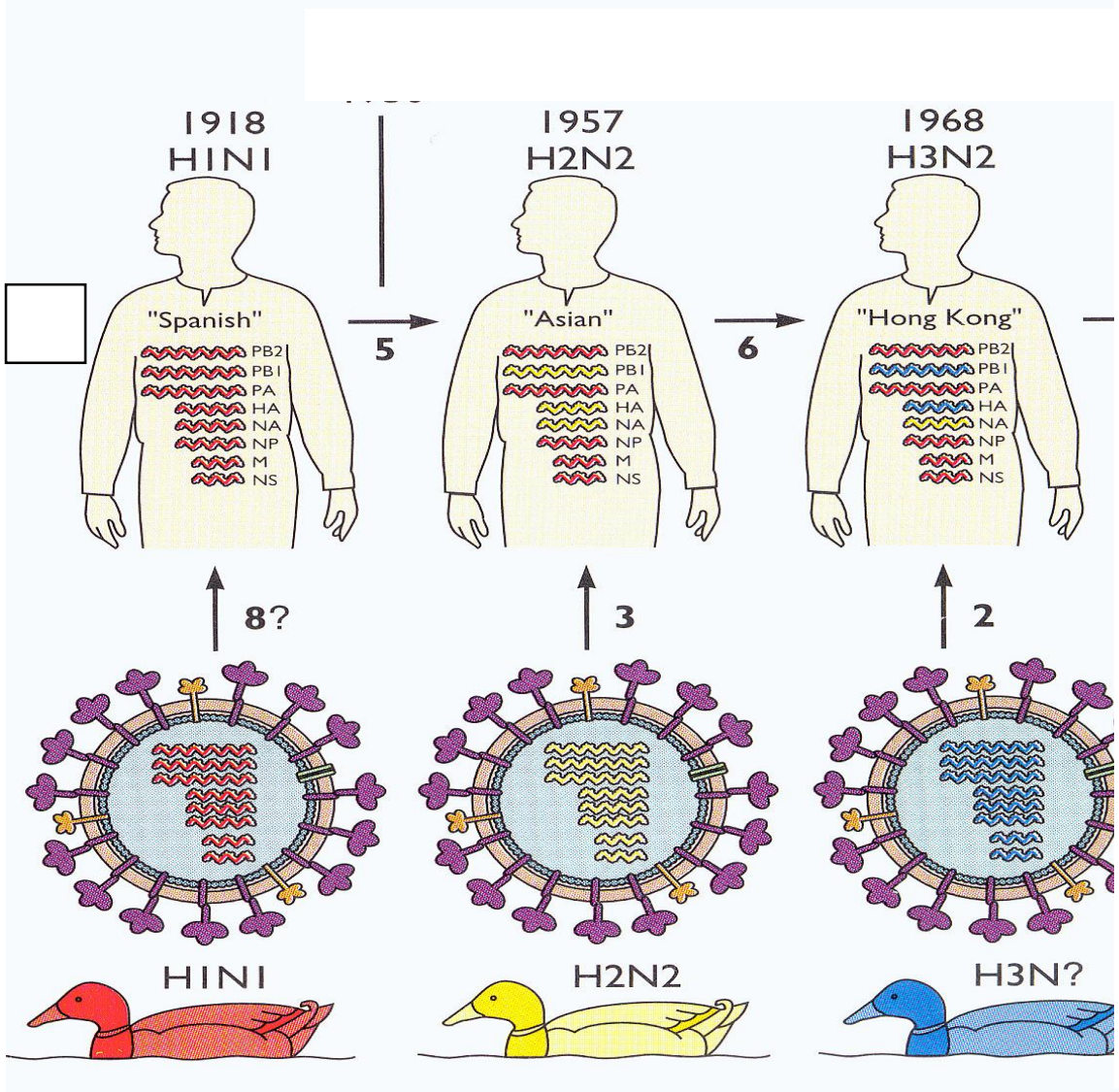


Graeme Laver on Tyron Island

- 1960's: Mystery of where novel influenza new H2 and N2 antigens found in H2N2 pandemic virus comes from →
- Graeme Laver & Robert Webster walking on a beach in New South Wales notice dead "mutton birds" → can migratory birds carry flu viruses ?
- Request funding for a field trip to the Great Barrier Reef to sample mutton birds and other migratory birds for influenza !!!
- Find evidence of N antibodies in serum from sampled birds (immuno-precipitation test done on the island itself !!). Subsequently identified to be N2

RG Webster "Flu Hunter" 2018

The origin of pandemic influenza viruses

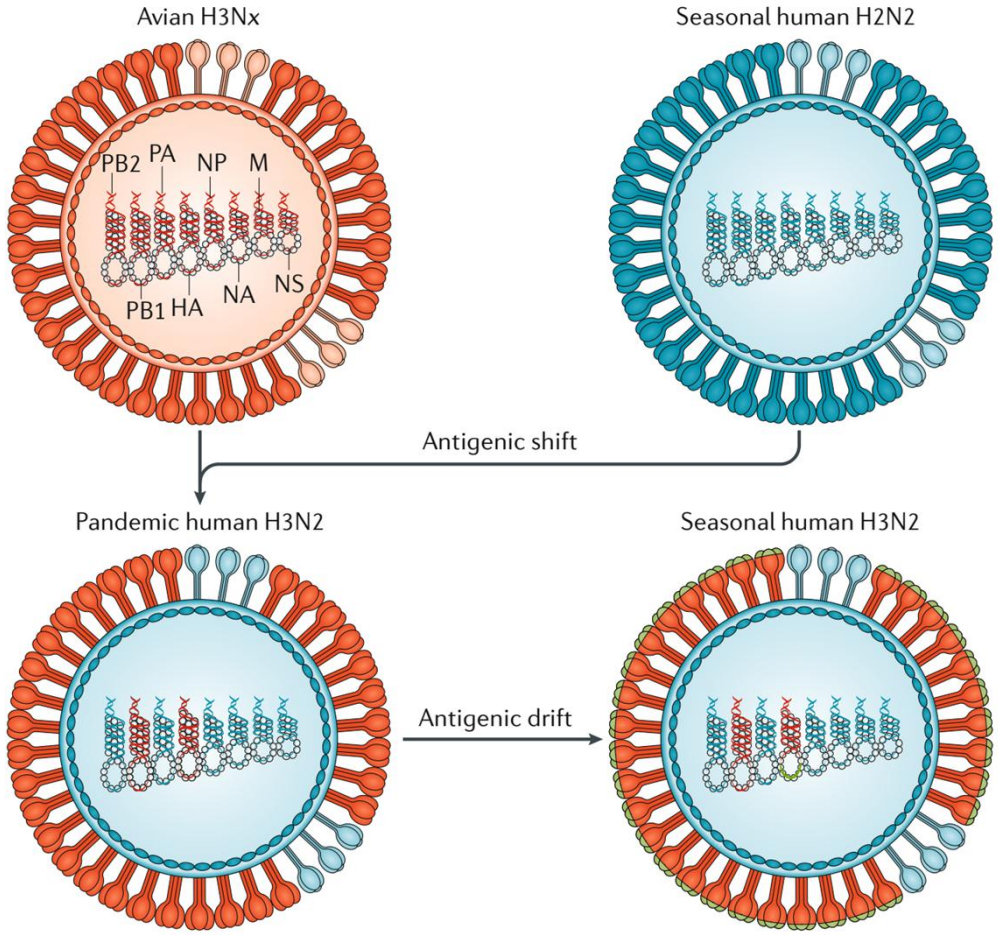


- Pandemic influenza have novel HA and/or NA. Not derived by “antigenic drift”. Introduced from animal reservoir.
- Antigenically different from circulating human seasonal influenza viruses.

- No / low immunity in the human population → potential to spread rapidly in humans, if such a virus has efficient transmissibility in human

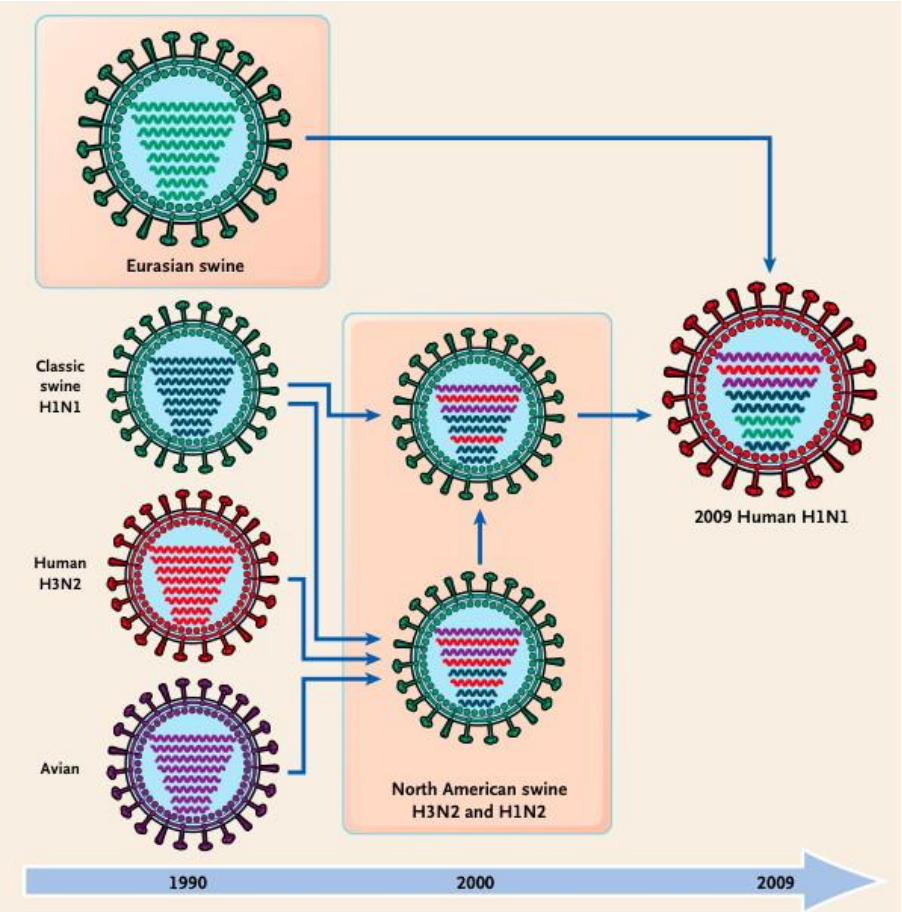
Virus genetic reassortment

- When two influenza viruses infect the same cell, the gene segments may “shuffle” and new gene combinations may arise



Krammer F et al. Nat Rev Disease Primers 2018: 4; 3

Reassortments leading to the emergence of the 2009 pandemic virus



Trifonov et al NEJM 2009

- 2009 pandemic virus had gene segments derived from Classical swine, avian and human influenza viruses
- The reassortment and emergence took place in swine in Central America



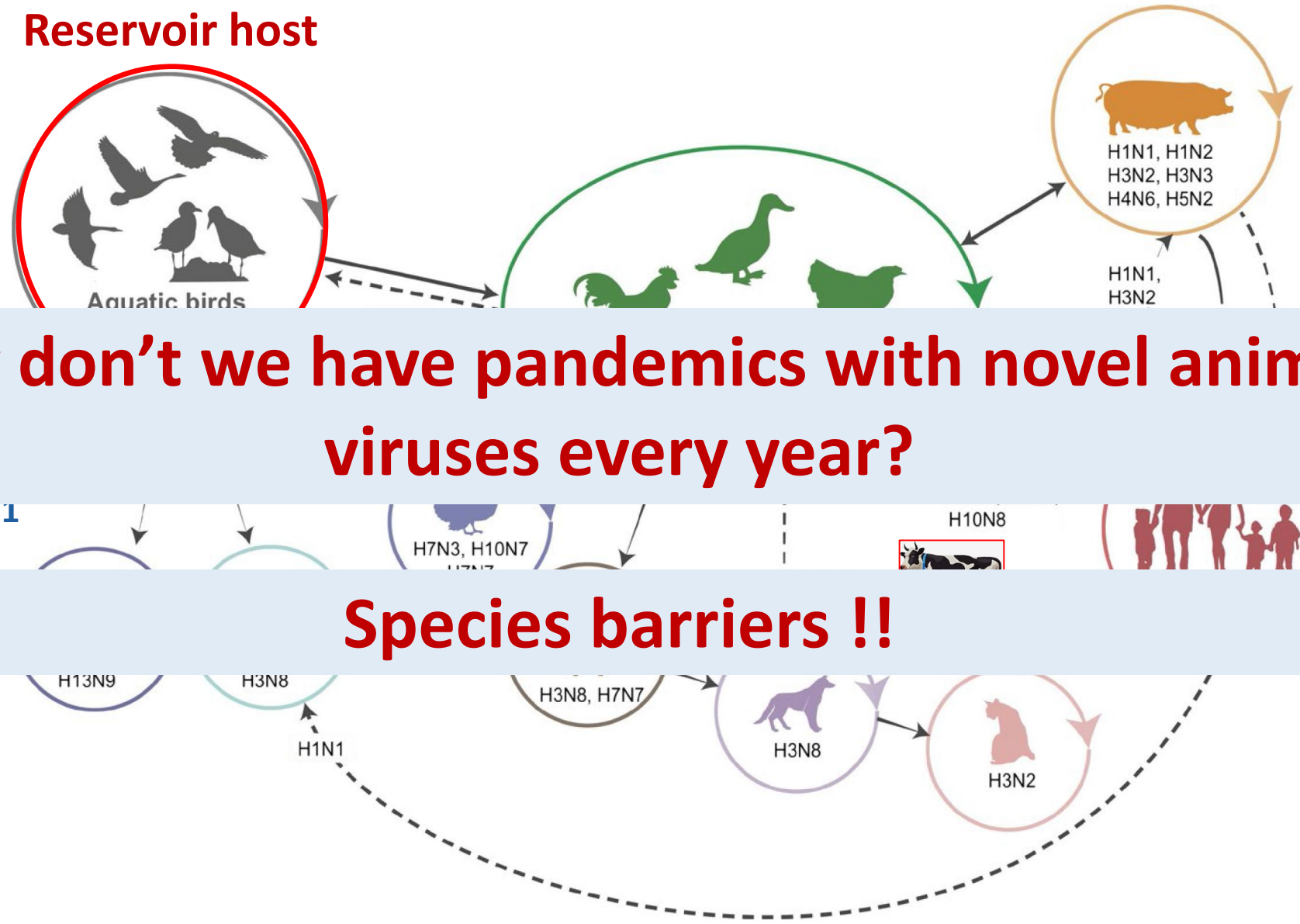
Mena, Nelson et al. Elife 2016.

Influenza A viruses infect a wide range of hosts

HxNy

x= 1-16, y=1-9

16 x 9 = 144 combinations

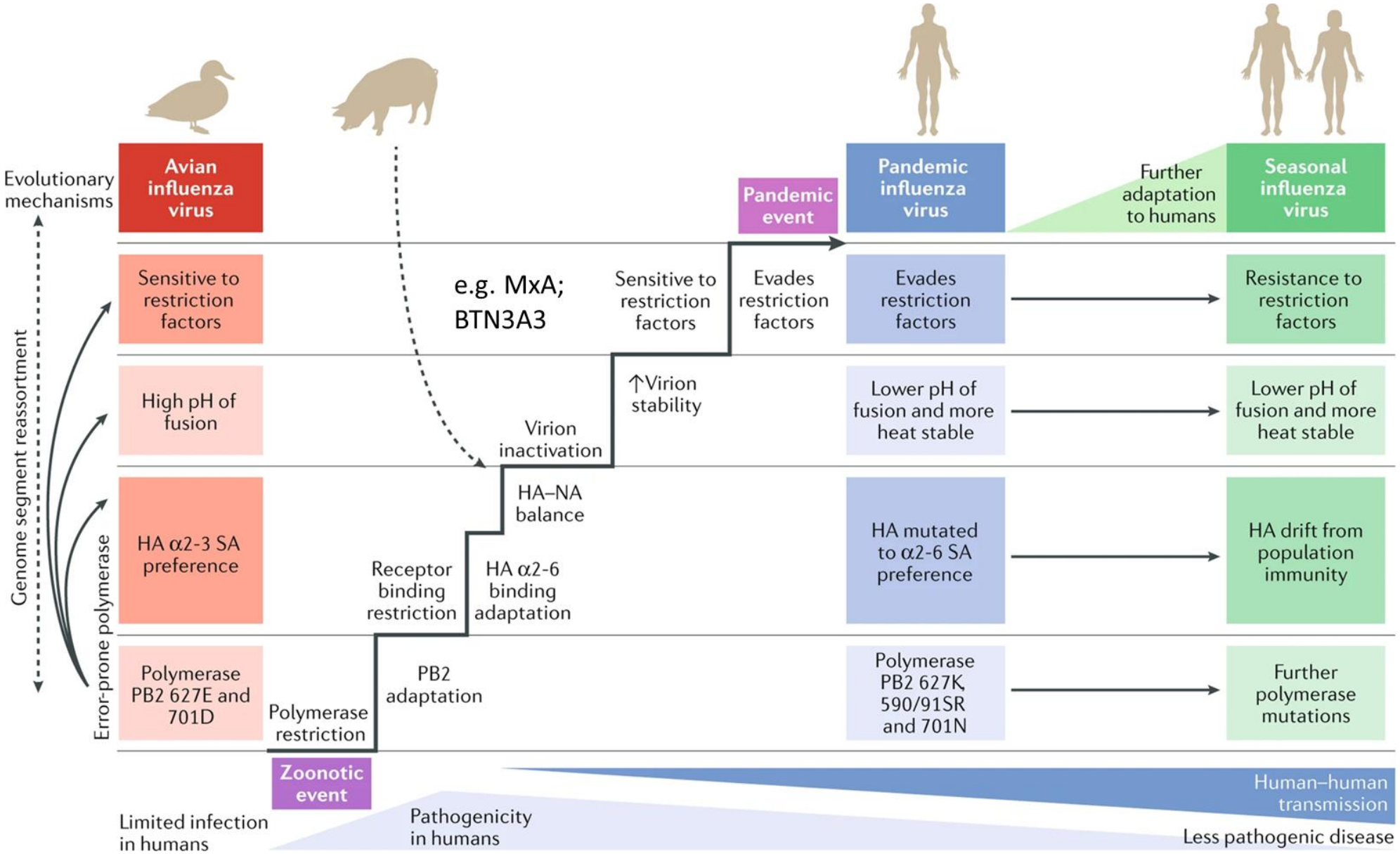


Why don't we have pandemics with novel animal viruses every year?

Species barriers !!

Joseph et al. Influenza Other Respir Viruses 2017

Adaptations required to acquire efficient transmission in humans



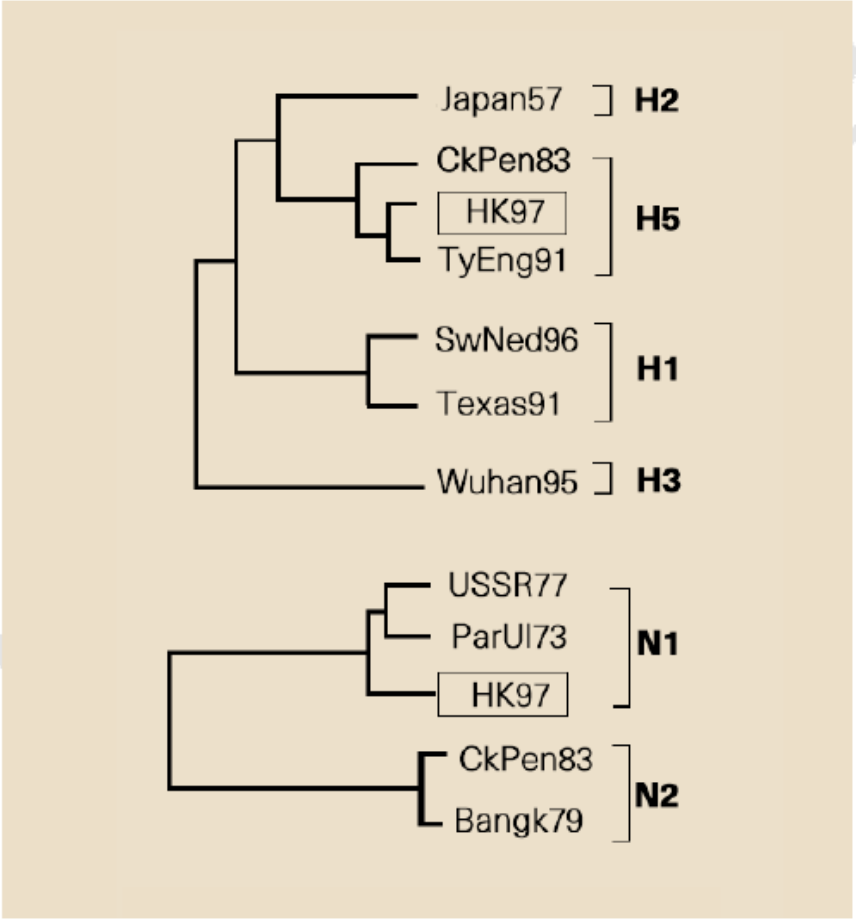
Long JS et al Nature Micro Rev 2019; 17: 67

Natural human infections with avian influenza viruses – prior to 1997

- 1957: Adult male returning to the USA following overseas travel → unwell → hepatitis → HPAI H7N7 isolated from blood (DeLay et al Public Health Rep 1967; 82: 615-20)
- H7N7 virus isolated from eye of a 43 year old woman with self-limited conjunctivitis who had tended ducks that mixed freely with waterfowl in a lake (Kurtz et al Lancet 1996: 348: 901-2)
- H7N7 virus isolated from a seal handler with conjunctivtis who handled seals infected with H7N7 virus (Webster et al NEJM 1981; 304: 911
- H7N7 conjunctivitis following laboratory infection (Taylor HR & Turner AJ Brit J Ophthalmology 1977: 61: 86-88)

Three year old boy dies with influenza, Hong Kong 1997

- 21 May 1997; 3 year old boy died after a five day illness with severe pneumonia, acute respiratory distress syndrome and Reye's syndrome. No underlying diseases
- Influenza A virus was isolated from a tracheal aspirate but could not be subtyped as a human H1 or H3 virus
- Isolate sent to multiple influenza reference laboratories for characterization.
- Jan de Jong (Erasmus, Rotterdam) arrives in Hong Kong …… to visit the public health laboratory
- Reports that HPAI H5N1 was identified by antigenic analysis and (later) confirmed by sequencing



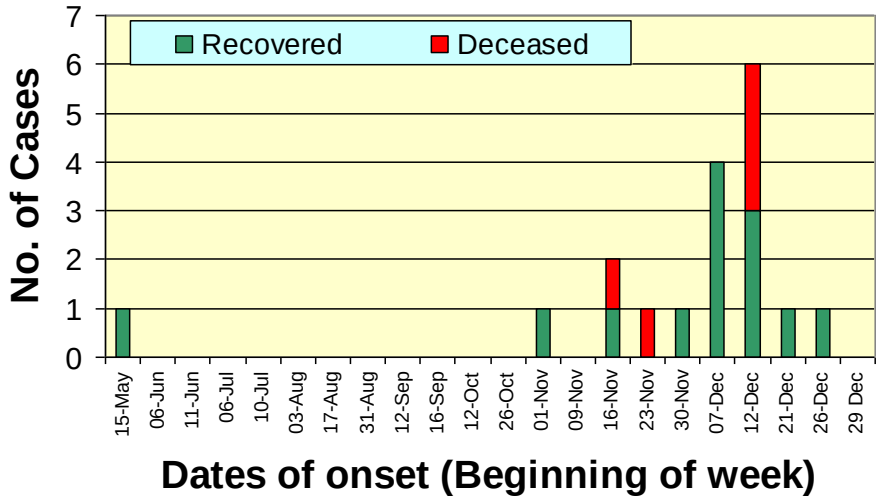
De Jong JC et al Nature 1997; 389: 554

The H5N1 incident in 1997 ……



- From November onwards, 17 more cases of H5N1 disease identified, 5 more deaths. Overall mortality 33%
- Outbreaks of avian flu in chicken farms in Hong Kong in March / April 1997

Epidemic Curve of Influenza A (H5N1)
Cases in HKSAR, May - Dec 1997



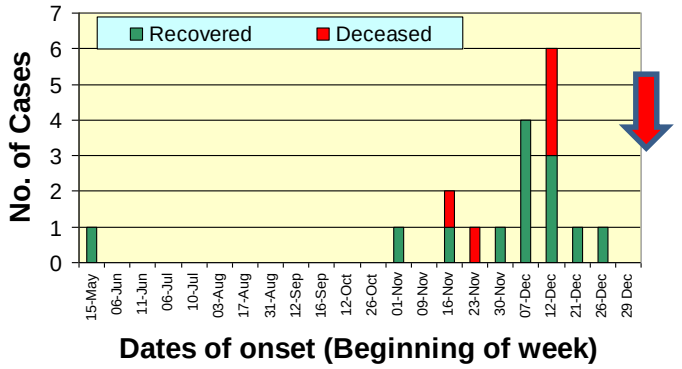
What will you do?

- ~ 20% of chicken in live poultry markets had H5N1 virus
  - *Shortridge et al 1999*
- Some (not all) patients with H5N1 disease had contact with live poultry markets – *Mounts et al 1999*
- Poultry workers in farms or markets not affected





Epidemic Curve of Influenza A (H5N1) Cases in HKSAR, May - Dec 1997



Case-Control Study of Risk Factors for Avian Influenza A (H5N1) Disease, Hong Kong, 1997

Mounts et al Journal of Infectious Dis 1999

Table 2. Activities and exposures associated with influenza A (H5N1) disease.

Activity and exposure	No. (%) with reported exposure			
	Case patients (n = 15)	Controls (n = 41)	OR (95% CI)	P[a]
Exposure to poultry				
Exposed to live poultry in market[b]	9/14 (64)	11/38 (29)	4.5 (1.2–21.7)	.045
Consumed poultry in restaurant	6/12 (50)	9/31 (29)	2.9 (0.6–14.9)	.375
Consumed poultry organs or poultry	1/14 (7)	4/38 (11)	0.6 (0.0–7.5)	.999
Consumed undercooked poultry products	5/11 (45)	10/29 (34)	1.9 (0.4–11.2)	.707
Household member cooked poultry products	2/13 (15)	11/33 (33)	0.2 (0.0–1.3)	.193
Household member in poultry industry	0/14	0/38	Undefined	NA
Exposure to human illness				
Anyone in flat had influenza-like illness[c]	5/15 (33)	15/40 (38)	0.8 (0.2–2.8)	.999
Contact with known H5N1 case	1/13 (8)	0/35	+Inf (0.1 to +Inf)[d]	.500
Other exposure				
Travel outside Hong Kong	0/14	3/37 (8)	0.0 (0.0–4.9)	.800
Selected outdoor activities[e]	2/14 (14)	8/37 (22)	0.0 (0.0–2.5)	.225
Live birds in home	6/12 (50)	14/31 (45)	1.4 (0.3–6.4)	.901
Other animals in home	2/13 (15)	1/33 (3)	2.5 (0.1–97.4)	.999
Played in indoor playground	0/15	13/41 (32)	0.0 (0.0–0.5)	.013
Household uses soap to clean[f]	5/14 (36)	25/38 (66)	0.3 (0.1–1.0)	.058

NOTE. OR, odds ratio; CI, confidence interval; NA, not available.

Risk Factors for Influenza A(H7N9) Disease—China, 2013

Liu B. et al. CID 2014

Bo Liu,[1,a] Fiona Havers,[2,a] Enfu Chen,[3,a] Zhengan Yuan,[4,a] Hui Yuan,[12] Jianming Ou,[5] Mei Shang,[6] Kai Kang,[7] Kaiju Liao,[1] Fuqiang Liu,[8] Dan Li,[1] Hua Ding,[9] Lei Zhou,[1] Weiping Zhu,[10] Fan Ding,[1] Peng Zhang,[11] Xiaoye Wang,[1] Jianyi Yao,[1] Nijuan Xiang,[1] Suizan Zhou,[6] Xiaoqin Liu,[12] Ying Song,[6] Hualin Su,[13] Rui Wang,[1] Jian Cai,[3] Yang Cao,[1] Xianjun Wang,[14] Tian Bai,[15] Jianjun Wang,[16] Zijian Feng,[1] Yanping Zhang,[1] Marc-Alain Widdowson,[17] and Qun Li[1]

Case-control study among H7N9 patients (n=89) with age, sex, and neighborhood-matched controls (n=339):

- Visited LBM (past 10 day): 67% patients versus 35% controls (mOR=5.4; 95% CI, 3.0–9.7)
- Visited market but no direct contact with poultry: 33% patients versus 26% controls (mOR=3.0; 95% CI, 1.6-5.7).
- No poultry contact and not in any location with poultry: 14% of cases versus 29% of controls

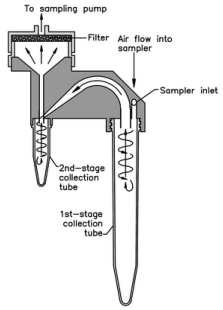






Infectious avian influenza viruses detectable in air in live poultry markets



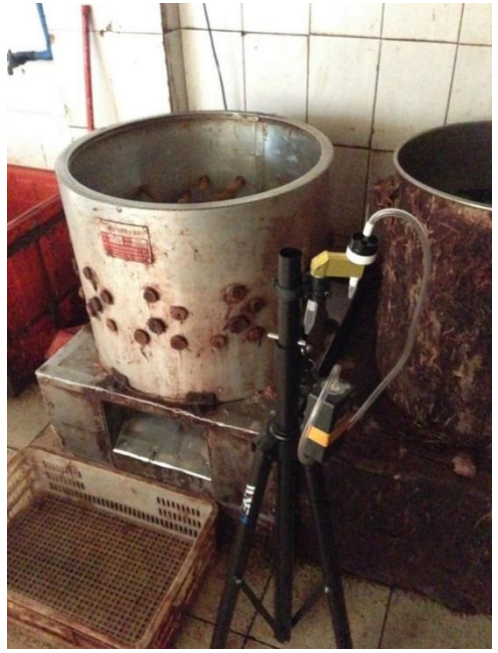


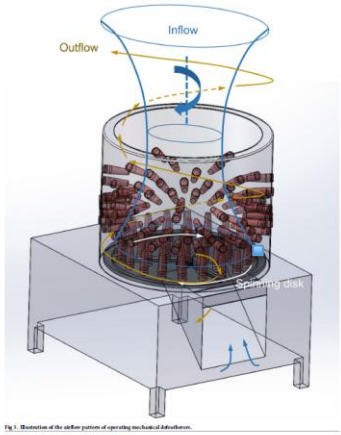
NIOSH cyclone air sampler

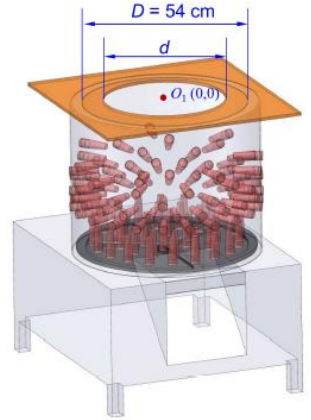
Infectious airborne particles in poultry markets

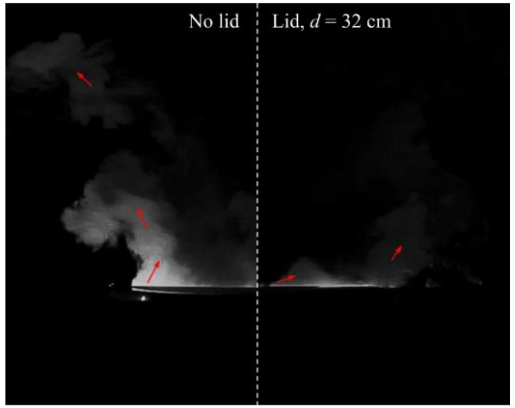
Zhou et al 2016

Major source of infectious airborne particles is the defeathering machine

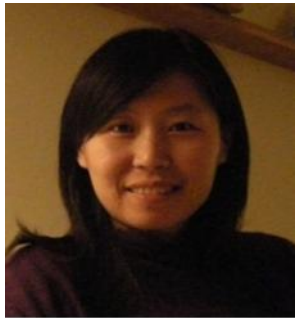








Wei J et al PLoS One 2019



H Yen

Detection of avian influenza virus in chicken carcasses by virus culture in Guangzhou

	Dressed poultry carcasses. No live poultry in stalls	Super-markets
Oropharyngeal swabs	44/121 (36%)	0/62 (0%)
Cloacal swabs	38/120 (32%)	0/62 (0%)
Visceral cavity	23/118 (20%)	0/23 (0%)

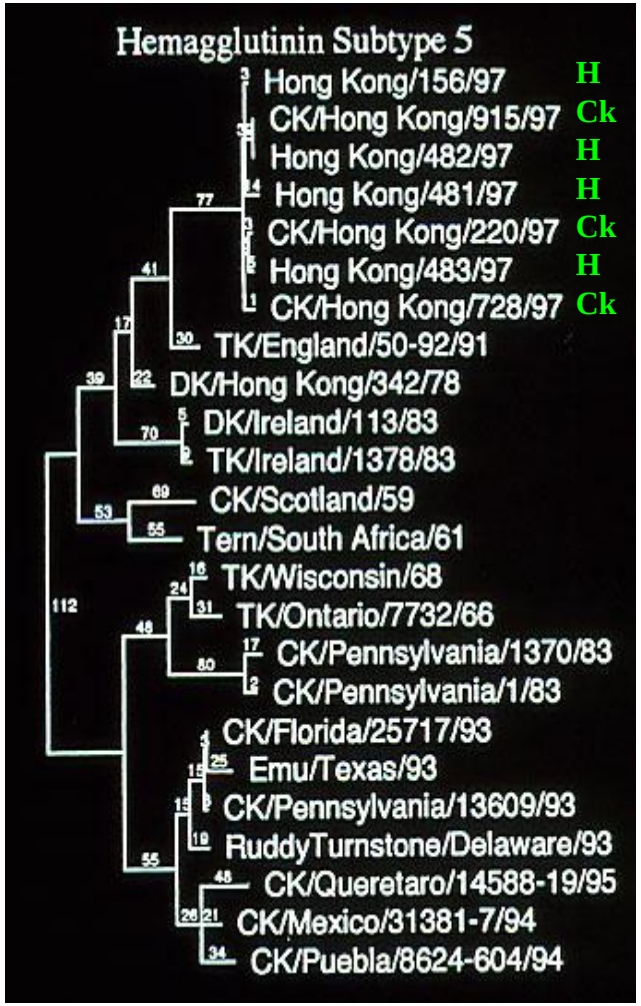
Conclusion

- Chicken carcasses from wholesale markets contaminated with infectious virus
- Although cooking will kill virus, infectious virus may contaminate hands, fomites and other foods in kitchen --> transfer to human resp tract
- Chicken carcasses from vertically integrated poultry systems "clean"

Mao X ---- H Yen EID 2017

The H5N1 incident in 1997

- Human H5N1 viruses were genetically identical to the virus causing disease in the chicken farms

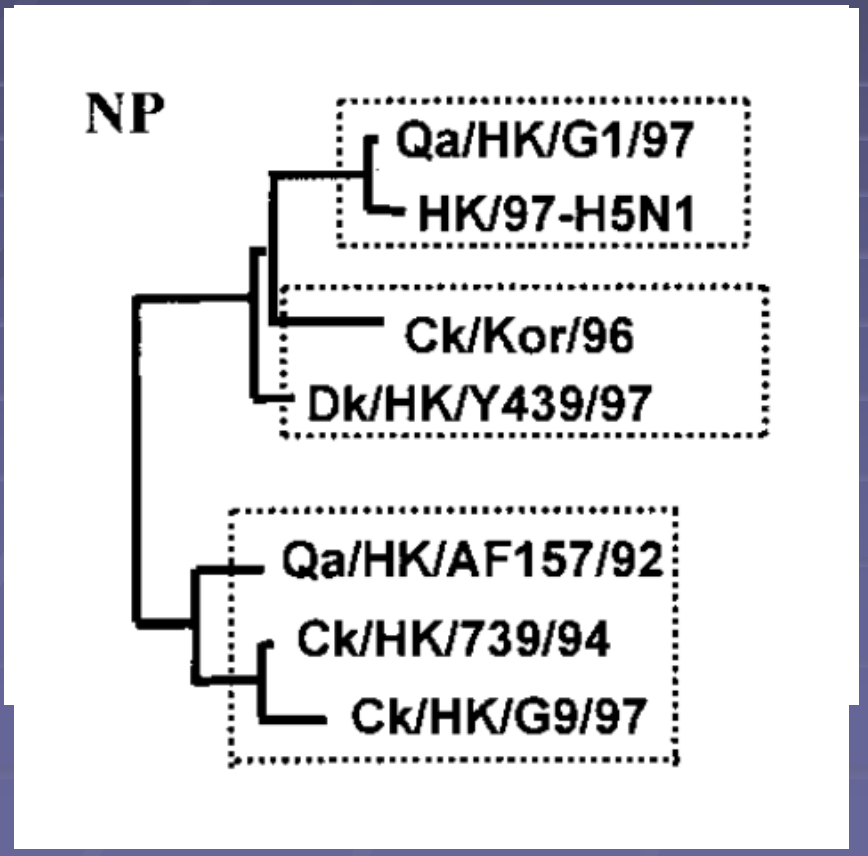


Subbarao et al 1998
Claas et al 1998

H5N1 Hong Kong 1997: Transmission in humans

- No convincing human - human transmission through social contact
  - *Katz et al 1999*
- Human - human transmission documented in health care setting. But transmission was inefficient
  - 2 health care workers nursing a critically ill patient with H5N1 were infected
  - *Bridges et al 2000*

Where did the HK/H5N1 1997 come from?



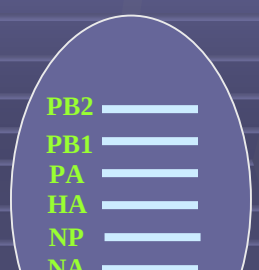




H5N1 Gs/GD/96-like

Xu et al 1999

H9N2 G1-like

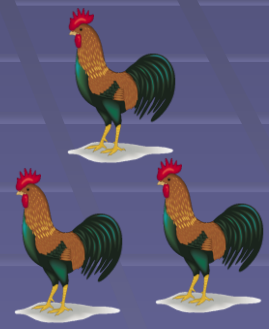


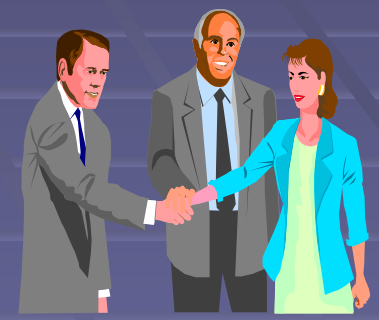


H6N1 W312-like









H5N1/97

Humans and Chicken

Guan et al 1999

Hoffman et al 2000



Not detected after 1997

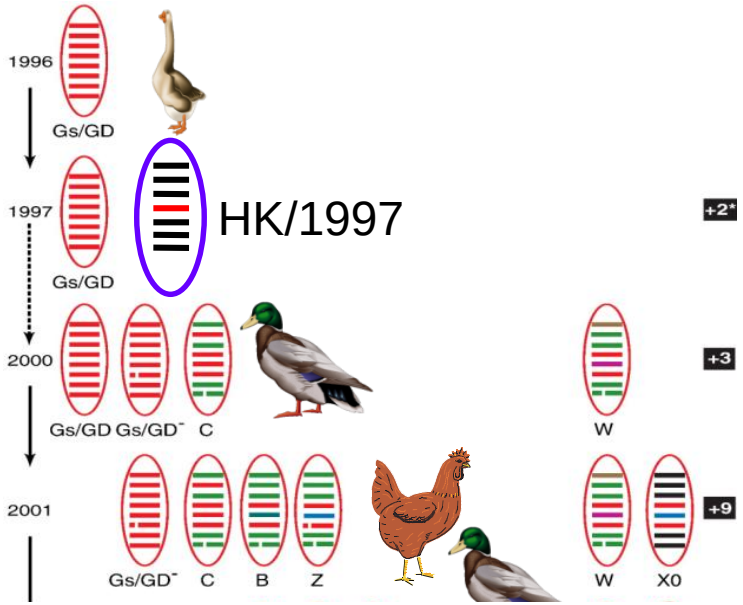
Active surveillance

Avian flu H5N1-Post 1997

- 1999 Gs/Gd H5N1 detected in imported geese
- 2000 Reassortment in ducks
- 2001 Outbreaks in live poultry markets
- 2002 Farm outbreaks
- 2002 Wild birds









Proc Natl Acad Sci, USA 2002
Proc Natl Acad Sci, USA 2004

H5N1 Influenza Outbreak – Kowloon Park, Hong Kong

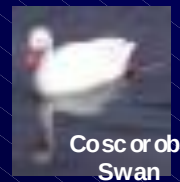

Puna Teal Duck

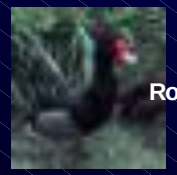

Wood Duck


Bar-headed Goose


Common Shel duck


Ruddy Duck


Coscoroba Swan


Rosybill Duck

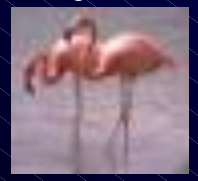

Red-crested Pochard


Chiloe Wigeion Duck


Victoria Crowned Pigeon


Ringed Teal Duck

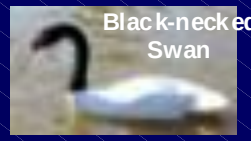

Hawaiian Goose


Greater Flamingo


Tufted Duck


Bahama Pintail

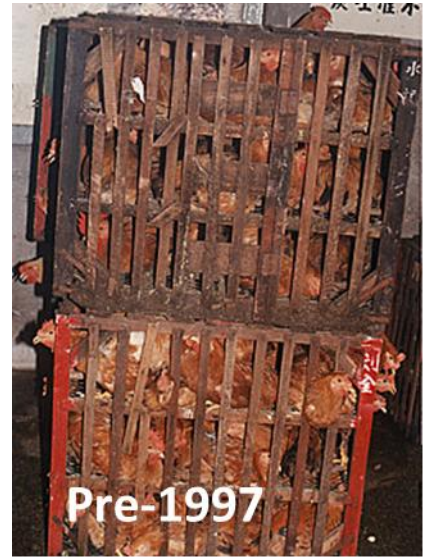

White-faced Whistling Duck


Black-necked Swan


Brazilian Duck

Measures to reduce risk of H5N1 in humans and poultry in Hong Kong

- Active surveillance in poultry in markets and farms
- Live poultry supply from certified mainland farms and testing at entry to HK
- Segregation of aquatic from terrestrial poultry
- Avian influenza Task force set up – multidisciplinary → "One Health" implementation before "One Health" became "fashionable"
- Enhance biosecurity in local farms
- Enhance biosecurity at Wholesale poultry market with separation / cleaning of poultry cages returning to farms







Threat from H5N1 in wild birds: Role of vaccination evaluated

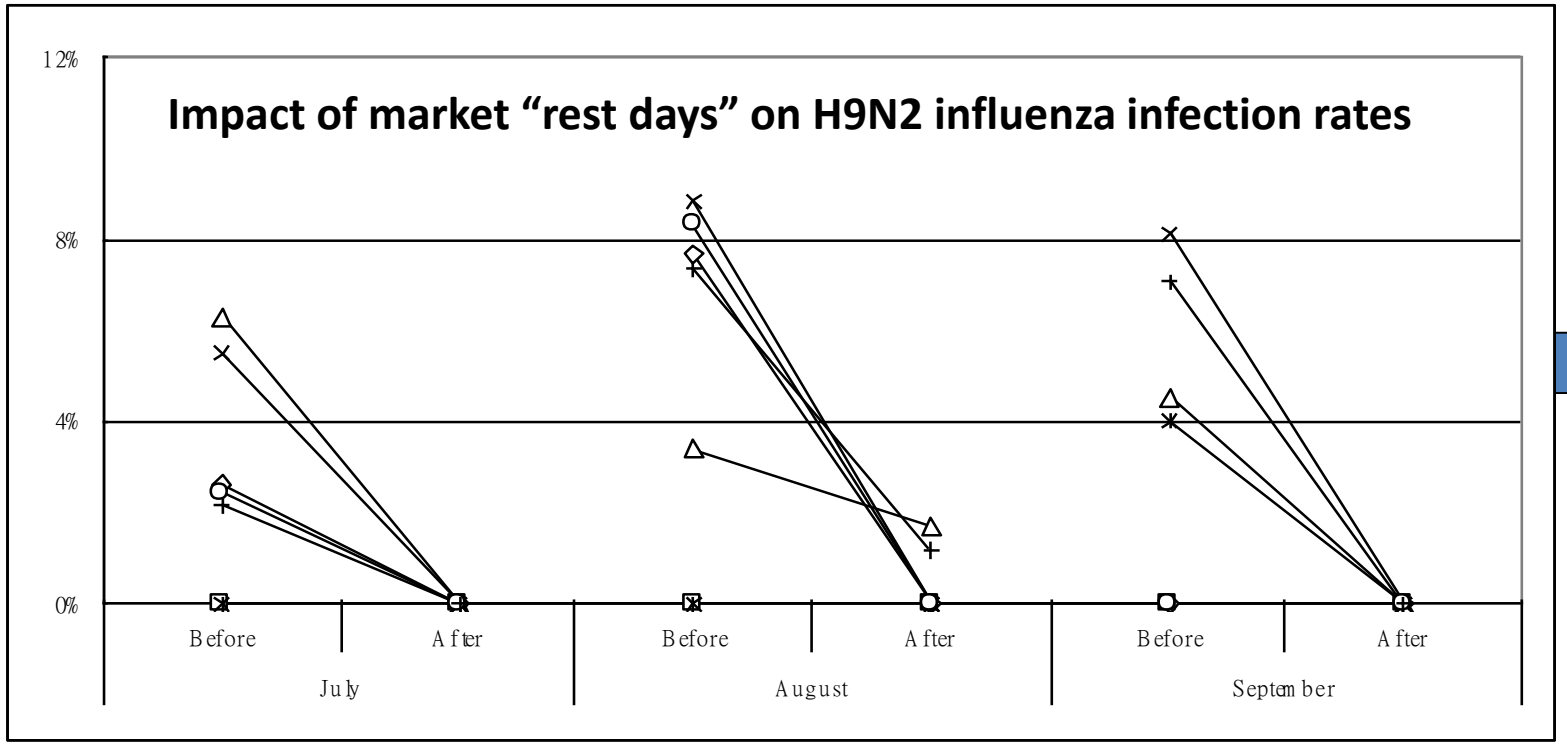




Poultry Vaccine concerns

- Vaccine may be seen as an alternative to bio-security
- Standardised potency: Bad vaccine is worse than no vaccine
- Protect from disease but mask asymptomatic transmission
- Vaccine may drive virus antigenic changes → need to monitor virus circulating in field and update vaccine if needed.
- Vaccine may increase risk of human adaptation ????
- Loss of ability to do serological monitoring poultry
  - ✓ DIVA (Differentiating infected from vaccinated animals)
  - ✓ Tagged unvaccinated sentinel in farms
- Export implications (not relevant for Hong Kong)

Virus transmission dynamics and critical intervention points





Retail poultry Market

Kung et al Avian Dis 2003; Lau et al EID 2007; Leung et al 2012;

Kung et al Emerg Infect Dis 2007; Samaan et al 2012; Indriani et al 2010

Risk for Infection with Highly Pathogenic Influenza A Virus (H5N1) in Chickens, Hong Kong, 2002

Emerg Infect Dis 2007; 13: 412-8

Nina Y. Kung,*†[1] Roger S. Morris,* Nigel R. Perkins,* Les D. Sims,‡ Trevor M. Ellis,‡ Lucy Bissett,‡ Mary Chow,‡ Ken F. Shortridge,† Yi Guan,† and Malik J.S. Peiris†

- **Visits to a farm by ≥1 persons from retail markets was a strong risk factor for infection in the farm**

Table 2. Descriptive analysis of farm area, standing population of chickens, and number of sheds of chicken farms included in survey, Hong Kong, 2002*

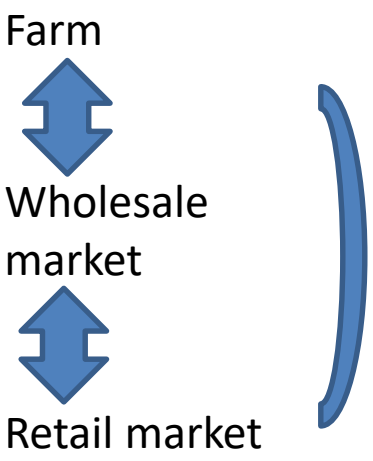
	Case (n = 16)					Control (n = 46)					
	Mean	Median	Max	Min	SD	Mean	Median	Max	Min	SD	p value
Farm area (m^2)	4,008	1,700	18,600	900	4,684	3,275	1,975	28,350	207	4,331	0.86
Chicken count (×1,000)	41.3	27.5	101.4	5.6	33.3	16.2	16.0	51	3.5	10.2	<0.001
Shed no.	7.4	5.5	20	2	5.9	8.0	7.5	19	1	4.4	0.65

*Max, maximum; Min, minimum; SD, standard deviation.

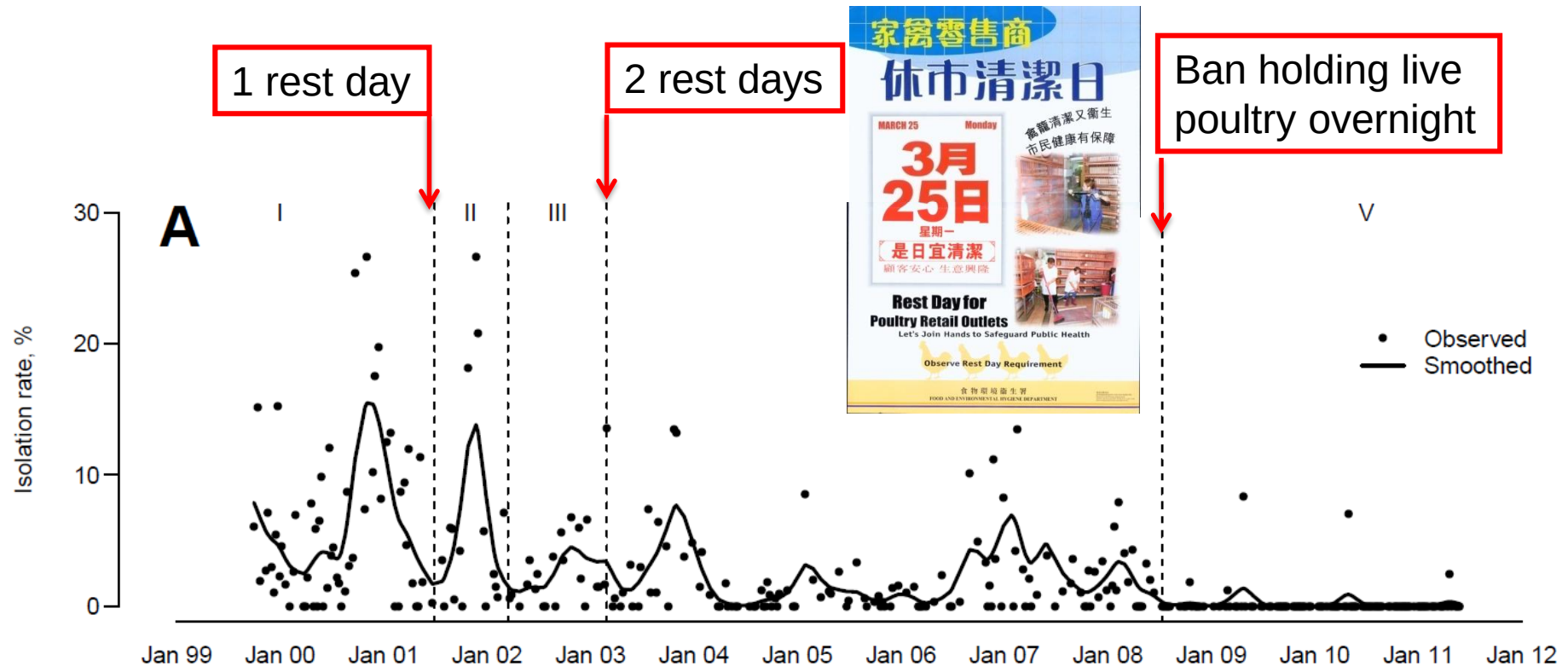
Table 4. Comparison of different multivariate models of risk factors for avian influenza type A virus (H5N1) infection among chicken farms, Hong Kong, 2002*

		OR (95% CI)		
Variable	Category	Model A	Model B	Model C
1	Owner lives off farm	37.04 (3.18–431.63)	12.64 (1.18–135.35)	45.84 (3.65–575.69)
2	Sell to retail markets	20.11 (1.47–274.98)	30.26 (2.26–405.09)	28.39 (2.30–350.40)
3	Highest death rate >30 d	17.37 (1.03–292.01)	20.51 (1.51–277.96)	24.28 (1.62–364.87)
4	Wild birds in feed trough	0.07 (0.01–0.85)		
5	Chicken count		1.07 (1.01–1.12)	
6	Relative in poultry industry			19.41 (1.46–257.74)
Cox and Snell R^2		0.42	0.46	0.43
Nagelkerke R^2		0.63	0.68	0.65
Significance of Hosmer and Lemeshow test		0.91	0.67	0.82
Degrees of freedom		3	8	3

*OR, odds ratio; CI, confidence interval.



No further cases of severe avian influenza disease acquired in Hong Kong since 1997

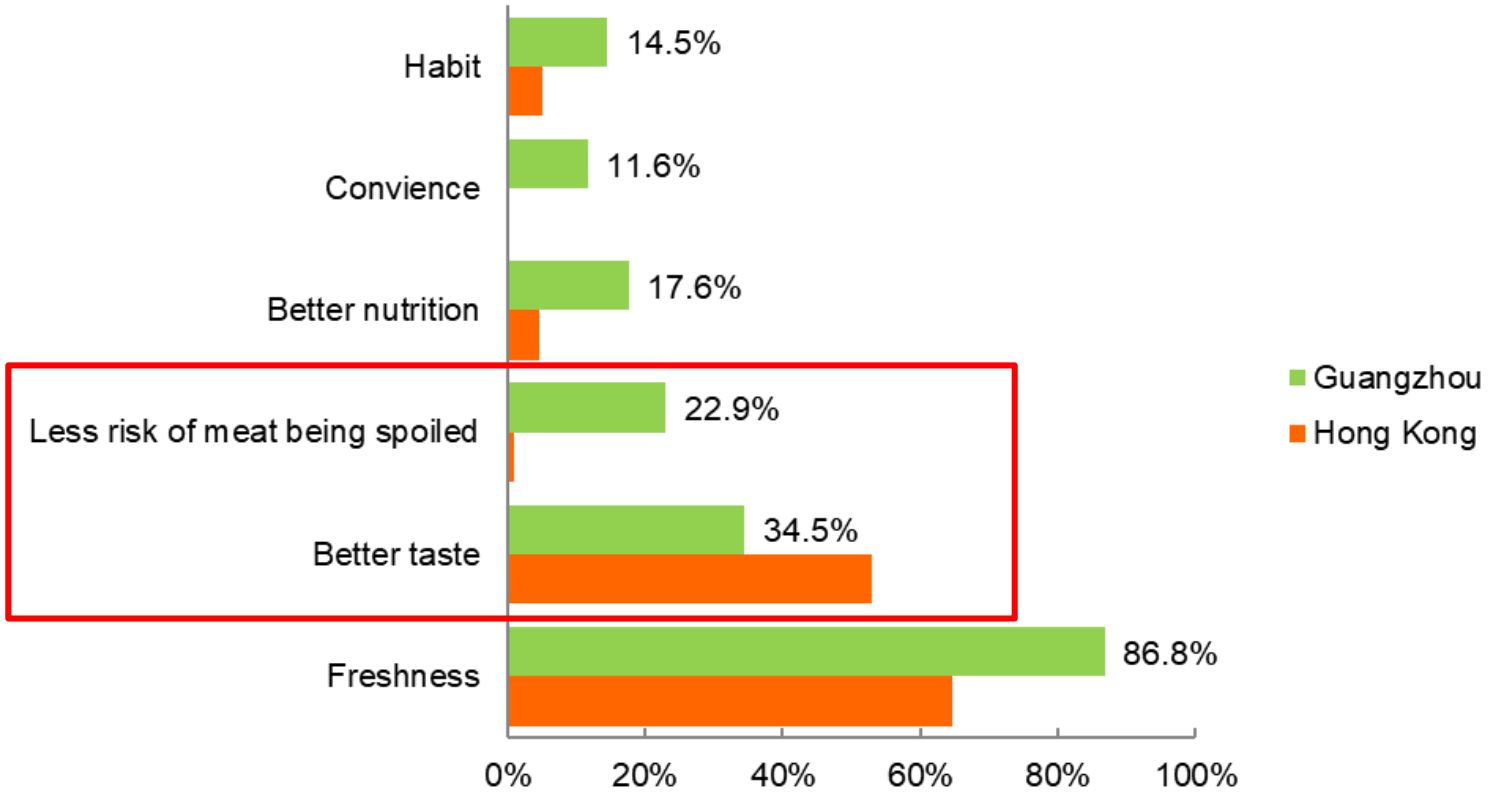


Leung et al EID 2012

Preferring to purchase live vs frozen / killed poultry (surveyed 2005-6)

- 949 (53.9%) of 1760 respondents in Hong Kong survey and 847 (54.6%)of the 1550 respondents in Guangzhou survey indicated preference for buying live poultry.

Of those who preferred to buy live poultry, the reasons for wishing to buy poultry live were:

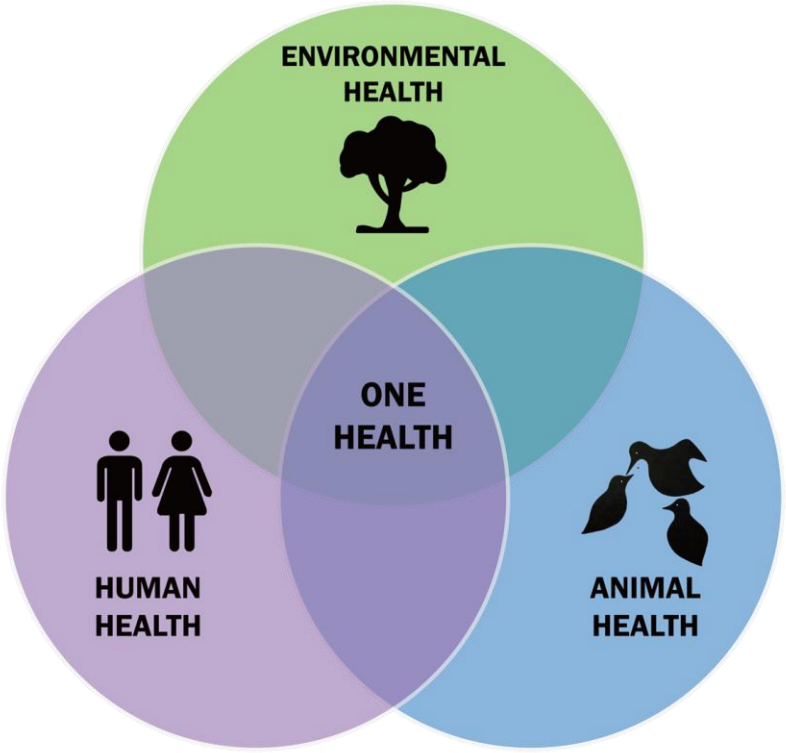


Liao, Fielding, Cowling et al - unpublished

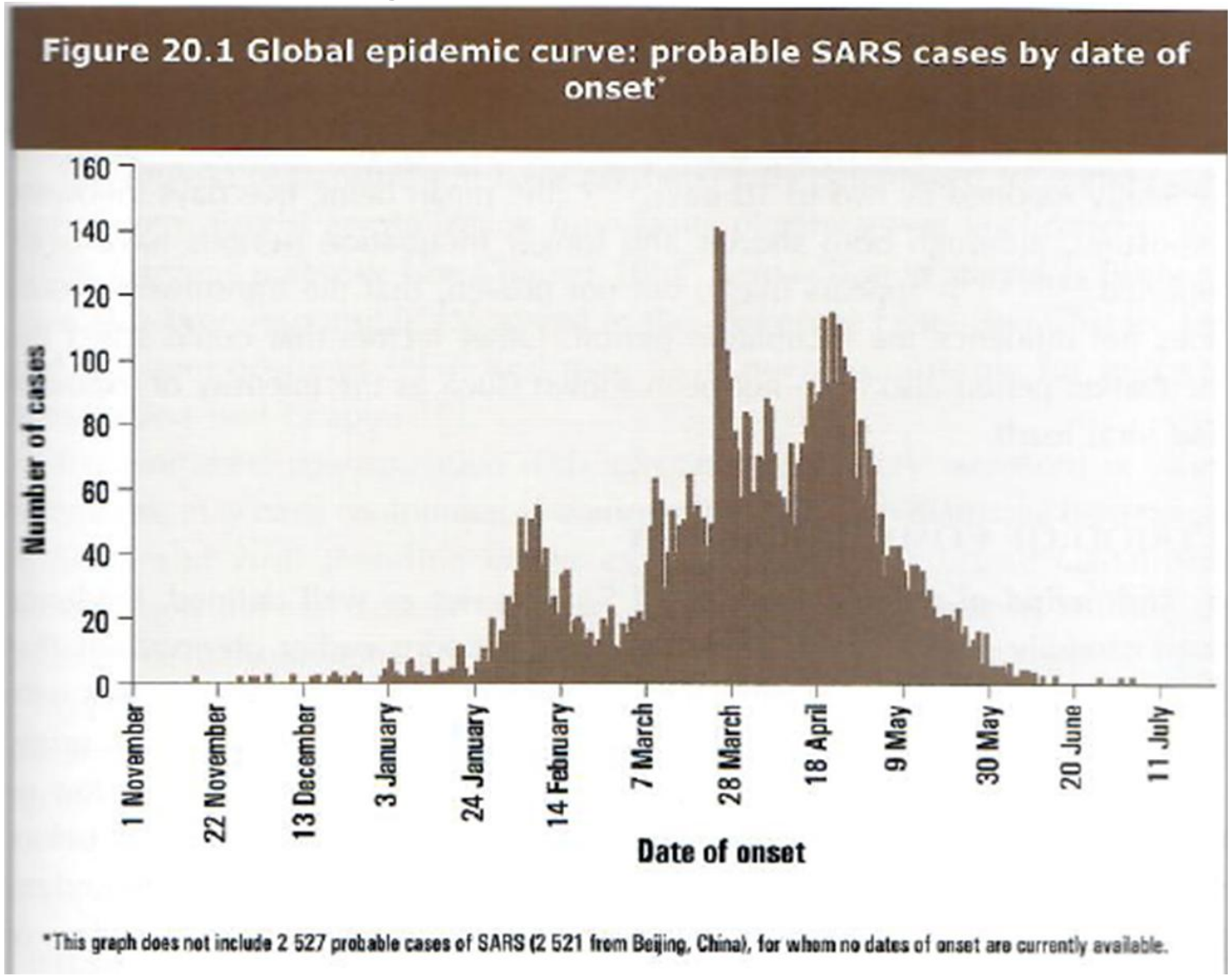
One Health

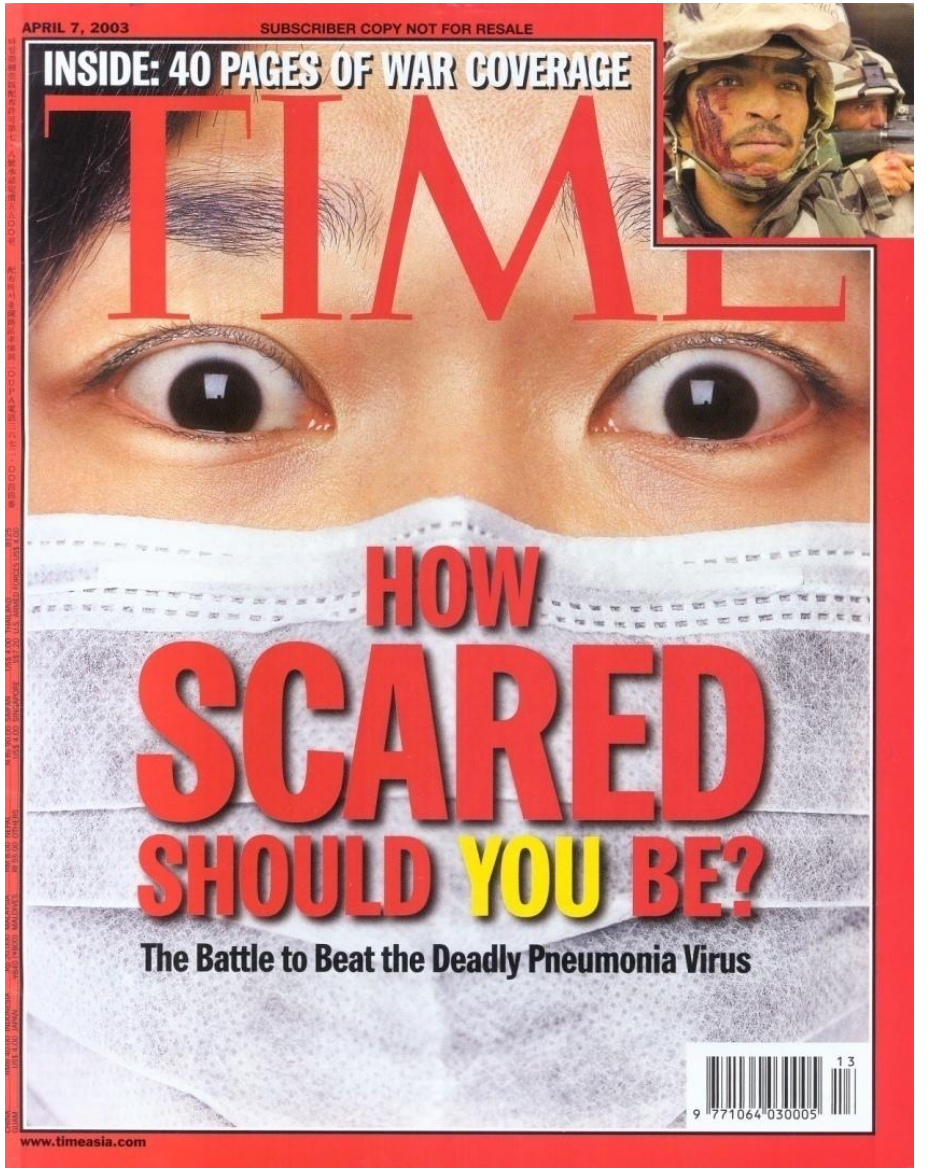
(One Health High Level Expert Panel definition)

- One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health *(“well-being”)* of people, animals and ecosystems.
- It recognizes the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-dependent.
- The approach mobilizes multiple sectors, disciplines and communities at varying levels of society to work together to foster well-being and tackle threats to health and ecosystems, while addressing the collective need for clean water, energy and air, safe and nutritious food, taking action on climate change, and contributing to sustainable development.

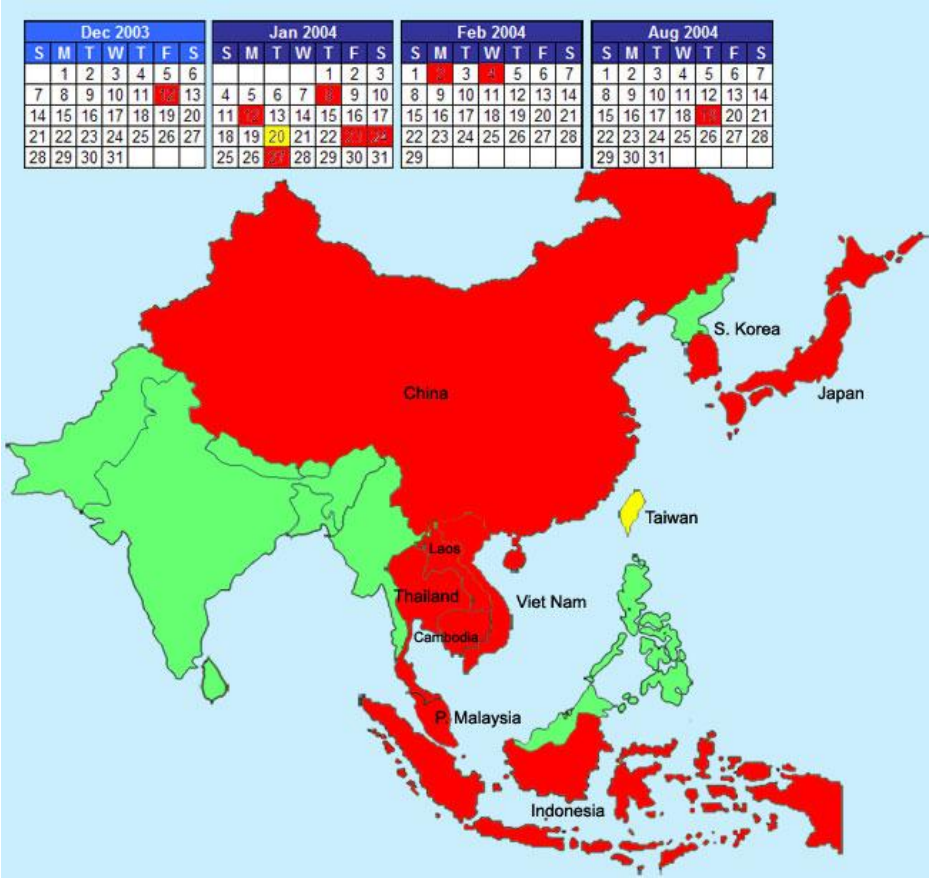


Re-emergence of human H5N1 disease: Imported cases in February 2003





Severe pneumonia cases investigated as suspected SARS in Dec 2003/Jan 2004 in Vietnam and Thailand found to be H5N1 → subsequently, poultry found to be infected in 9 countries in Asia



	Report of first case	Human cases / deaths		
		2003	2004	2005
Vietnam	Jan 2004	3/3	29/20	61/19
Thailand	Jan 2004		17/12	5/2
Cambodia	Feb 2005			4/4
Indonesia	July 2005			19/12
China	Nov 2005			8/5

Live poultry markets









Domestic ducks



Backyard poutlry



The real world







What would this poultry farmer do?

The avian flu paradox:

Exposure = Disease

X



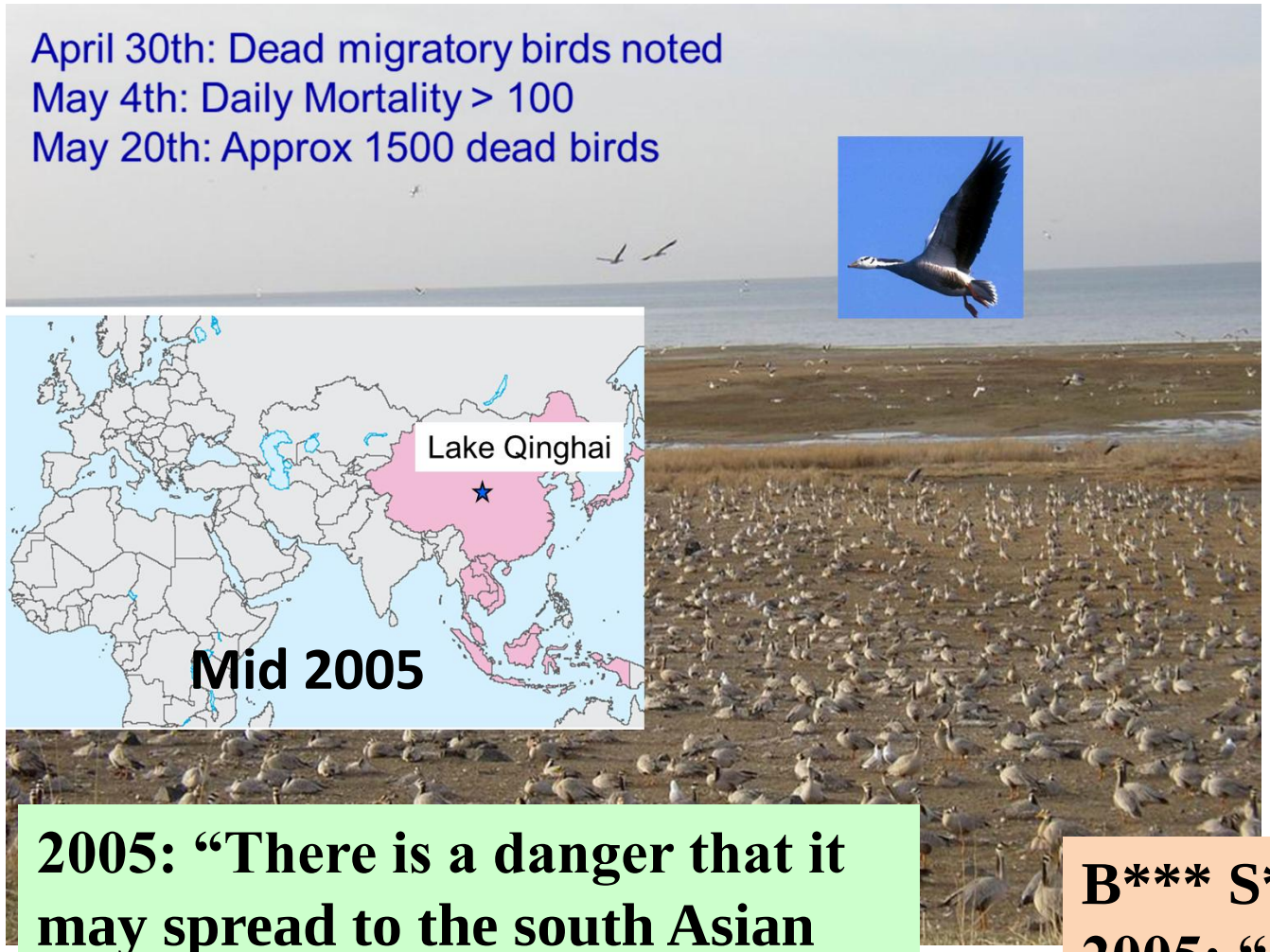
JP/Apriadi Gunawan

POULTRY OFFERING: People from the Karo ethnic group gather Monday at the North Sumatra provincial legislative council in Medan. They brought chickens to be slaughtered and eaten with members of the council to prove their area is safe from bird flu, despite a string of deaths recently from the disease in their North Sumatra village.

Another man dies in Sumatra village

- Exposure necessary, but not sufficient
- Disease is highly stochastic

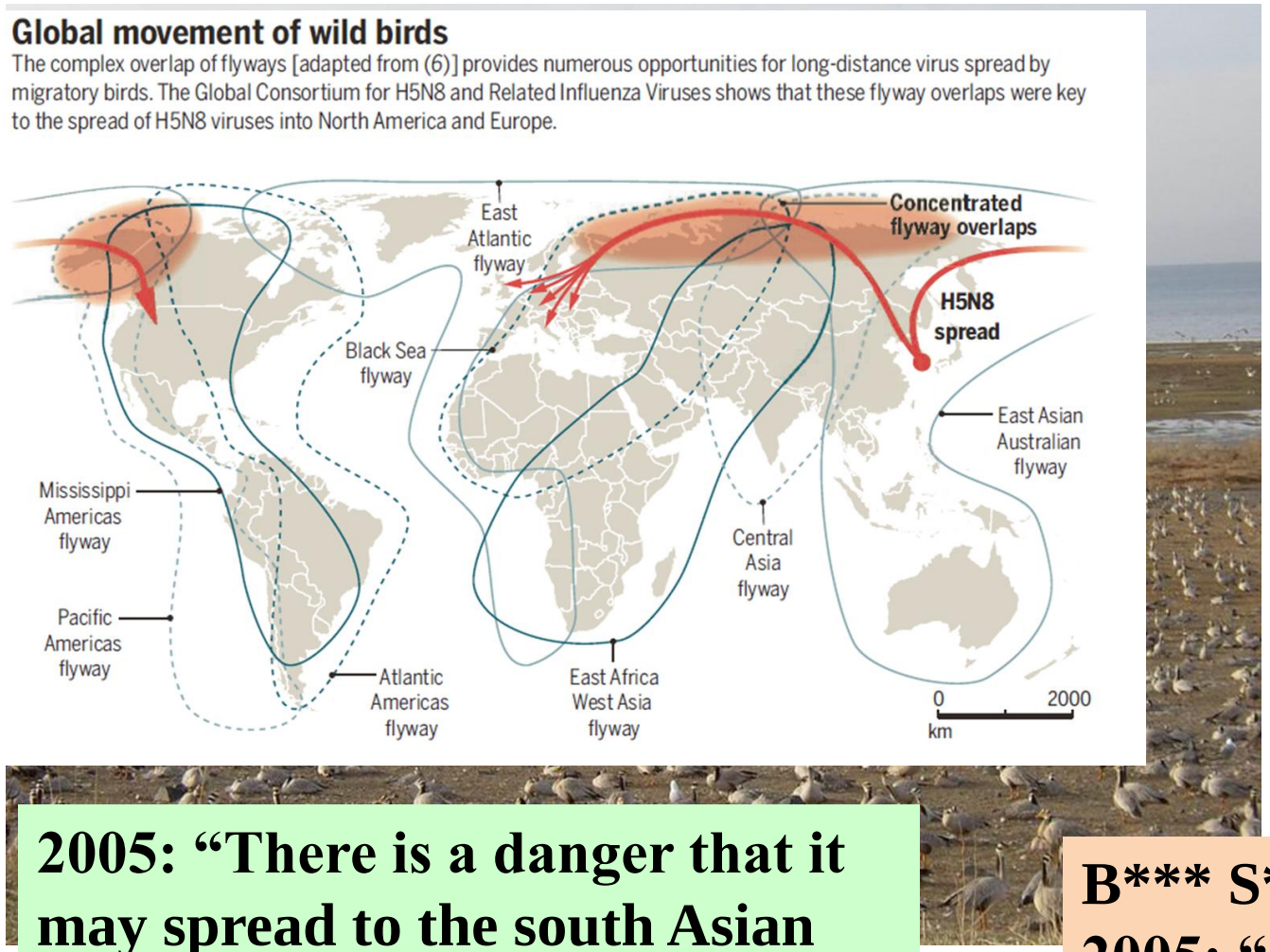
H5N1 outbreak in Qinghai Lake 2005

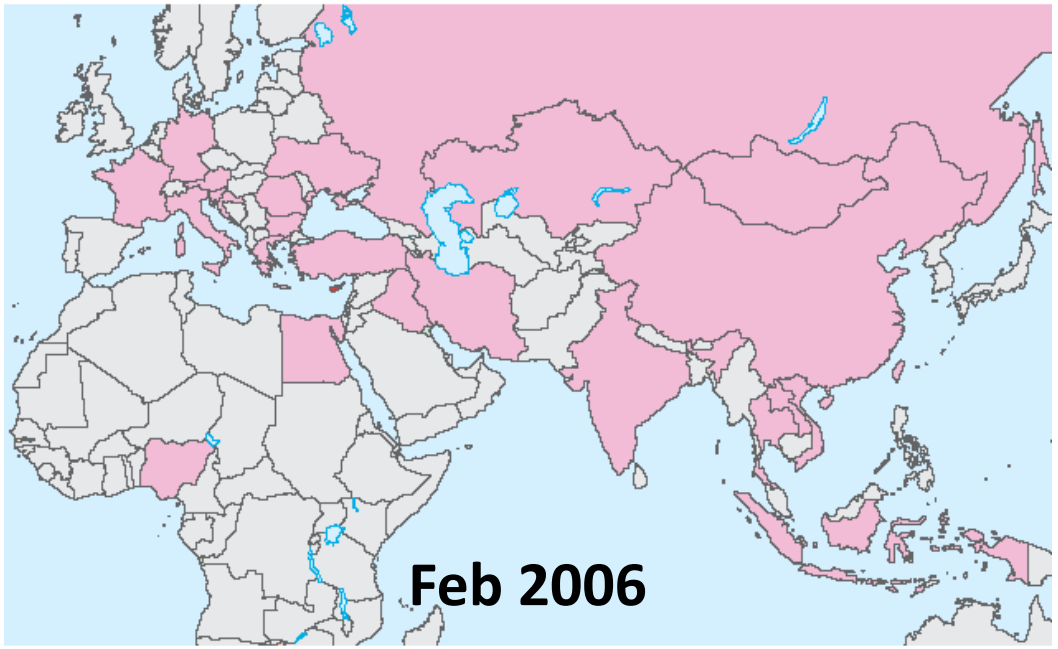


2005: "There is a danger that it may spread to the south Asian subcontinent …. and Europe"
- Chen et al Nature July 2005

B* S***
2005: "Sick birds do not fly !!! Migratory birds will not spread H5N1"**

H5N1 outbreak in Qinghai Lake 2005

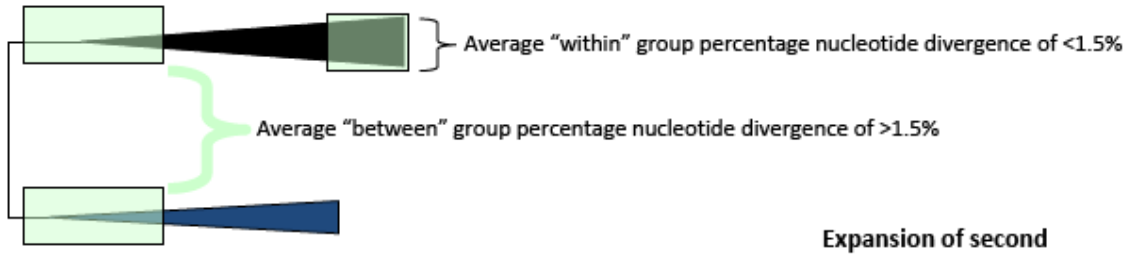




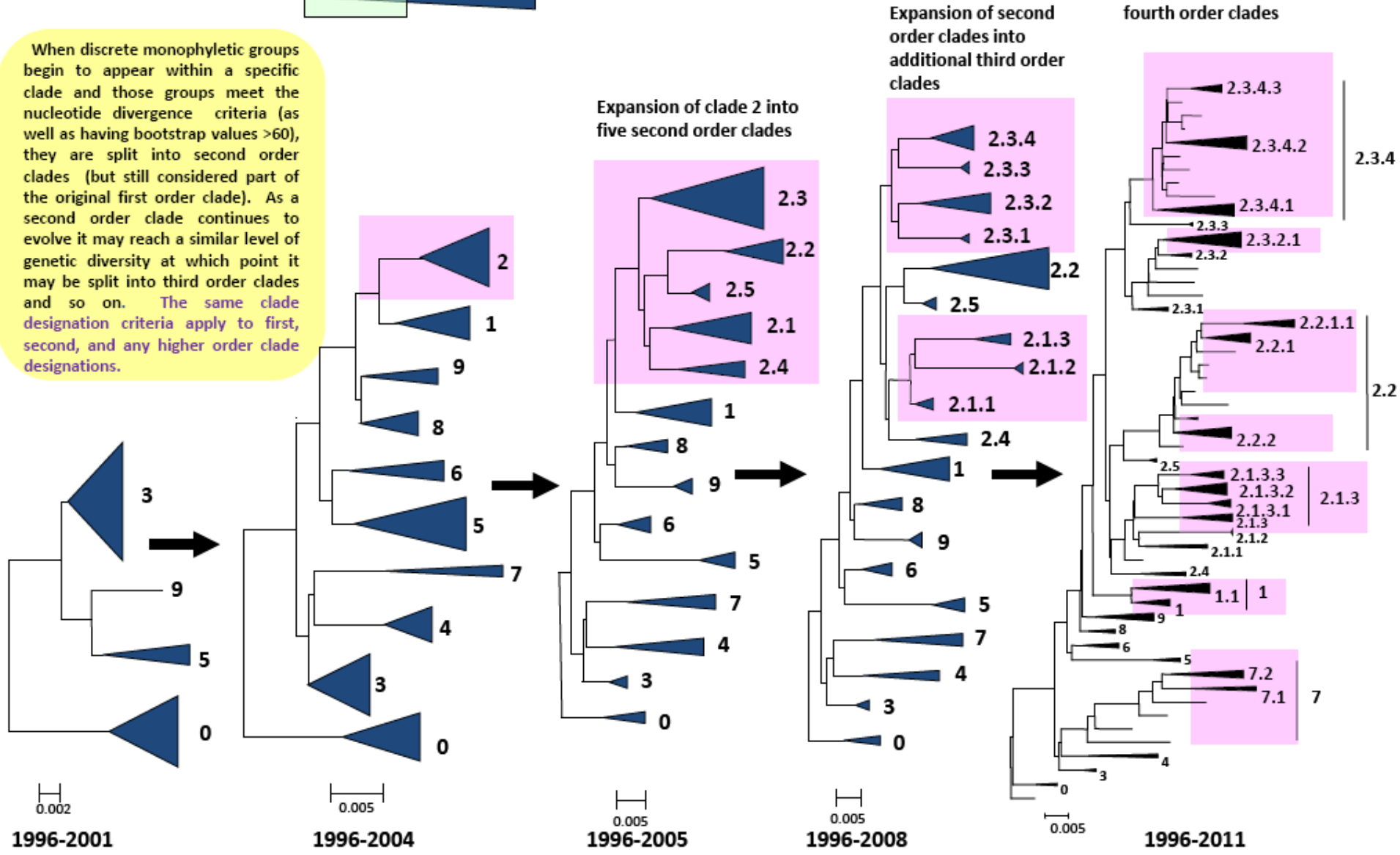
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Evolution of the Asian H5 Hemagglutinin

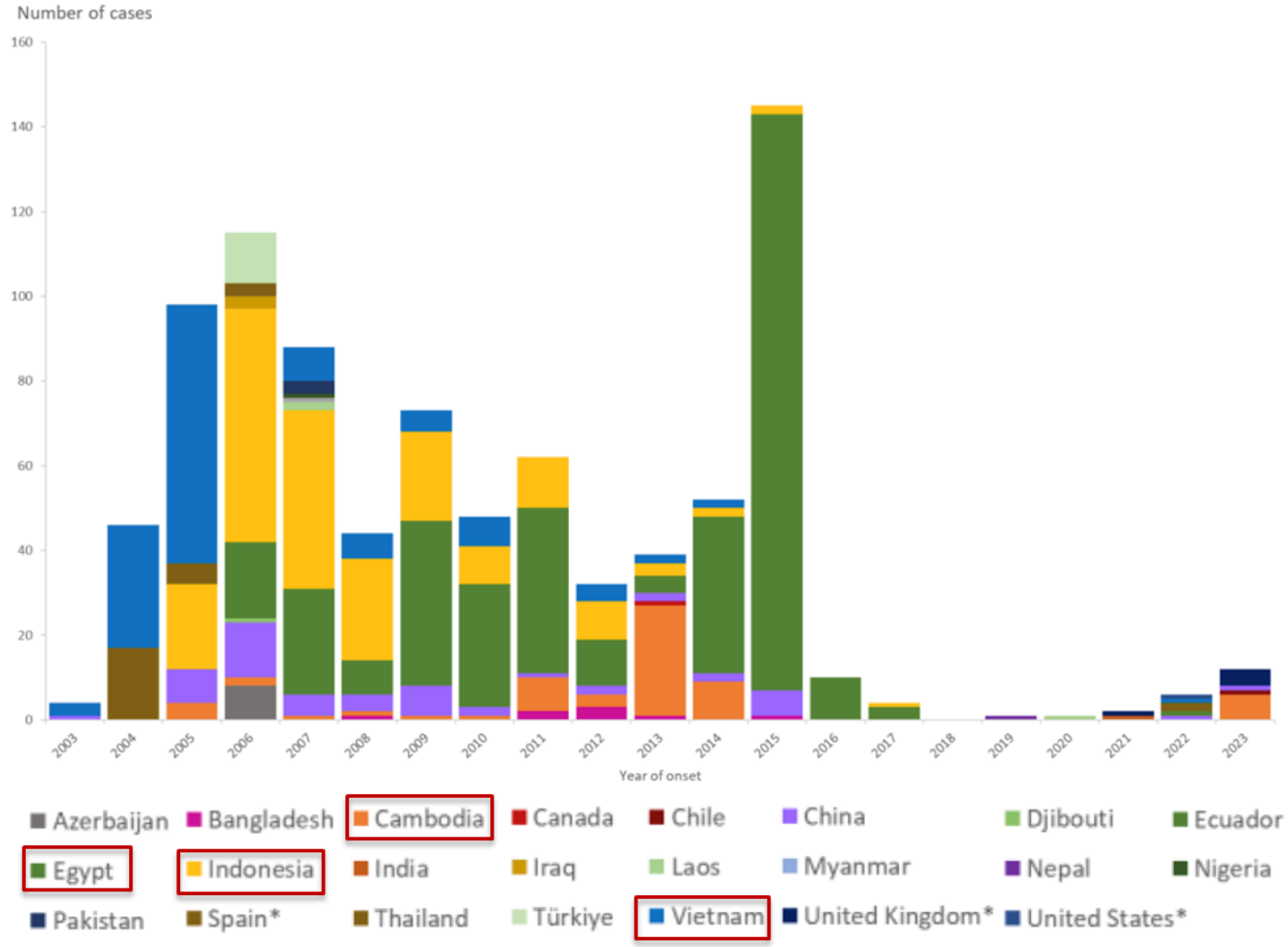


When discrete monophyletic groups begin to appear within a specific clade and those groups meet the nucleotide divergence criteria (as well as having bootstrap values >60), they are split into second order clades (but still considered part of the original first order clade). As a second order clade continues to evolve it may reach a similar level of genetic diversity at which point it may be split into third order clades and so on. The same clade designation criteria apply to first, second, and any higher order clade designations.



HPAI H5N1 infections in humans: 2003-2023

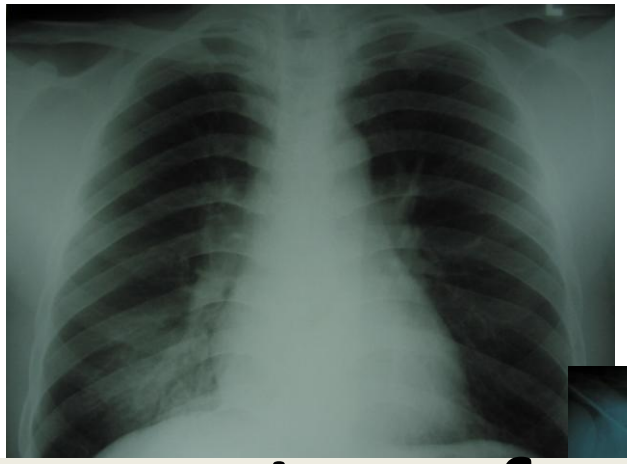
Confirmed cases = 882
Fatal cases = 461 (52%)



Hong Kong 1997: H5N1 Clinical presentation

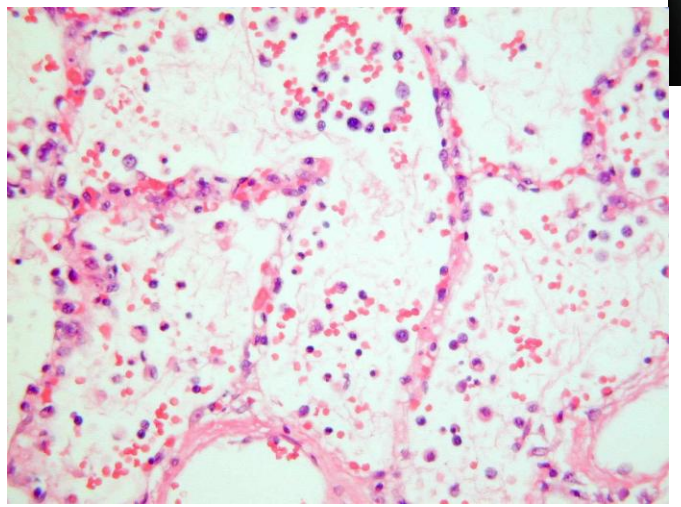
- 18 cases, 11 severe, 6 fatal
- Age range 1 – 60 years
- Mild URTI-like (7 patients; < 6 years)
- Severe viral pneumonia (n=11)
  - age >6 years with one exception
  - No s
  - Seve
    illne
  - Leukopenia / Lymphopenia
  - 2/3rd have deranged liver function tests, diarrhoea / vomiting
  - Death associated with Acute Respiratory Distress Syndrome and multi-organ dysfunction

Yuen et al Lancet 1998

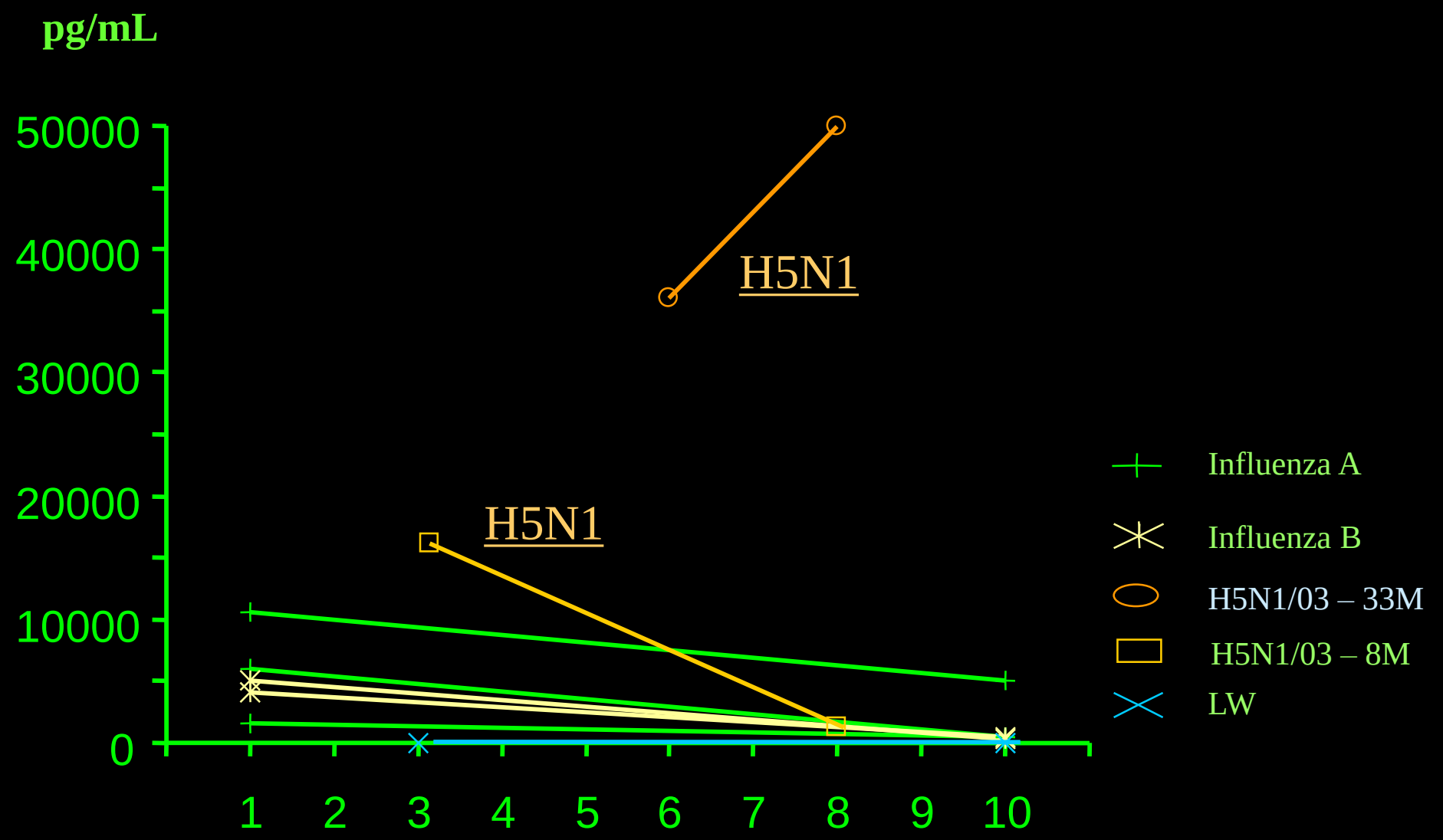




Why the clinical severity of human H5N1 disease ?

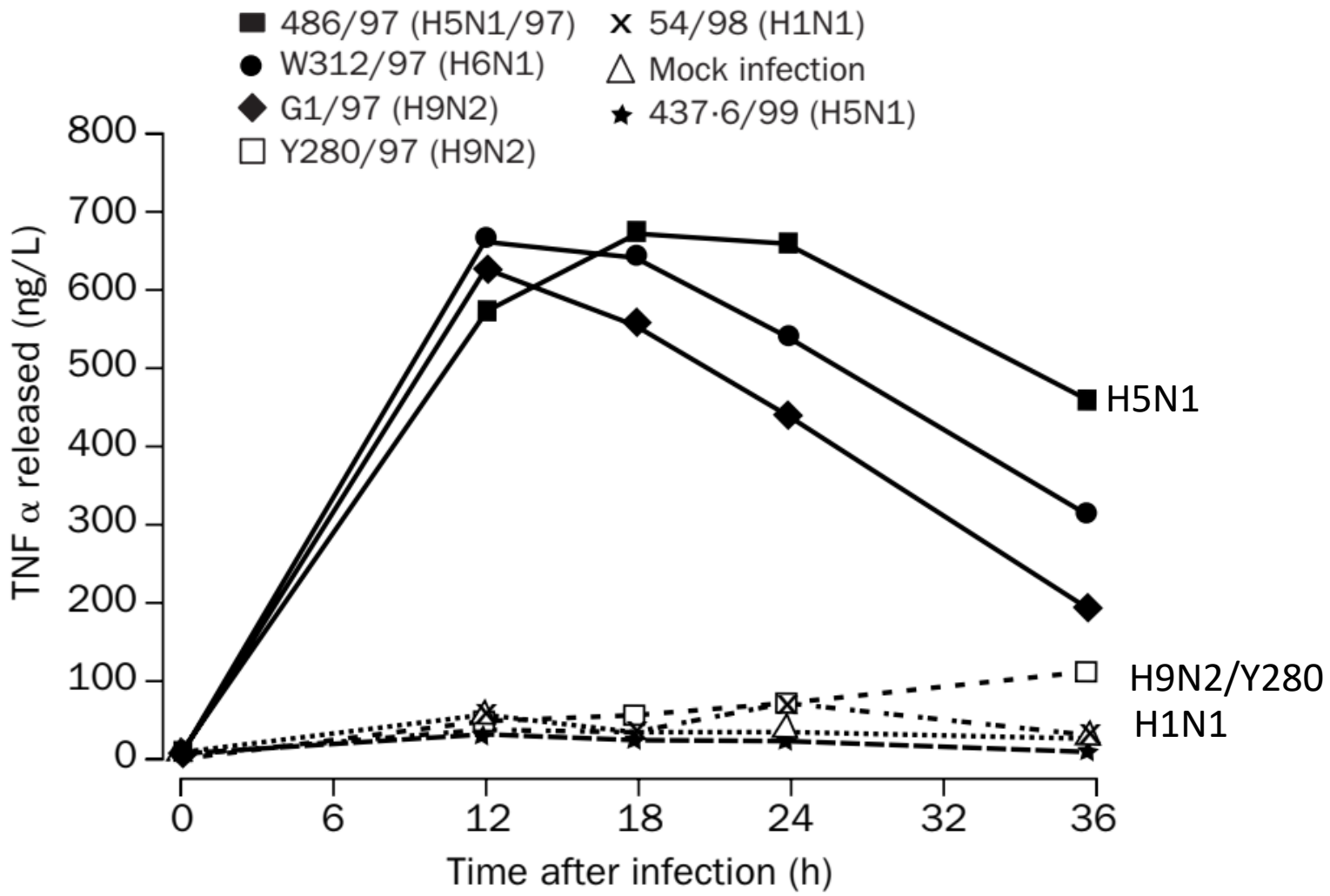


Serum IP-10 levels in patients with H5N1/03 and H3N2 infection



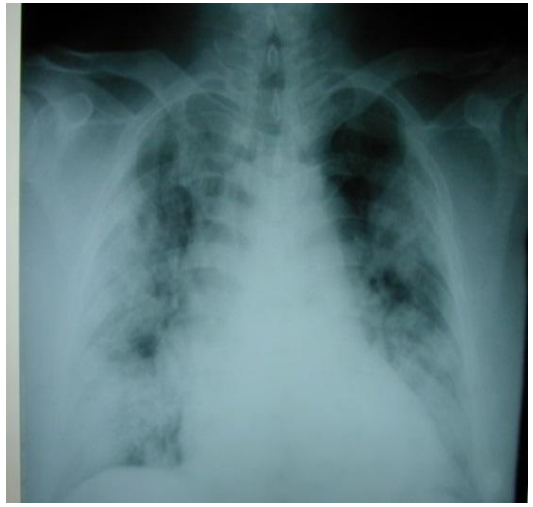
Peiris et al Lancet 2004; 363: 617-9

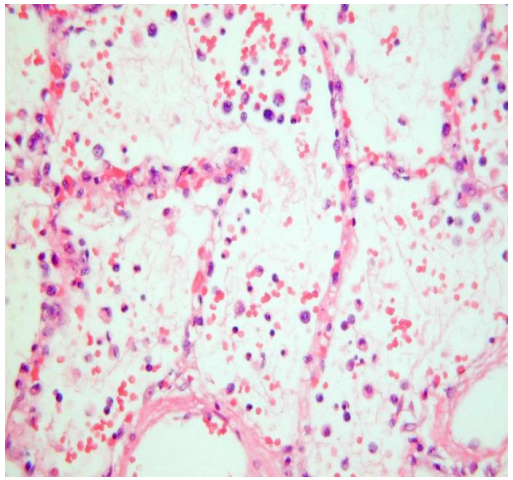
Human primary macrophages infection with H5N1/97 or seasonal H1N1: TNFα in culture supernatants of

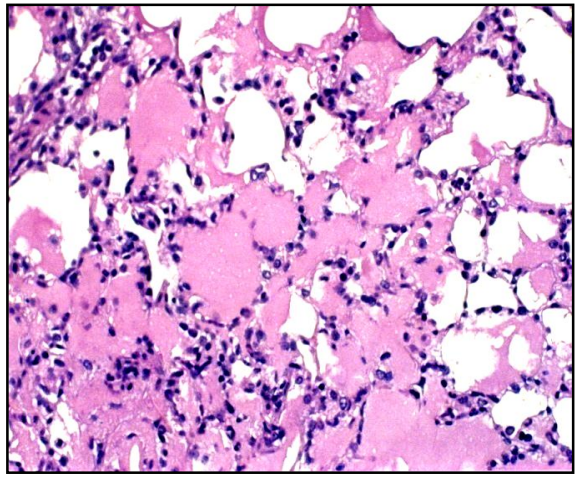


Cheung et al Lancet 2002; 360:1831-7

How does H5N1 cause severe respiratory distress syndrome (ARDS)?



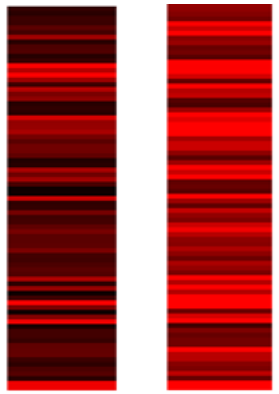






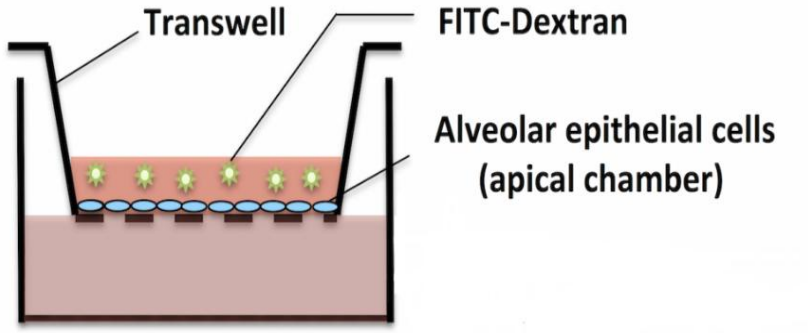
H5N1: Case fatality: 52%
Antivirals alone do not assure survival

Cheung CY et al Lancet 2002
Peiris JSM et al Lancet 2004
Lee SM et al J Infect Dis 2008
Hui KP, et al. J Immunol. 2009
Hui KP et al J Infect Dis 2011
De Jong et al Nat Med 2006



H5N1 dysregulates host responses
→ Aggravates lung injury

- Innate immune pathways dysregulated include TNF signaling; Type I IFN signaling; Jak-Stat signaling; Apoptosis; Cox-2
- Quantitative rather than qualitative



Alveolar fluid is cleared via specialised ion channels


Michael Chan

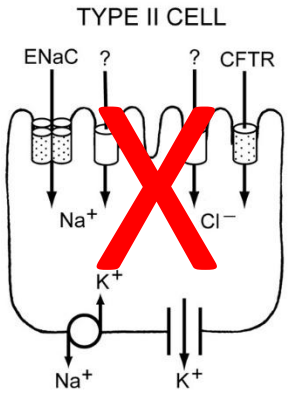
H5N1 virus infected lung cell

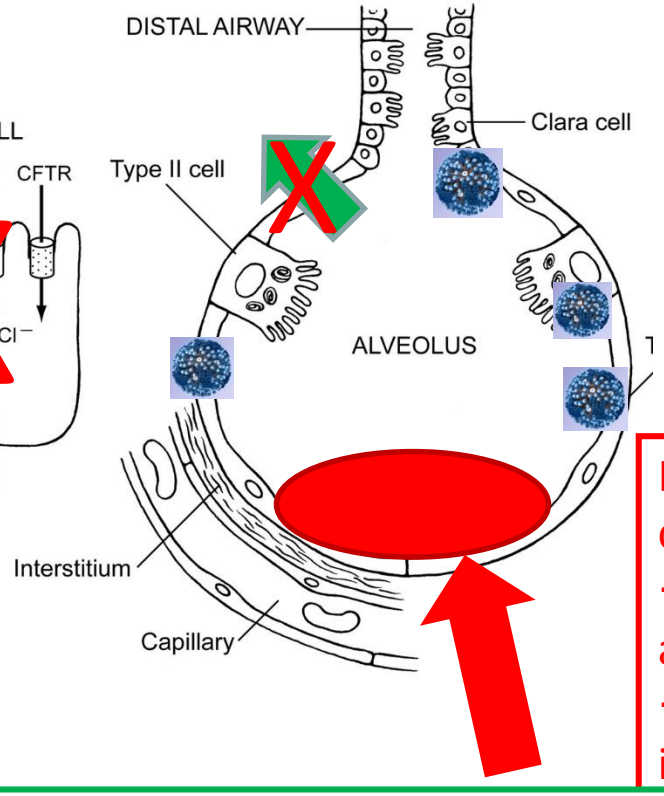
↓

Soluble mediators

↓

Reduces expression ion channels

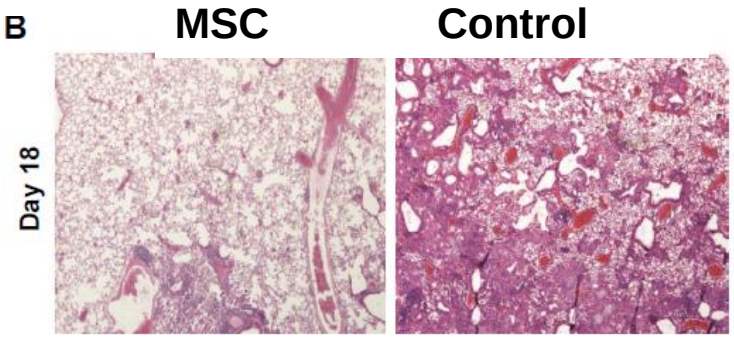




Impaired fluid clearance → Fluid accumulation → Acute lung injury



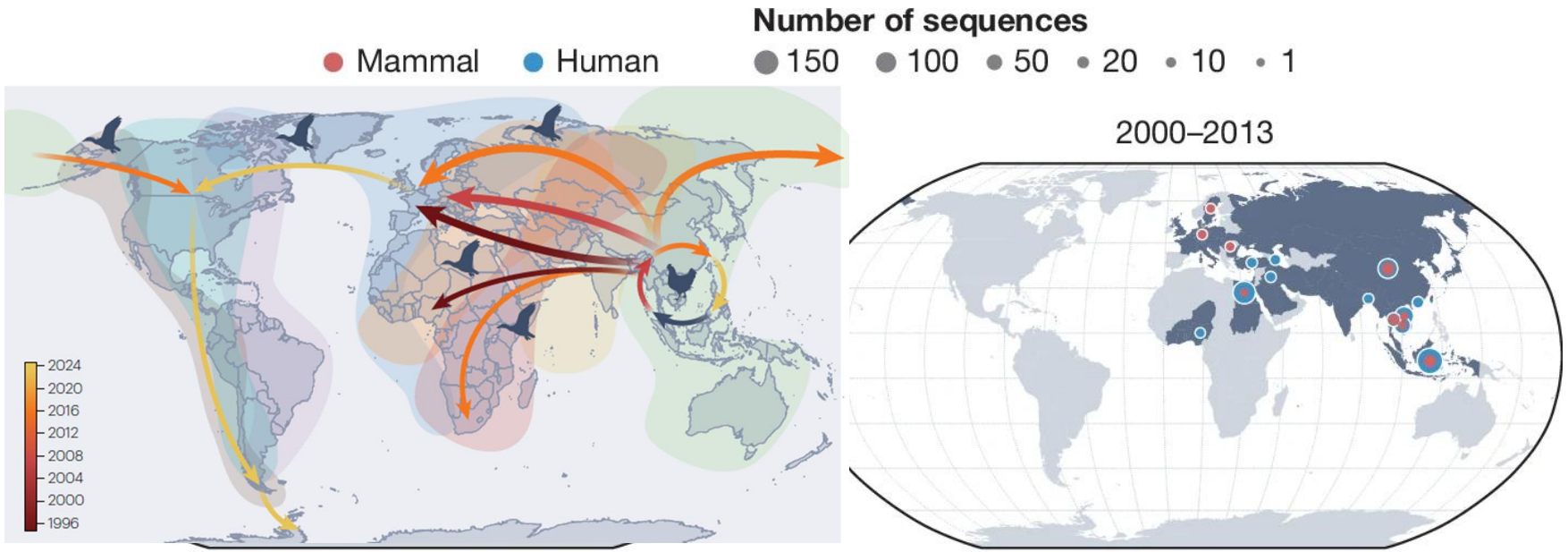
Mesenchymal stromal cell therapy reversed loss of ion channels, improved lung pathology and survival



Exploring small molecule therapies and MSC microvesicle therapy to reverse fluid accumulation

Chan M et al PNAS 2016; Loy et al JID 2019

Dissemination of avian Flu A(H5N1)



Wild bird spread

- 2005-8: Clade 2,2
- 2009-2010: Clade 2.3.2.1 (H5N8)
- 2014-2015: Clades 2.3.4.4 & 2.3.2.1c
- 2016-2017 & 2020-23: clade 2.3.4.4b

Prior to 2020, H5Nx did not persist in Europe

Lasted only 1 year

Clade 2.3.4.4b
Long stalk NA

Outbreaks in fur farms in Spain & Finland

Clade 2.3.2.1a S Asia
Clade 2.3.2.1c/e Indonesia SE Asia, Cambodia

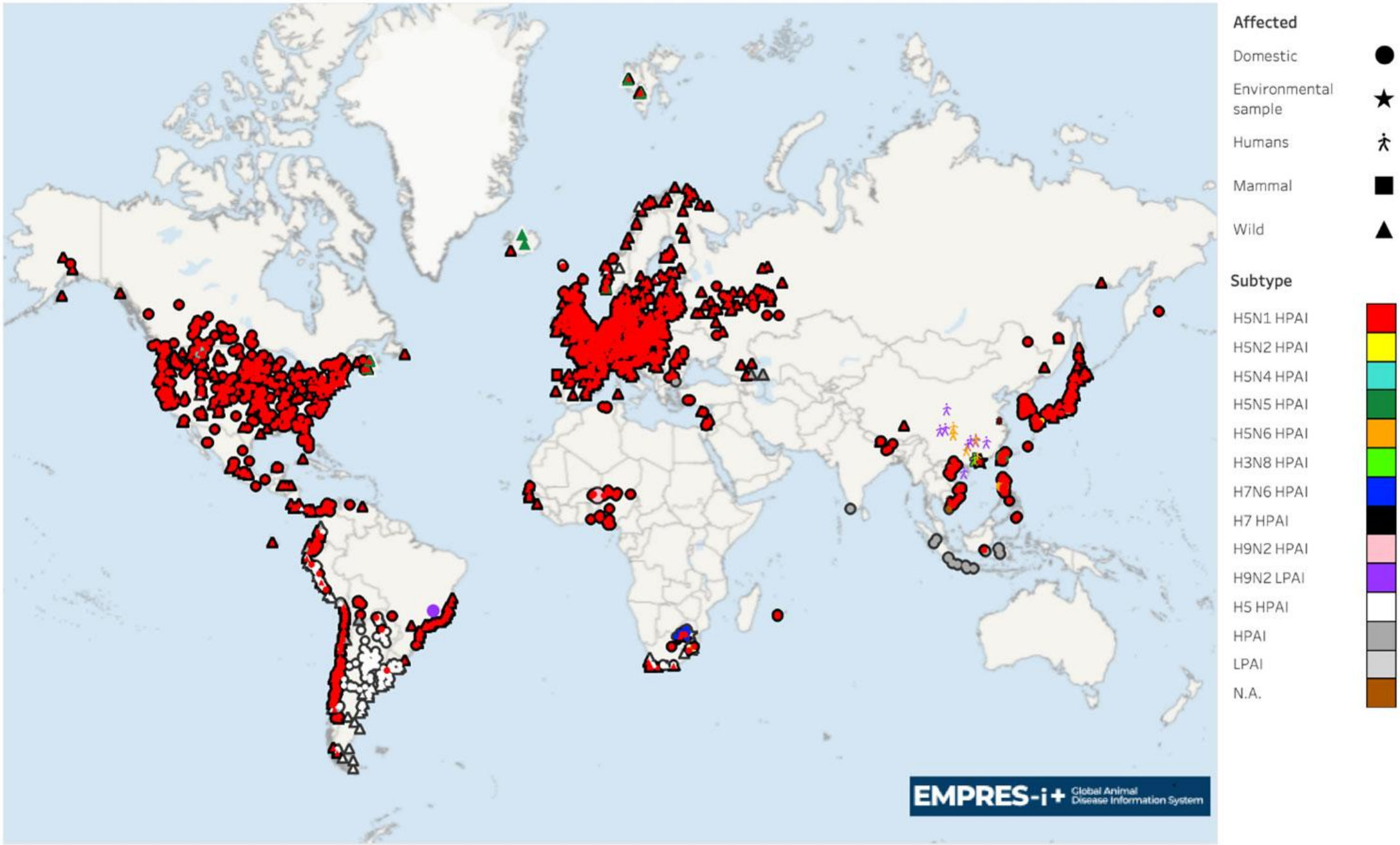
Peacock T et al Nature 2025

Marine mammals
S America

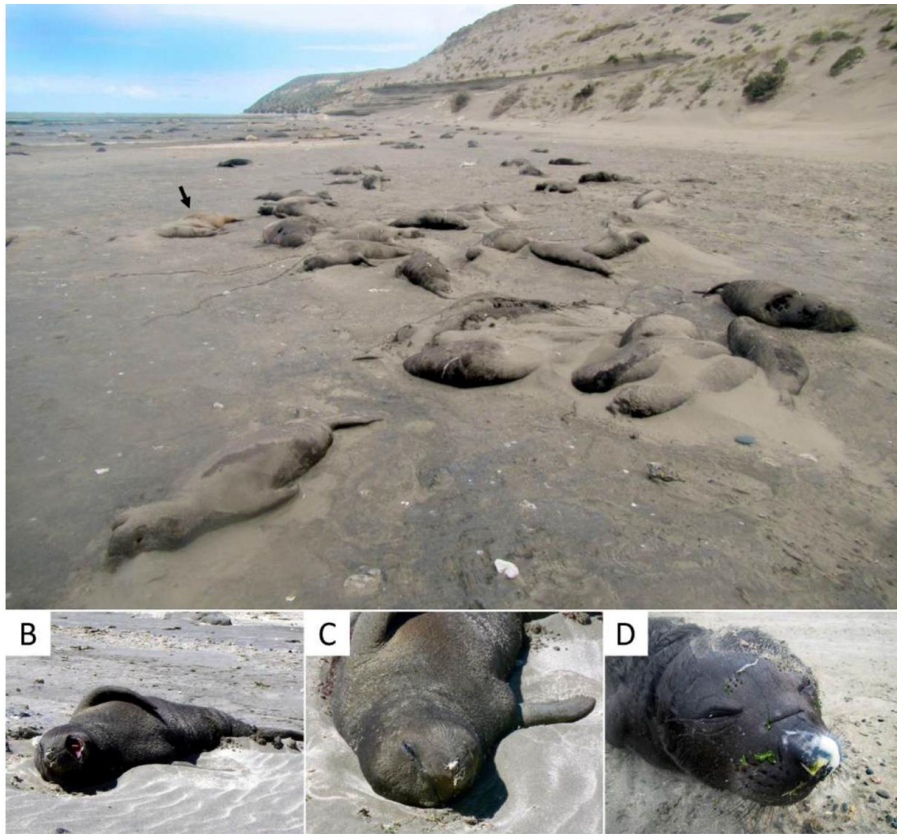
Challenges for HPAI control

- H5N1 outbreaks prior to 1997:
  - Wild bird LPAI→ infects poultry→ mutates to HPAI→ localised outbreaks→ depopulation and control (“stamping out”) was effective. International trade restrictions are critical.
- Post 1997 Goose/Guangdong/69 lineage H5N1 HPAI
  - Local poultry trade based spread PLUS Long range spread via migratory birds. Different scale of challenge. Old solutions may no longer be sufficient

Confirmed Avian influenza events worldwide from 1 October 2022 to 30 September 2023



Outbreak of clade 2.3.4.4b H5N1 in aquatic birds and mammals in South America





>30,000 sea lions; >17,000 elephant seals, many sea bird species killed
Mammalian adaptive mutations appearing PB2 Q591K; D701N etc
Likely mammal to mammal transmission

Uhart MM et al. Nature Comms 2024;15:9516

The Texas veterinarian who helped identify bird flu H5N1 in cows

The first calls that Dr. Barb Petersen received in early March were from dairy owners worried about crows, pigeons and other birds dying on their Texas farms. Then came word that barn cats — half of them on one farm — had died suddenly.

Within days, she was hearing about sick cows with unusual symptoms: high fevers, reluctance to eat and much less milk. Tests for typical illnesses came back negative.

Petersen, …. collected samples from cats and cows and sent them to Dr. Drew Magstadt, a friend from college who now works at the veterinary diagnostic laboratory at Iowa State University.

The samples tested positive for A(H5N1). Influenza never before seen in cattle.



This photo provided by Sunrise Veterinary Service in May 2024 shows Barbara Petersen. The first calls the Amarillo veterinarian received in early March 2024 were from dairy owners worried about crows, pigeons and other birds dying on their Texas farms. Then came word that barn cats _ half of them on one farm _ had died suddenly. Within days, she was hearing about sick cows with unusual symptoms: high fevers, reluctance to eat and much less milk. (Sunrise Veterinary Service via AP)

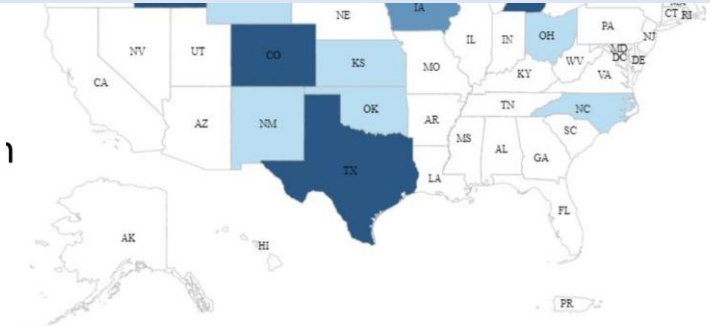
Jonel Aleccia
https://apnews.com/article/bird-flu-dairy-farmers-barb-petersen-07bd5accb01441bc5d279464271d2371

H5N1 outbreak in diary cattle, USA

- Texas diary herd: fall in milk production & mastitis → 25 March 2024 H5N1 detected in milk samples, very high infectious virus titre (>10^{7-9} /mL)
- By end 2024 >190 herds in 14 states.
- From affected farms: Humans, cats, raccoons, opossums, wild birds, poultry

How is this virus spreading?

- **Within herds**
- **Across states**

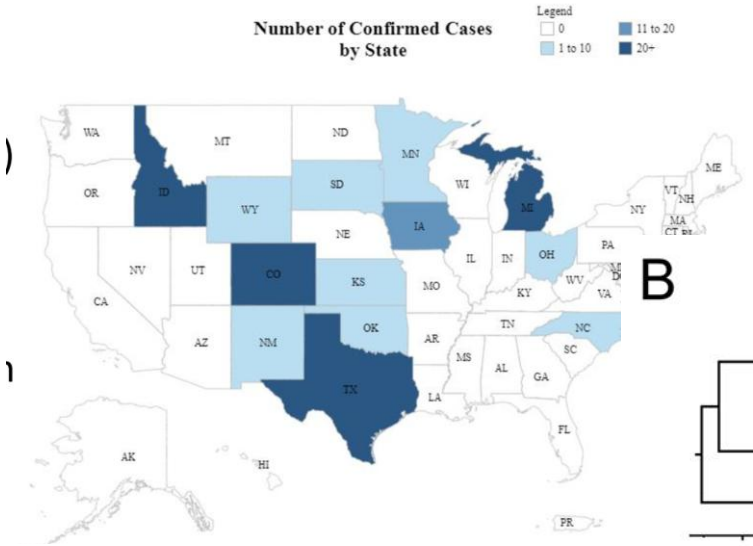


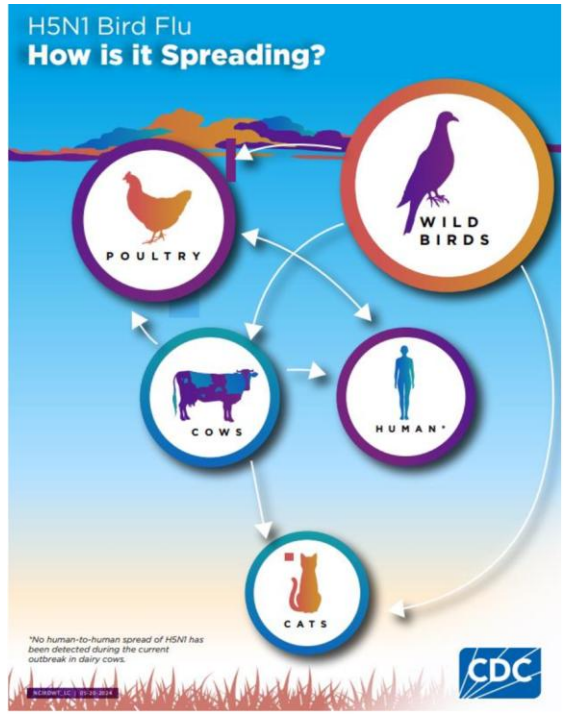
H5N1 outbreak in diary cattle, USA

- Texas diary herd: fall in milk production & mastitis → 25 March 2024 H5N1 detected in milk samples, very high infectious virus titre (>10^{7-9} /mL)
- By end 2024 >190 herds in 14 states.
- From affected farms: Humans, cats, raccoons, opossums, wild birds, poultry
- Initially, single spillover to cows, B3.13 genotype (probably late 2023). Spread from one diary farm to multiple states in USA.

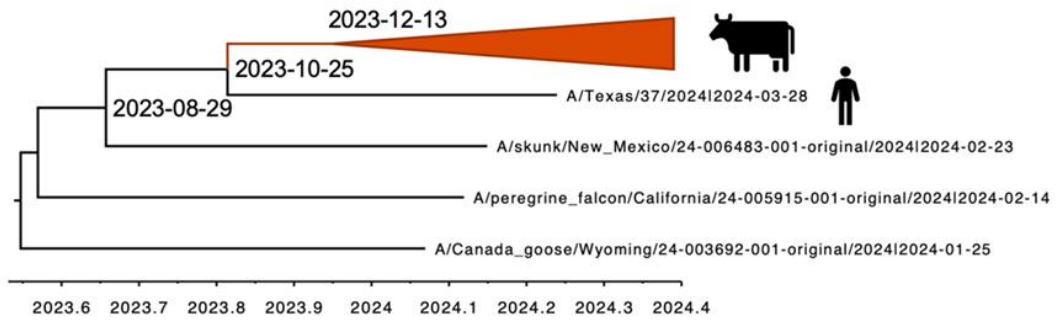




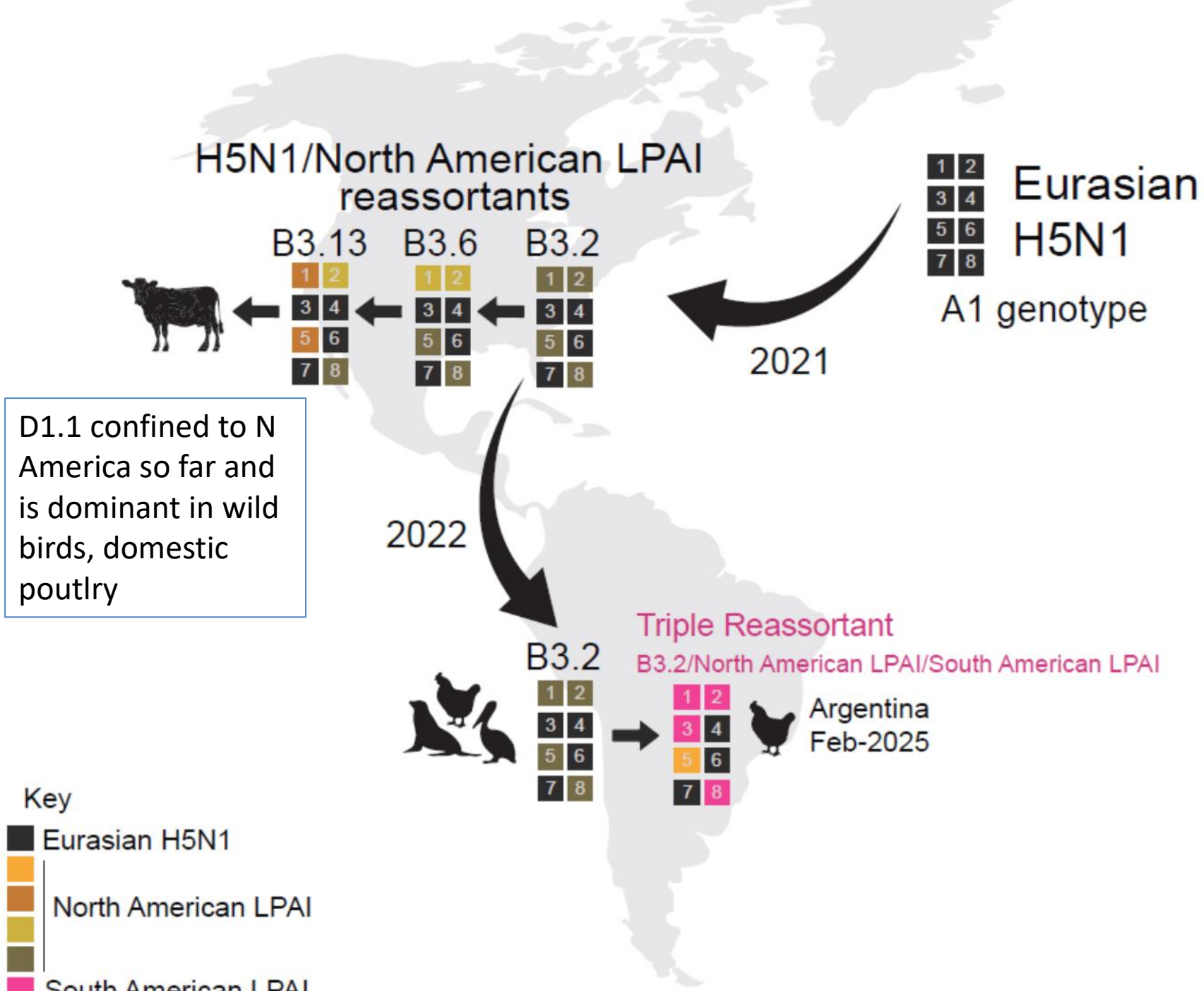




B



Nguyen TQ et al. bioRxiv May 1 2024

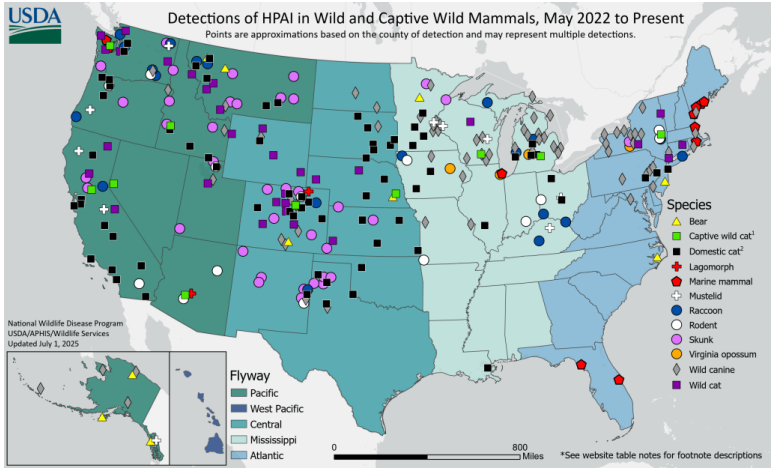


D1.1 confined to N America so far and is dominant in wild birds, domestic poutlry

Vanstreels RET et al bioRxiV 2025 https://www.biorxiv.org/lookup/doi/10.1101/2025.05.23.655175?__cf_chl_tk=2Q05ND43duRVd5QAv5QBDX9Ru1Bnr9JeOxAt.hKWI18-1752116677-1.0.1.1-vxzVFuJW8GPpD..lVpasVc33hy5k6HBwdlAI7nz4Sps

Current Status in USA (Part 1)

- 13,421 wild birds; 174.8 million poultry, mainly D1.1
- Since first report in cows on 24th March 2024, 1,074 dairy farms in 17 States of USA.
- Mostly derived from single B3.13 spill-over
- But two independent spill-overs of D1.1 to cows (Jan 2025)(Nevada, Arizona) → one human case with D1.3 genotype (poultry exposure)
- Over 200 species of mammals affected including wild mammals (foxes, bears, seals), stray of domestic cats & dogs, farmed animals (cows, goats, mink)



https://www.cdc.gov/bird-flu/situation-summary/index.html

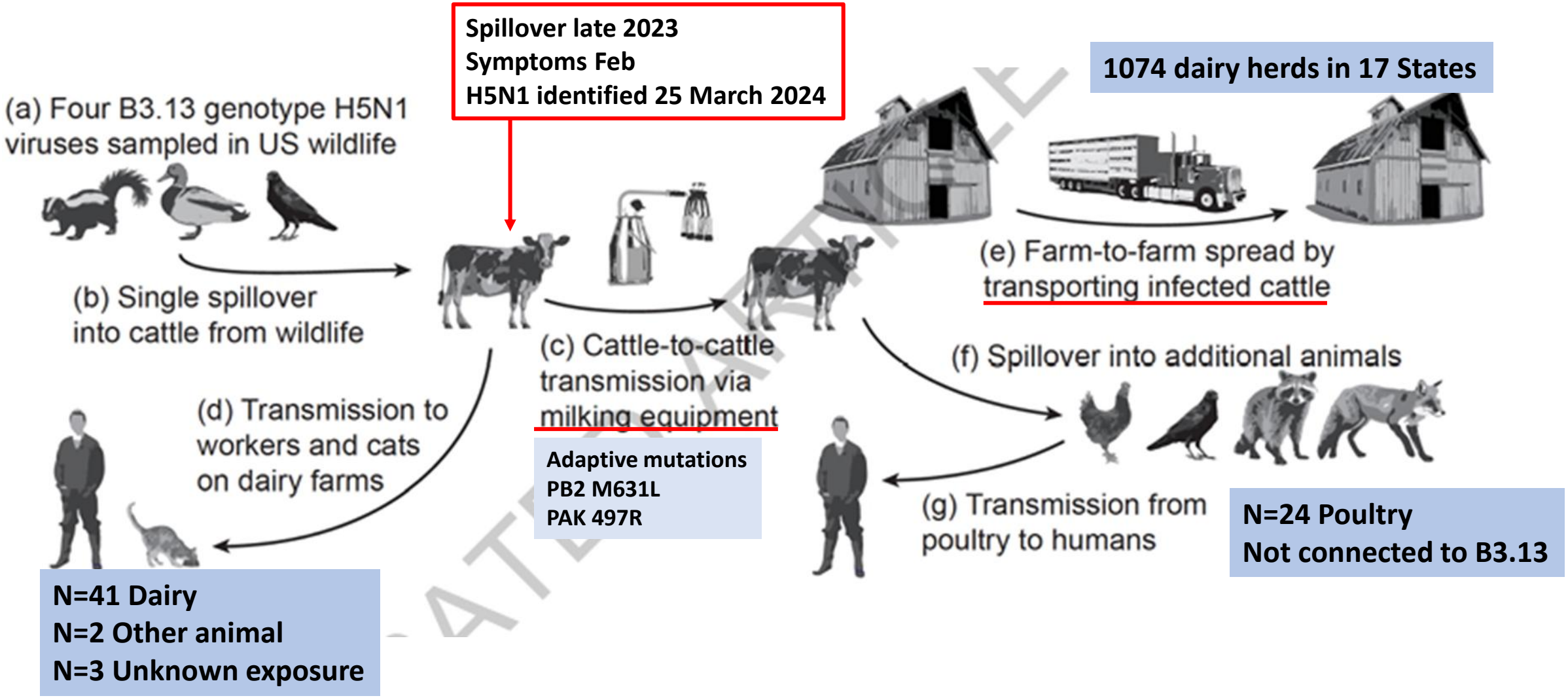
https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/mammals

Why are dairy cow outbreaks not reported elsewhere in the World?

- Dairy cow mammary glands experimentally inoculated with H5N1 B3.13 or a current European H5N1 wild bird isolate led to necrotizing mastitis, reduction of milk production and high fever for both H5N1 isolates.
- In European H5N1 virus inoculated cows, mammalian adaptive mutation E627K emerged in the viral polymerase basic protein 2 (PB2) after intramammary replication of H5N1. Not in B3.13 inoculated cows.

Halwe et al Nature. 2025 Jan;637(8047):903-912.

How did influenza A (H5N1) clade 2.3.4.4b (B3.13) emerge and spread in dairy cows in USA?



Peacock T et al Nature 2025

Current Status in USA (Part 2)

- Human cases: 70 (with one death) in 14 States (6 cases detected via National Flu Surveillance System). All except one are B3.13
  - 41 had dairy cattle exposure (serology suggests more undetected cases; not all H5N1 infections lead to antibody response (*Levine et al EID 2025*)
  - 24 poultry farm / culling exposure
  - 2 other animal exposure
  - 3 with unknown source
  - (6 of these cases detected via National Flu surveillance system)

https://www.cdc.gov/bird-flu/situation-summary/index.html

Human infection with H5N1 in US, Canada, Mexico (bovine or avian exposure)



Uyeki TM et al NEJM 2024

	Total
Mild	67/71 (94%)
Severe	2/71 (2.8%)
Fatal	2/71 (2.8%)

Why North American H5N1 cases appear mild?

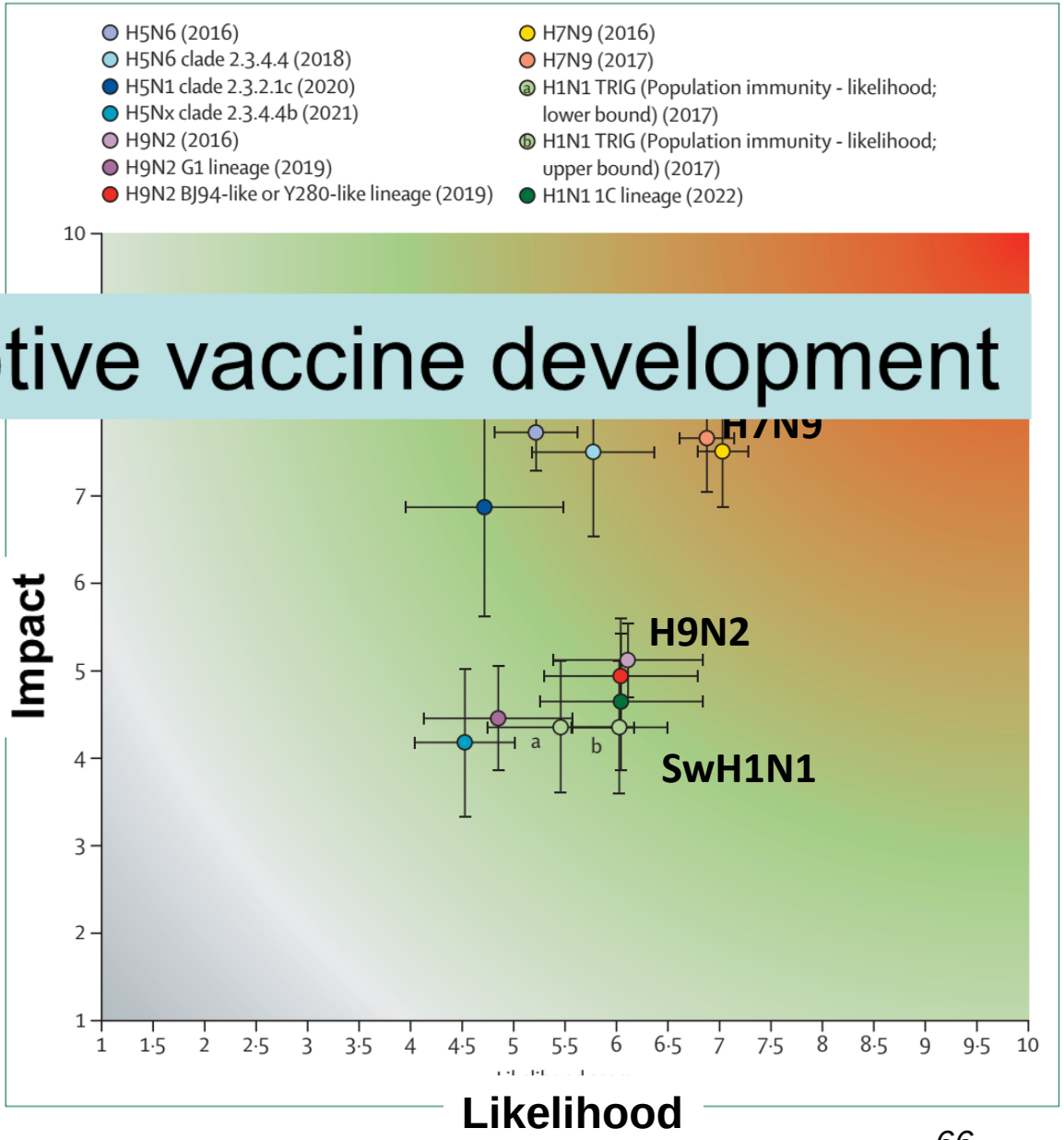
- More intensive detection of mild cases??
- Route of exposure via conjunctiva ?

Framework for assessing influenza virus pandemic risk
WHO Tool for Pandemic Risk Assessment (TIPRA)

Assess likelihood
- Receptor binding
- Tx in animal model
- Population immunity
- Human infection
- Genomic Characteristics
- Infection in animals
- Geographic distribution in animals

Assess impact
- Disease severity
- Population immunity
- Antiviral susceptibility
- Viral genomics
- Receptor binding
- Human infection

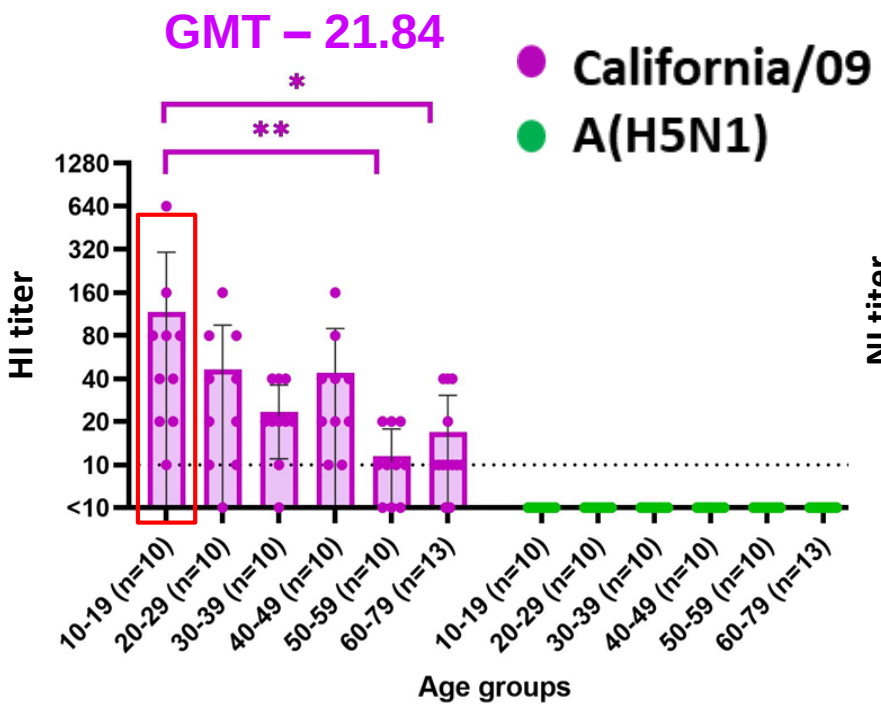
Prioritize pre-emptive vaccine development



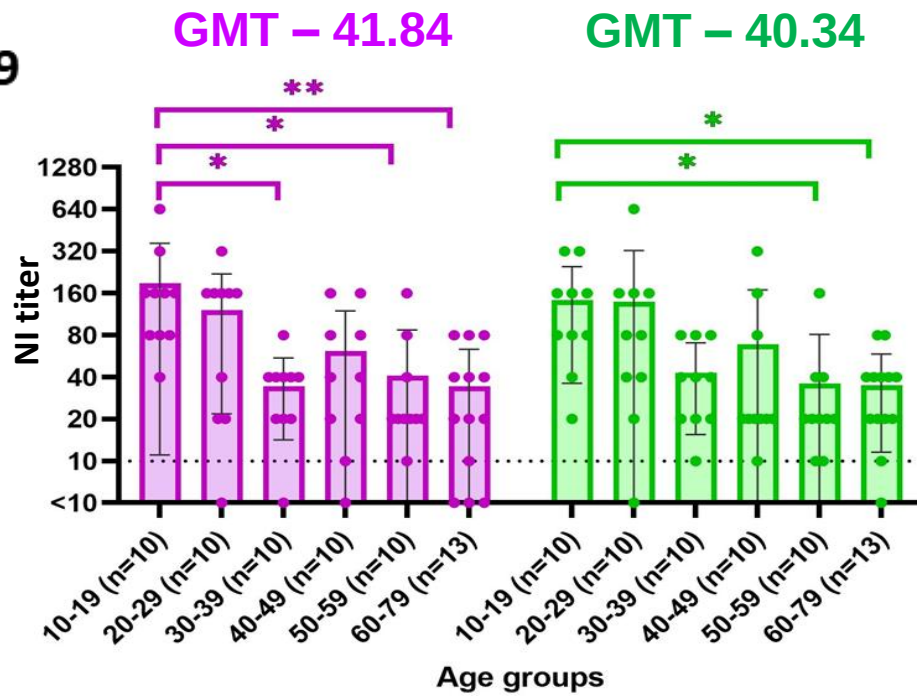
Yamaji R et al Lancet Microbe 2024

Protective antibody responses in age-stratified healthy sera from 2020

Haemagglutination Inhibition (HI) titer



Neuraminidase inhibition (NI) antibody titer



Kruskal-Walli's test,*p < 0.033, **p < 0.002,*p<0.001**

Used recombinant H6N1 virus with N1 from A/Cal/09 and A(H5N1), to avoid steric interference from HA binding antibodies as recommended by Eichelberger in J Virol Methods 2014

A(H5N1) from A/Palatea minor/Hong Kong/HKUMPT-2022 (clade 2.3.4.4b)

Daulagala P, et al. Emerg Infect Dis. 2024;30:168-171.

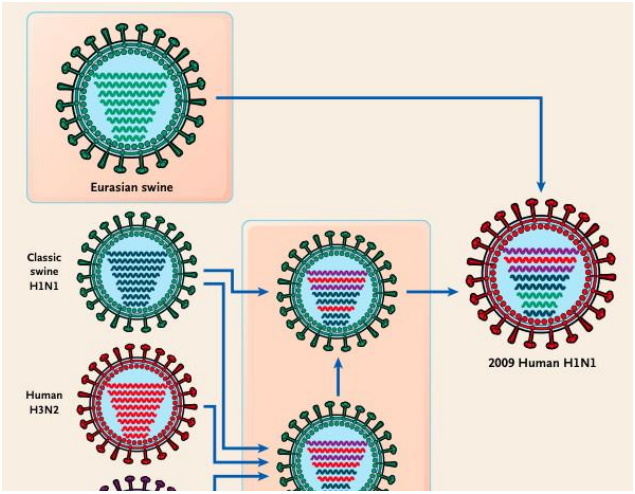


Ferret anti-sera raised against California/09 showed cross-reactive NAI titres against A(H5N1) virus

Post-infection ferret anti-sera	NI antibody titer measured using Rg-A(H6N1) viruses		
	Homologous virus A(H1N1)	California/09 A(H1N1)pdm09	Spoonbill/HK/22 A(H5N1)
A/USSR/90/1977	320	<10	<10
A/Chile/01/1983	320	<10	<10
A/Singapore/06/1986	320	<10	<10
A/Texas/36/1991	320	<10	<10
A/Brisbane/59/2007	1280	<10	<10
A/California/04/2009_pdm09	2560	2560	320-640*

***NI titer range detected in n=6 ferret anti-sera**

N1 NA of 2009 pdm H1N1 is



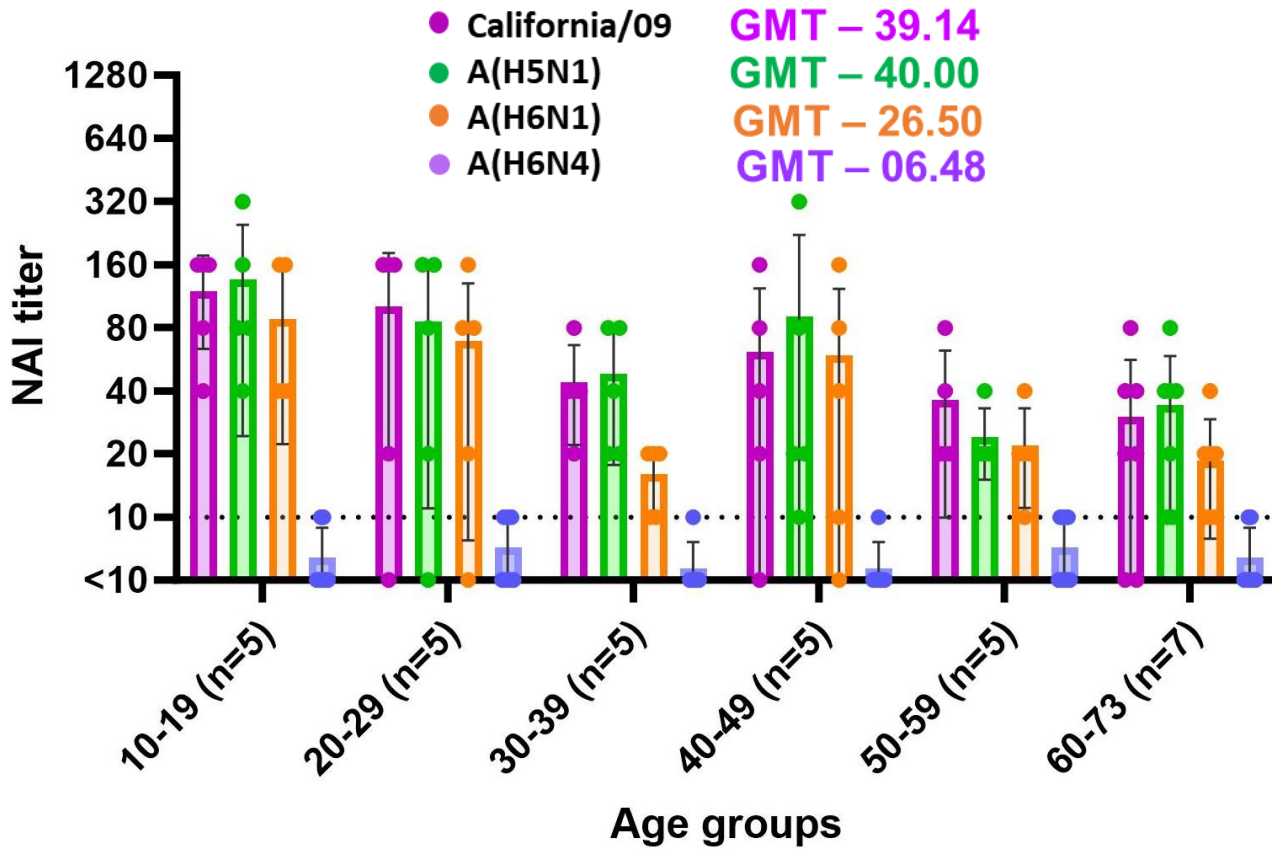
- ✓ N386K substitution on the NA contributed to a one-way antigenic drift from California/09 to Michigan/15.

Gao et al (mBio, 2019)

Daulagala P, et al. Emerg Infect Dis. 2024;30:168-171.

Daulagala et al – unpublished data

Healthy human sera from 2020 possessed cross-reactive NI response against N1 of A(H5N1) and N1 of A(H6N1) but not N4 of A(H6N4)



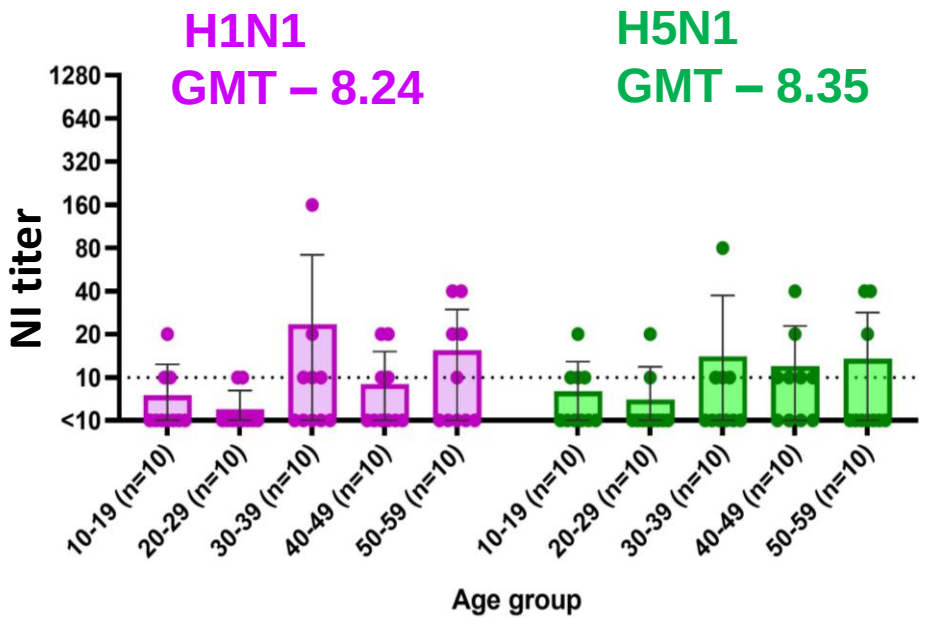
- Human population has no HI antibody to H5 but has high titers of NI antibody to N1 of H5N1 and H6N1 - NI antibodies conserved against avian origin viruses of same subtype.
- Adaptation in humans as seasonal influenza leads to progressive loss of cross reactivity.

A(H5N1) from A/Palatea minor/Hong Kong/HKUMPT-2022 (clade 2.3.4.4b)
A(H6N1) from A/Palatea minor/Hong Kong/HKUMPT-2006/2015
A(H6N4) from A/Anas acuta/Hong Kong/HKUMPT-2022

Daulagala P, et al. Emerg Infect Dis. 2024;30:168-171.

Limited NI reactivity towards A(H5N1) virus in healthy adult pre-pandemic sera collected in July 2009

- California/09
- A(H5N1)



Kruskal-Walli's test,*p < 0.033, **p < 0.002,***p<0.001

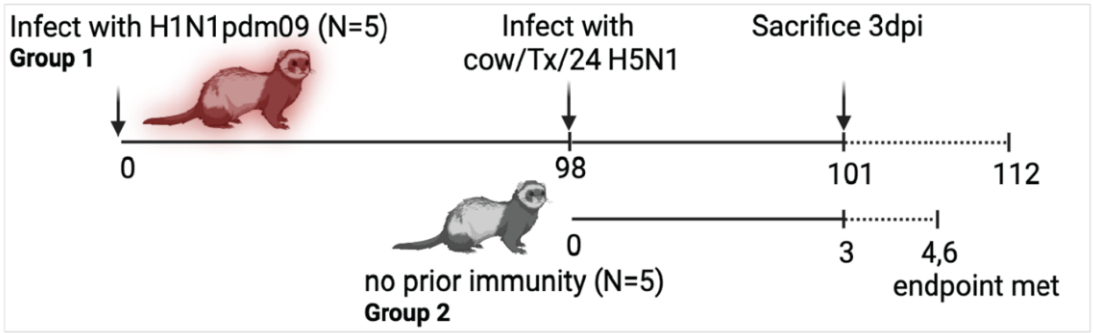
✓ 66% (33/50) showed NI titres below the detection limit (<1:10) to both California/09 and A(H5N1) virus

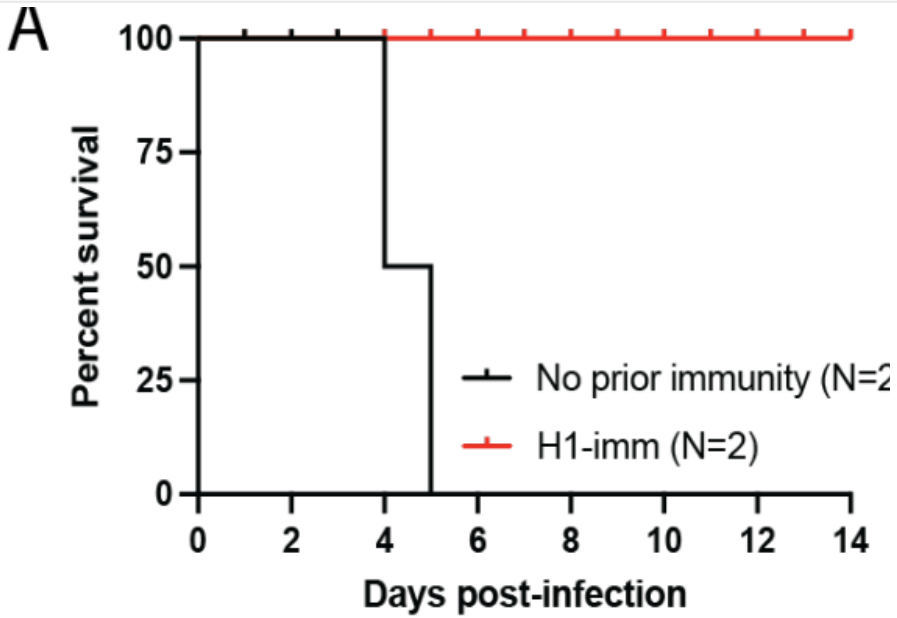
Higher % AA homology between NA of California/09 and the A(H5N1)

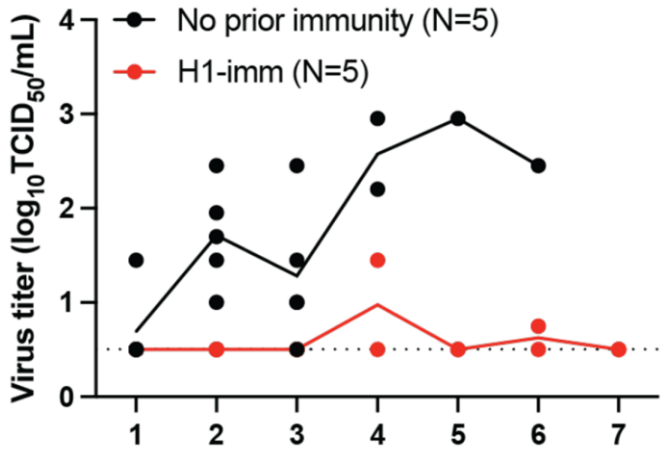
A(H1N1) strain	Amino acid differences compared to	Amino acid Homology (%)
A/USSR/90/1977	70	84.08
A/Chile/01/1983	68	84.58
A/Singapore/06/1986	68	84.58
A/Texas/36/1991	69	84.33
A/Brisbane/59/2007	76	83.83
A/California/04/2009	53	88.00

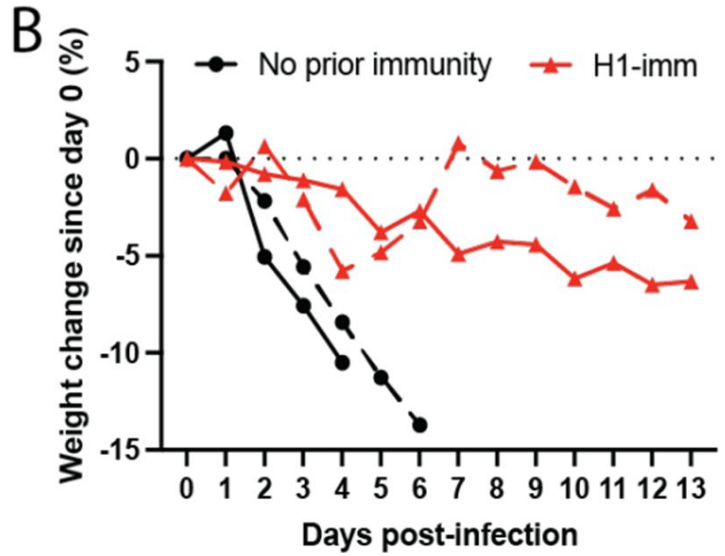
Daulagala P, et al. Emerg Infect Dis. 2024;30:168-171.

Ferrets with prior pH1N109 infection are protected from challenge with influenza A H5N1 (cow/Texas/24)









What is conferring this protection?

- NI antibodies
- Stalk binding antibodies
- T cell immunity

Le Sage V et al. EID 2025; 31(3):477-87

Similar results in Sun W et al EID 2025; 31(3)458-466 → also less protection vs H7N7 challenge

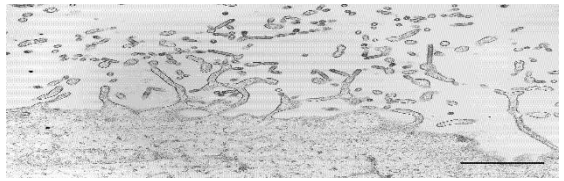
What vaccines may be available in the event of a H5N1 pandemic?

- Vaccines based on older (A/Vietnam/2004; A/Indonesia/2005) H5N1 viruses are already licensed and small stockpiles available. When used with adjuvant (MF59 or AS03), two or three doses provide some cross-reactive HI antibodies vs bovine H5N1 (*Khurana Nat Med 2024*)
- A/Astrakhan/3212/2020 (H5N1) had been identified as a candidate vaccine strain some years ago. MF59 adjuvanted subunit vaccine produced (CSL Seqirus). Cross reacts well with bovine H5N1. Used in farmworkers in Finland. 5 Million doses purchased for US National stockpile
- A/American Wigeon/South Carolina/22-000345-001 (H5N1) another WHO selected reference strain for vaccine production
- Candidate vaccines (conventional and RNA) are in development (*Furey C t al. Nature Comm 2024*).
- Vaccine for poultry (H5N2) has been developed (Zoetis) and received provisional approval, but not being used in USA.

Issues on developing H5N1 influenza vaccines using conventional technology at scale

- Current influenza vaccines are largely produced in embryonated hen eggs and smaller amounts as cell-culture based vaccines
- Current global capacity ~1.2 billion trivalent vaccines annually → 3.6 billion doses of monovalent vaccine.
- But current capacity is dedicated to producing seasonal flu vaccines, which are live saving.
- Some smaller vaccine manufacturers make flu vaccine half the year and other pediatric vaccines for rest of year.
- What is the trigger for a decision to stop making seasonal flu vaccine and convert to making H5N1 vaccine? Getting this decision wrong can be costly, both economically and in lives.
- RNA vaccines currently in phase 3 clinical trials for seasonal flu, pending licensure. IF licensed, can potentially switch to H5N1.
- Supply chain issues: vials, needles, syringes needed for pandemic response
- Who will get the vaccine first !!!!

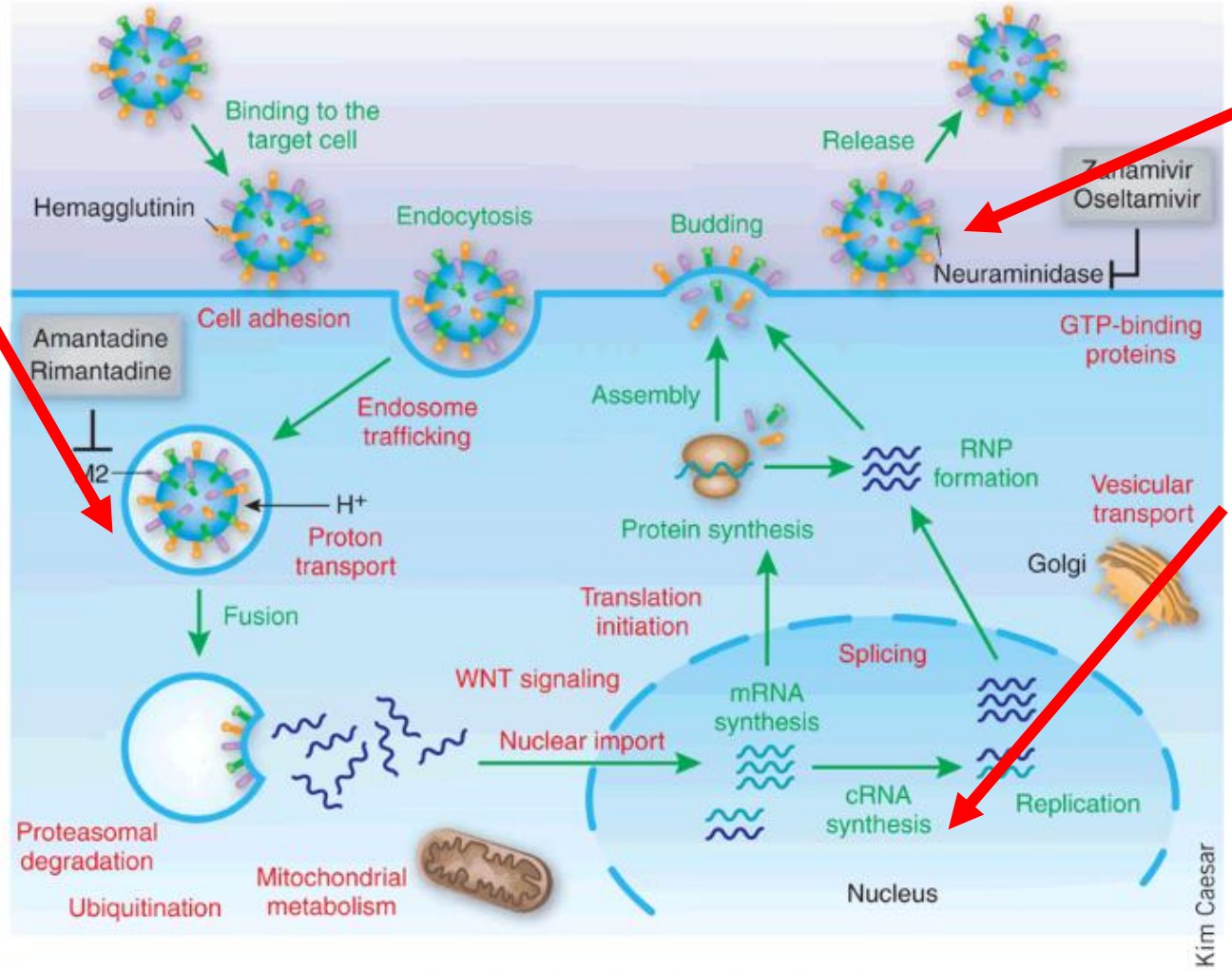
Influenza: Antivirals





Virus uncoating
Amantadine
Rimantadine
(most human seasonal viruses now resistant)

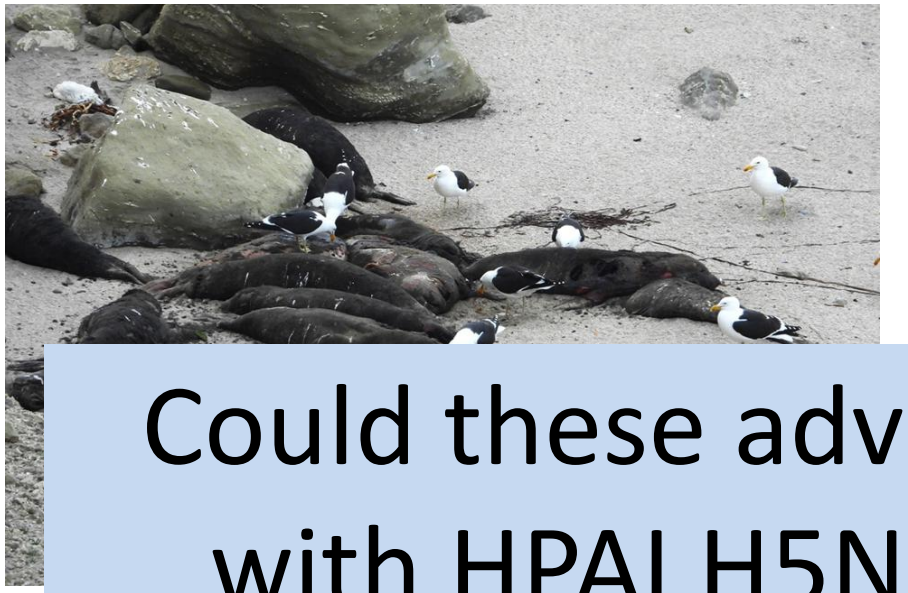
Clade 2.3.4.4b H5N1 viruses were sensitive; Some clade 2.3.2.1c viruses (Cambodia) were resistant



Neuraminidase inhibitors
Oseltamivir/zanamivir
Laninamivir (approved in Japan)

Replication inhibitors
T705 (Favipiravir)
Baloxavir marboxyl (Xofluza)

Baloxavir was superior to oseltamivir in experimental mouse study





Could these adverse global consequences with HPAI H5N1 have been prevented?

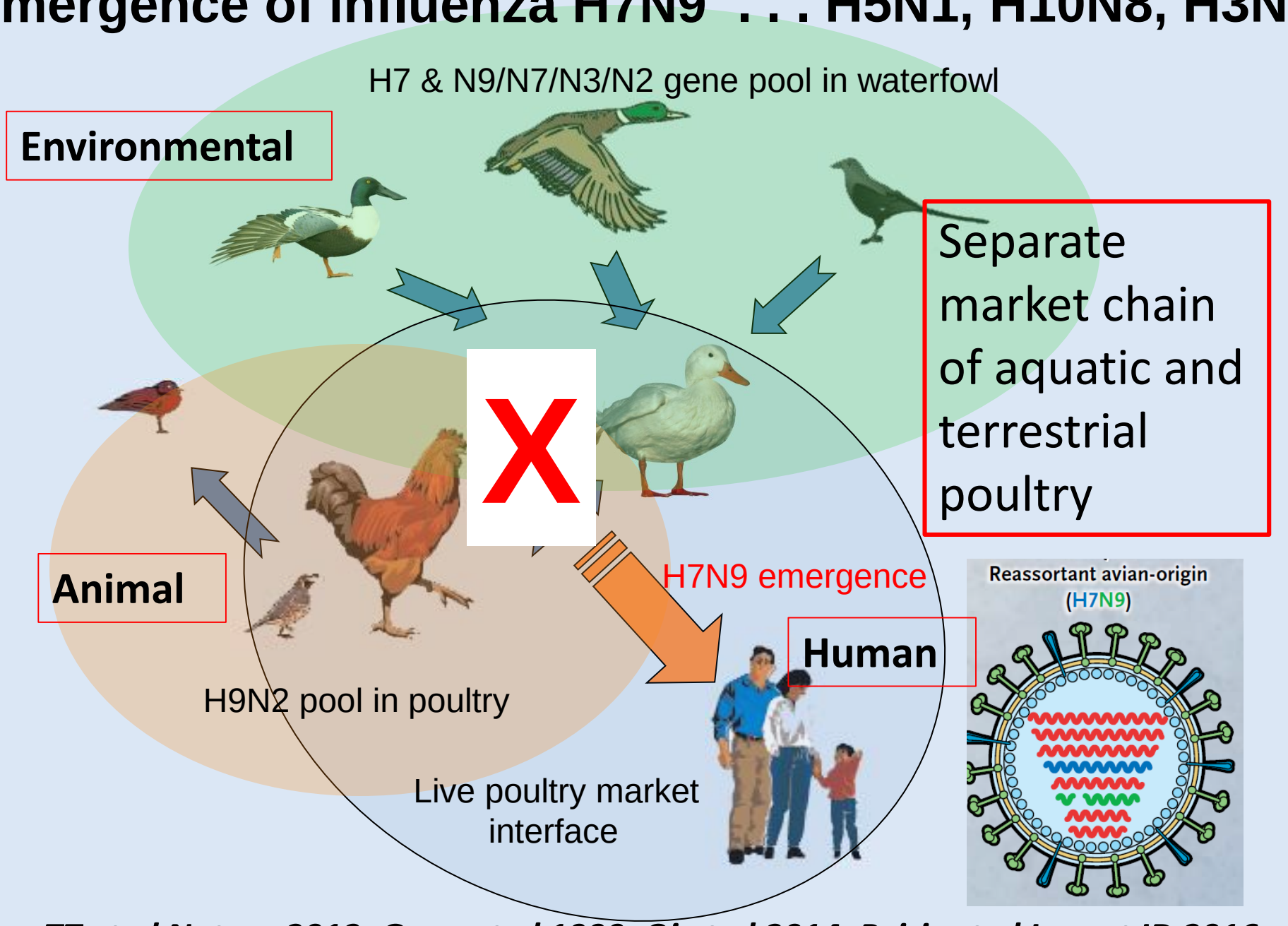
Ter
H5N1 when the virus invaded South America in 2023, like these elephant seals (Mirounga leonina) in Punta Delgada, Argentina. Image © Valeria Falabella.

Islands and Islas Malvinas, where two-thirds of the entire population lives. Image © Julia Emerit and Augustin Clessin.

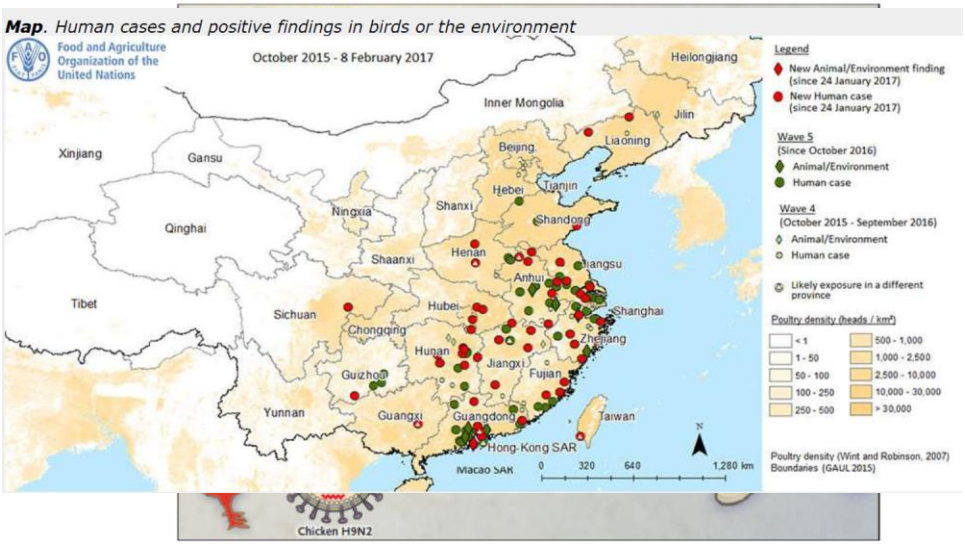


An estimated 17,000 elephant seal pups (Mirounga leonina) died when the H5N1 virus moved down from North and Central America to invade both the Atlantic and Pacific coasts of South America in 2023. Image © Marcela Uhart, UC Davis.

Emergence of influenza H7N9 . . . H5N1, H10N8, H3N8



Lam TT et al Nature 2013; Guan et al 1999; Qi et al 2014; Peiris et al Lancet ID 2016



Influenza A H7N9; First reported in Shanghai 2013

1567 human cases since then

615 deaths

But only one zoonotic case since 2017

Introduction of H5/H7 bivalent vaccine in 2017 reduced H7N9 virus circulation in poultry markets

– Wu et al EID 2019

Changes in virus receptor binding???

- Hou Y et al EMI 2024

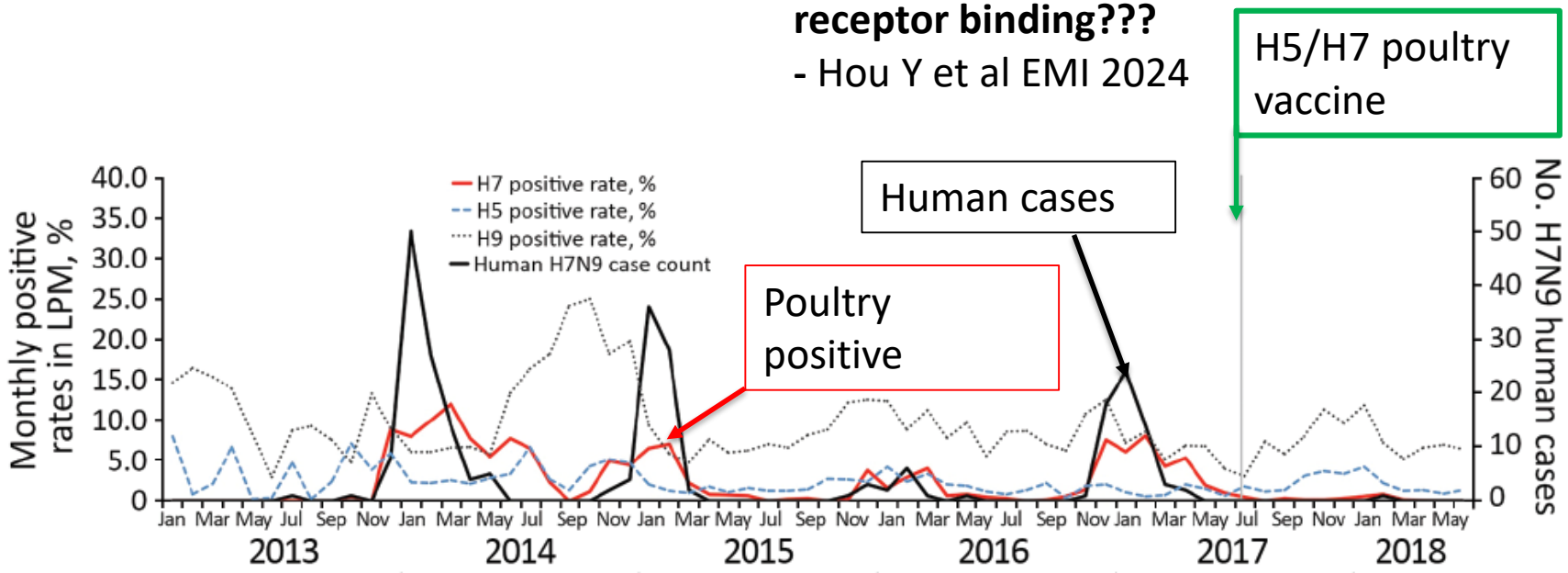
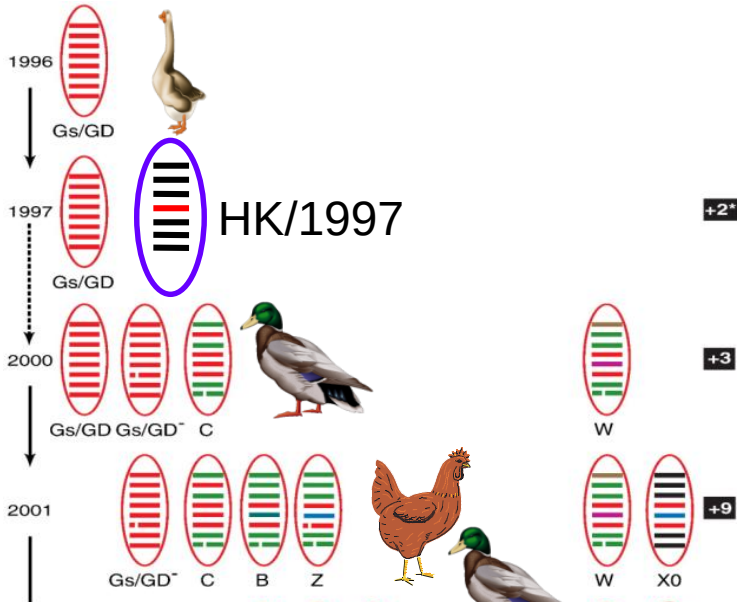


Figure 1. Monthly H5, H7, and H9 positive rates at live poultry markets (LPM) and human H7N9 cases in Guangdong Province, China, January 2013–June 2018. Vertical gray line shows the introduction (July 2017) of the bivalent H5/H7 vaccine in poultry.

Avian flu H5N1-Post 1997

- 1999 Gs/Gd H5N1 detected in imported geese
- 2000 Reassortment in ducks
- 2001 Outbreaks in live poultry markets
- 2002 Farm outbreaks
- 2002 Wild birds

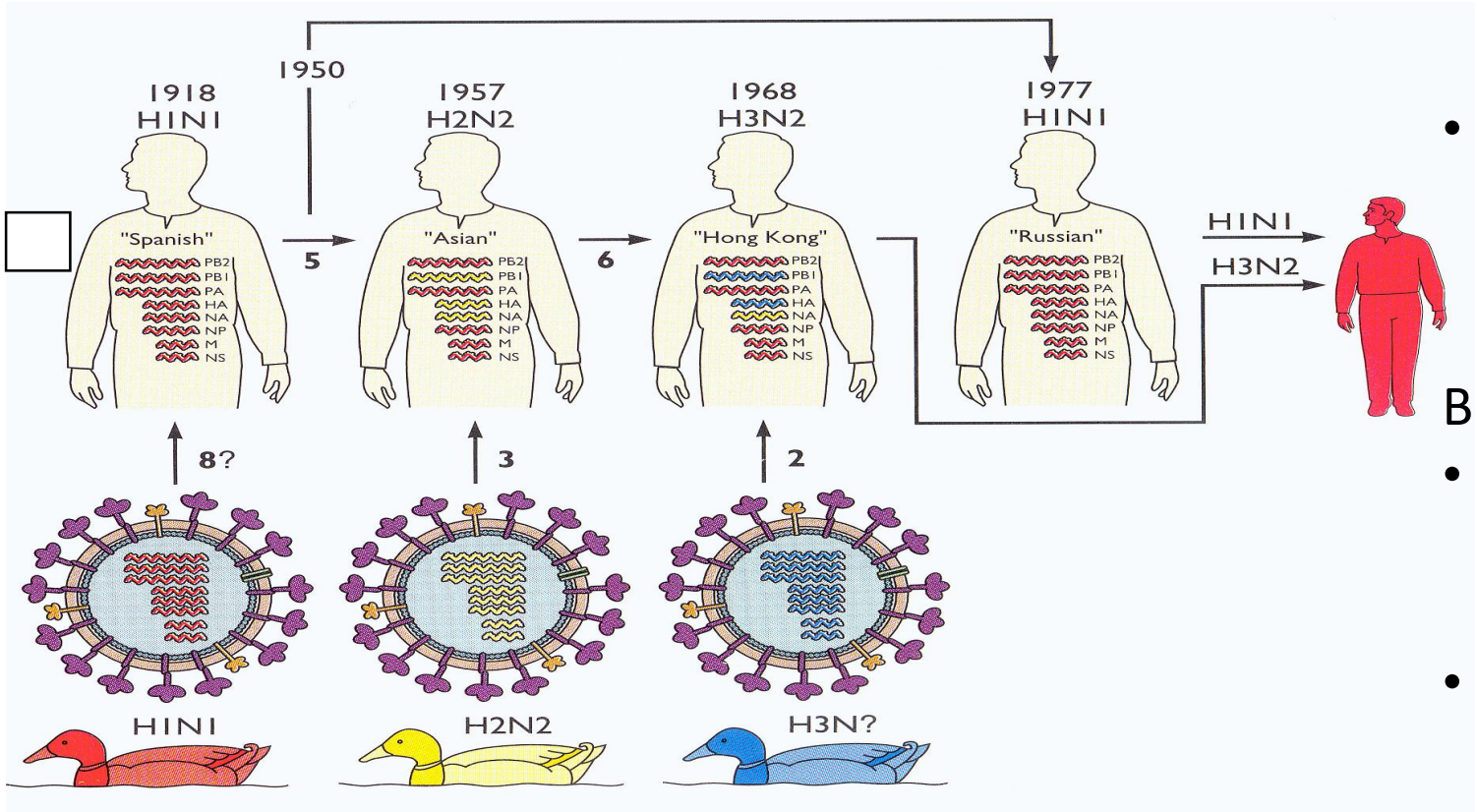






Proc Natl Acad Sci, USA 2002
Proc Natl Acad Sci, USA 2004

Will H5N1 cause the next pandemic?

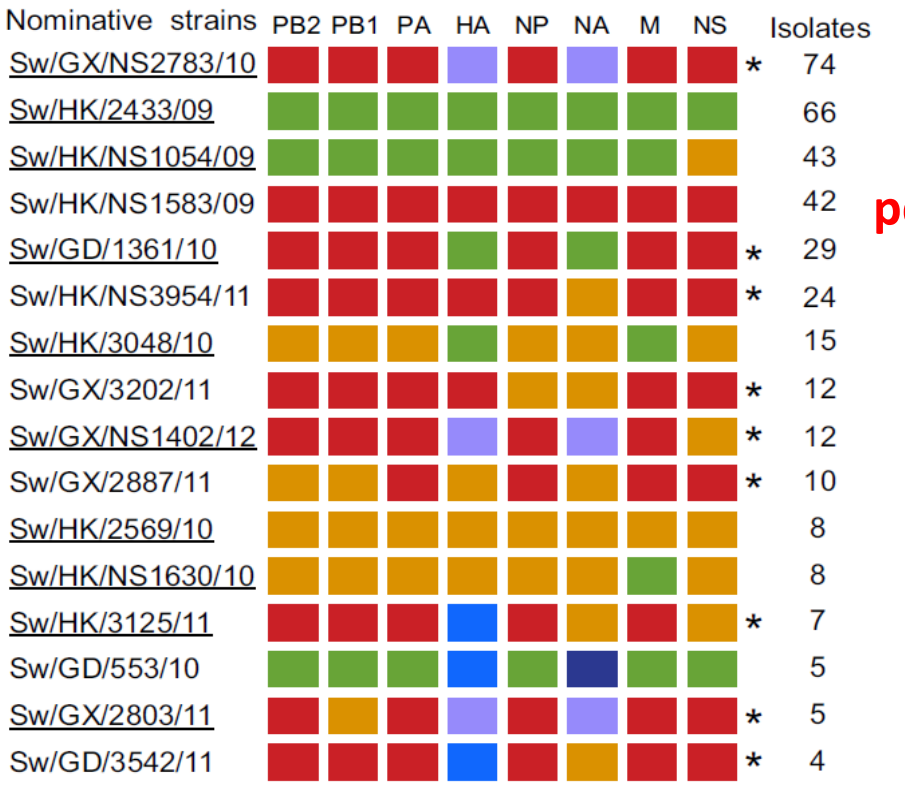


- Clade 2.3.4.4b viruses are unusually adapt at crossing species barriers, already transmitting in mammalian species (cows, marine mammals)

BUT

- Past pandemics were all caused by “low pathogenic” influenza viruses H1,2,3. Not HPAI
- Last pandemic pdmH1N1/2009 had avian origin genes. But these transited to humans via swine influenza viruses .
- Swine adapted influenza viruses have less host barriers to overcome

Genotypic diversity of swine influenza in China



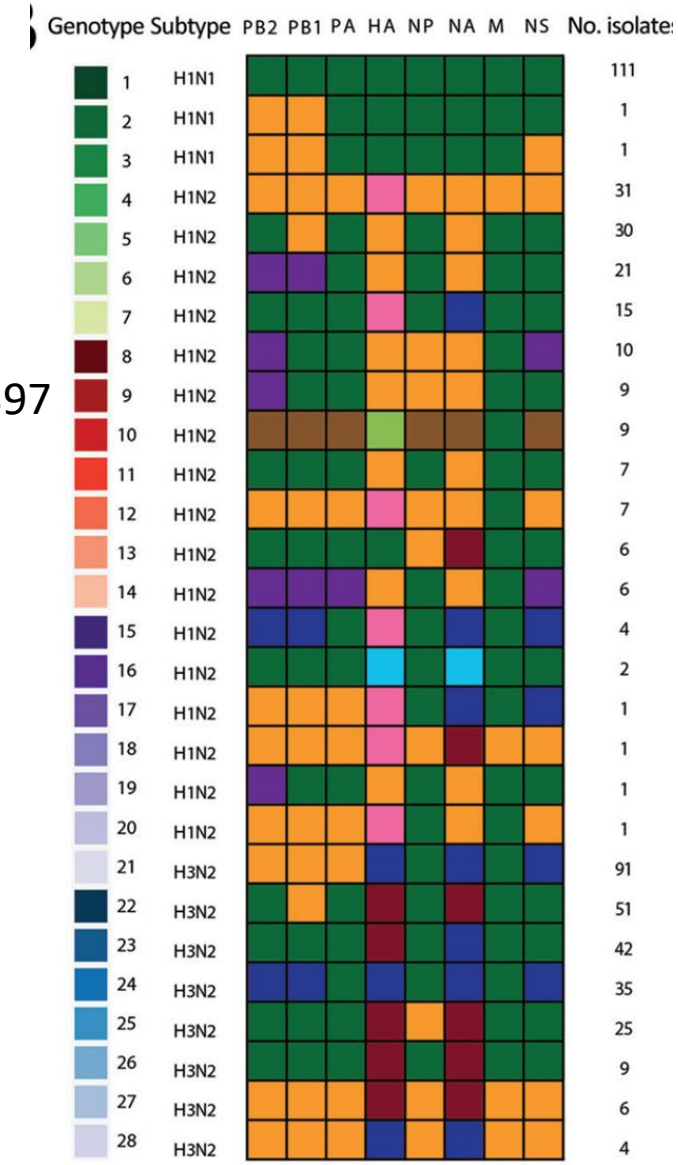
Liang et al, J Virol, 2014

pdm09

Genotypic diversity of swine influenza in Vietnam

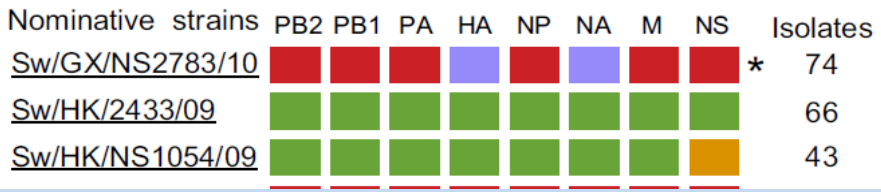
Cheung et al EID 2023; 29: 1397

Pdm09 Green



- Human pdmH1N1/09 virus internal genes are widespread in swine influenza viruses in Asia → well adapted to humans

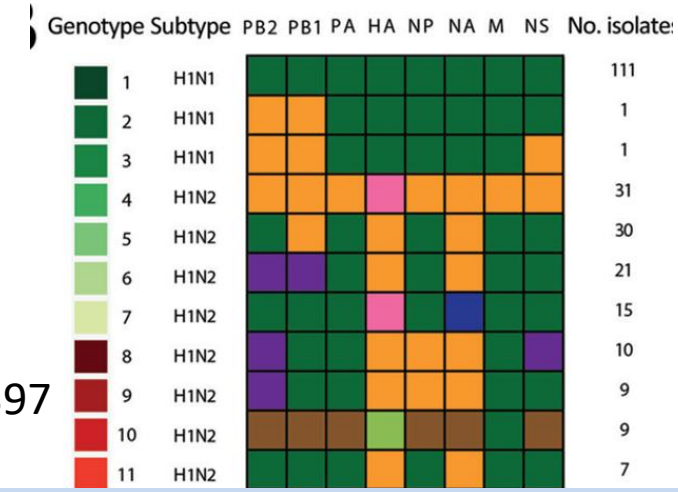
Genotypic diversity of swine influenza in China





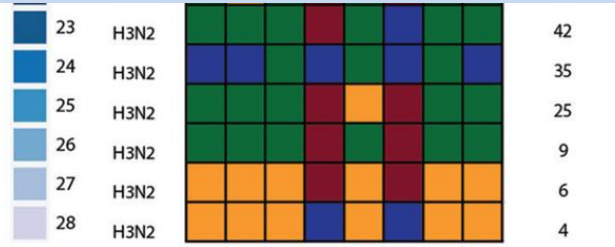
Liang et al, J Virol, 2014

Genotypic diversity of swine influenza in Vietnam



Cheung et al EID 2023; 29: 1397

- Next pandemic may also come via swine?
- Where will the avian genes come from? H5N1 or other avian virus
- Is H5N1 getting established in swine?



virus internal genes are widespread in swine influenza viruses in Asia → well adapted to humans

Can pigs be infected with HPAI H5N1 (B3.13)?

- Oro-respiratory infection of pigs resulted in productive replication of a bovine-derived HPAI H5N1 B3.13 virus.
- Infectious virus identified in the lower respiratory tract of infected pigs and sero-conversion was observed in most
- In one animal, emergence of a mutation in hemagglutinin (HA) previously associated with increased affinity for "mammalian-type" α2,6-linked sialic acid receptors, but this mutation did not reach consensus levels.
- Sentinel contact pigs remained sero-negative throughout the study, indicating lack of transmission.

Kwon T, Pathogenicity and transmissibility of bovine-derived HPAI H5N1 B3.13 virus in pigs. bioRxiv [Preprint]. 2025 Mar 7:2025.03.04.641414.

Questions !!!!

95-10=85