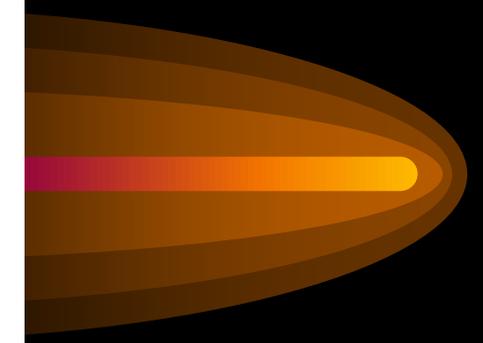
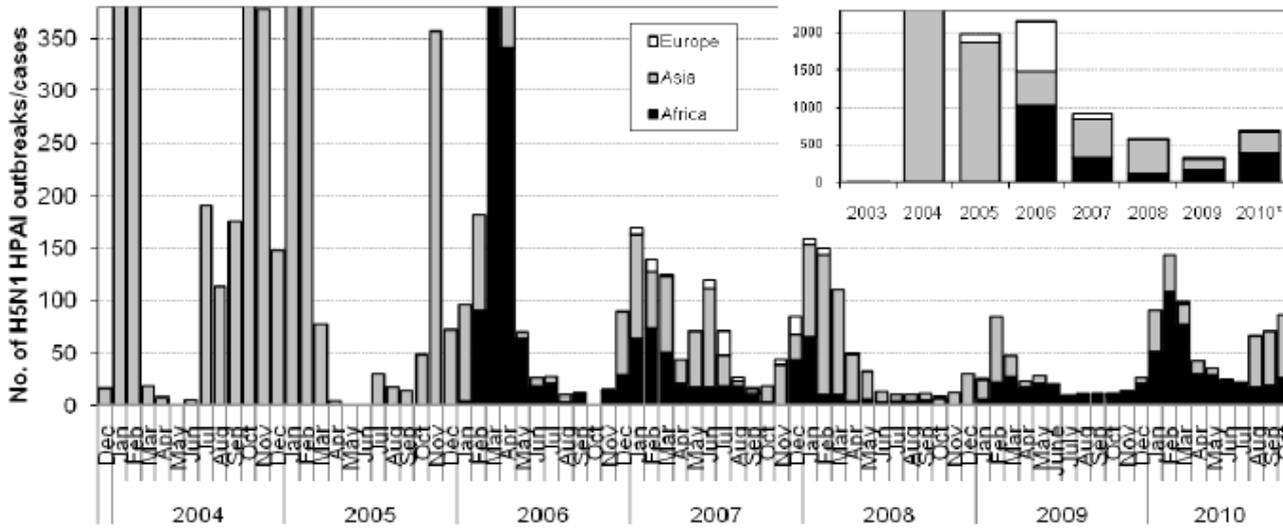


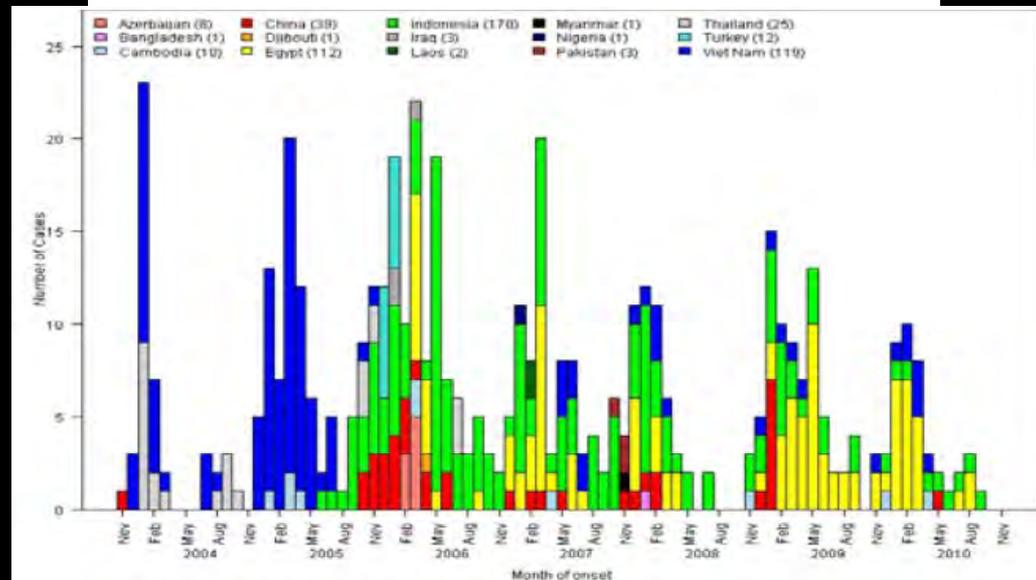
Highly pathogenic avian influenza H5N1



HPAI outbreaks by continent: excludes Indonesia



Human cases by country over time



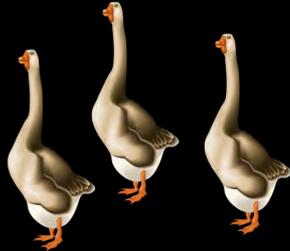
How well are we doing?

FAO EMPRES

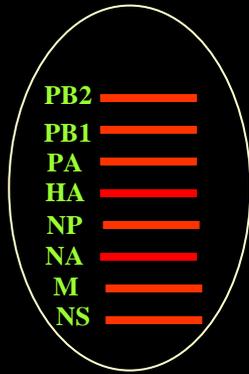
So what?

Subtype		Countries affected	Disease	Cases / deaths
H7N7, H7N2, H7N3	LPAI HPAI	USA, UK, Canada, Netherlands	Conjunctivitis ILI ARDS/Death	99 cases 1 death
H9N2	LPAI	Hong Kong, S. China	ILI	12 cases 0 deaths
H5N1	HPAI	15 countries	ILI, LRI, ARDS	518 cases 306 deaths (CFR 59%)

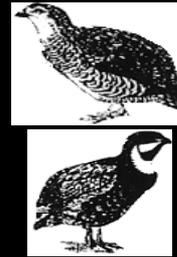
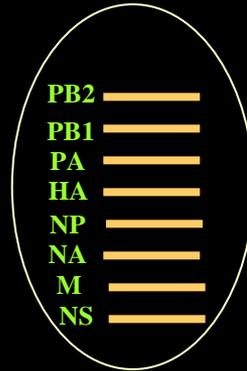
1996



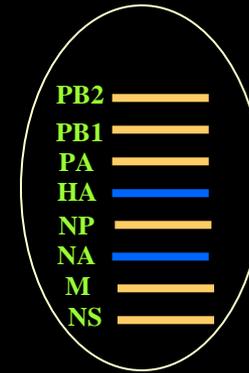
H5N1 Gs/GD/96-like



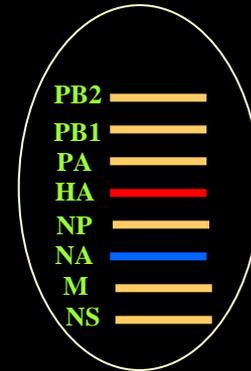
H9N2 G1-like



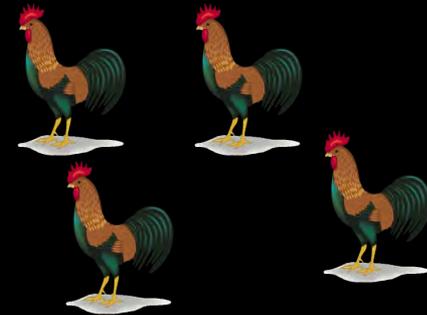
H6N1 W312-like



1997



H5N1/97



Xu, X et al., *Virology*, 1999

Guan, Y et al., *PNAS*, 1999

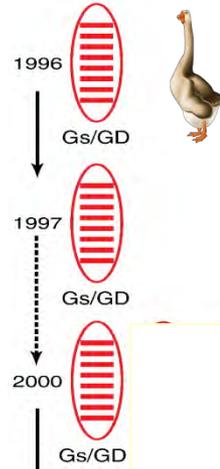
Hoffmann, E et al., *J. Virol.* 2000

Humans and Chicken

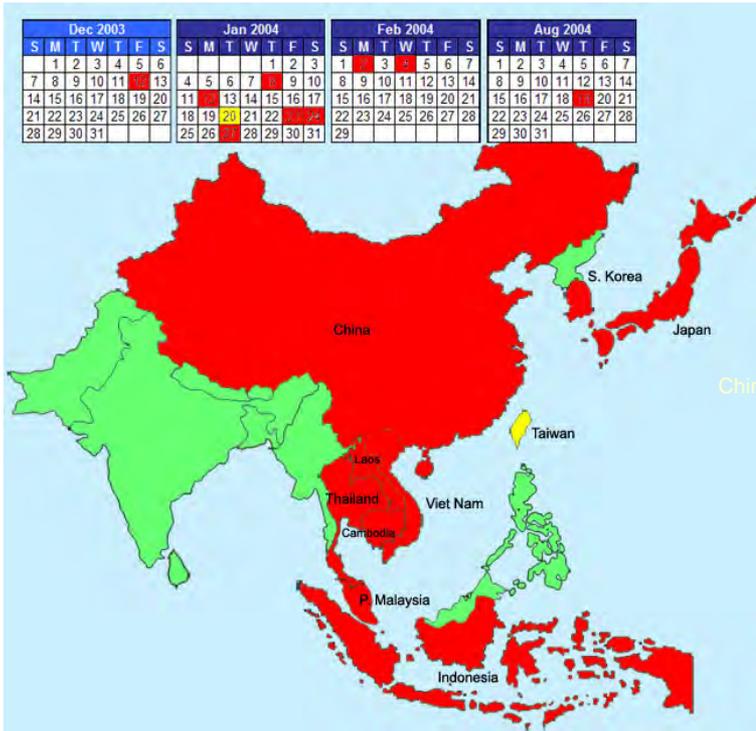
Not detected
after 1997

HPAI H5N1

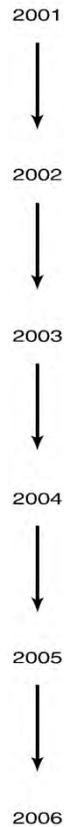
Duan et al Virology 2008



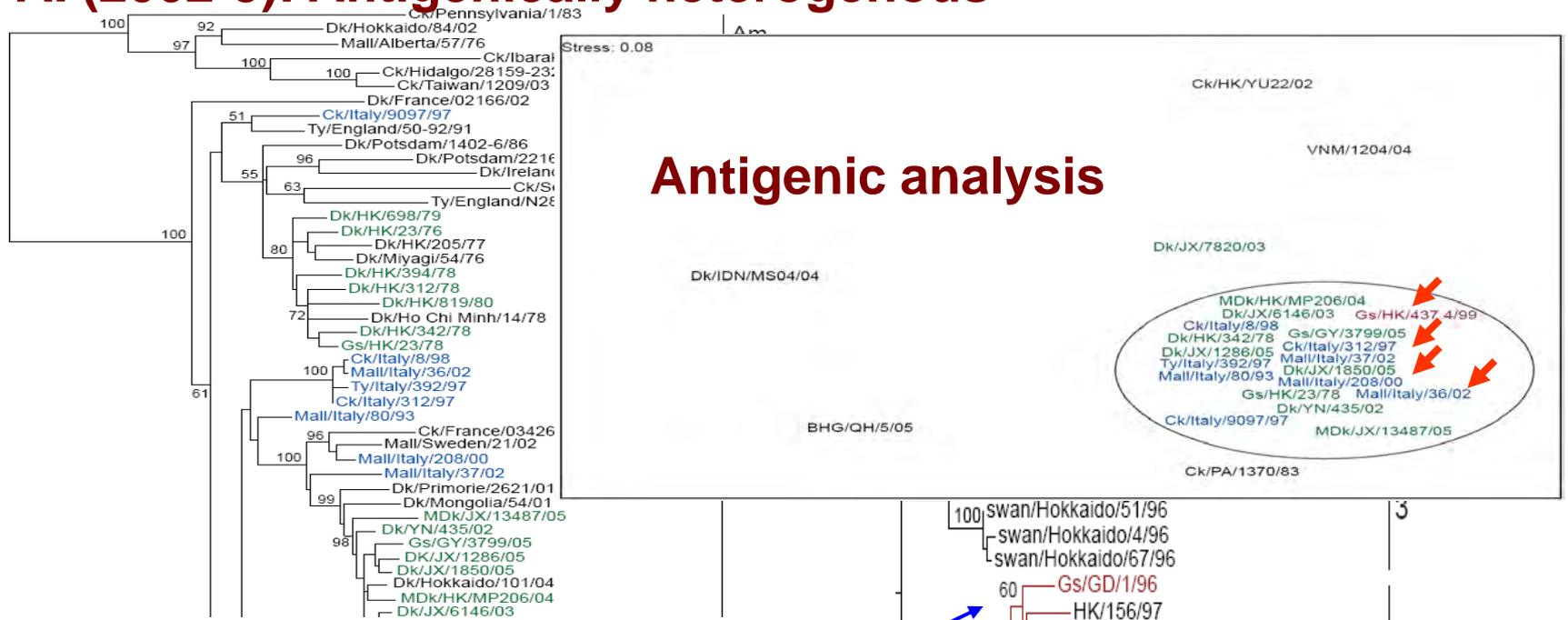
+2*



2003/2004

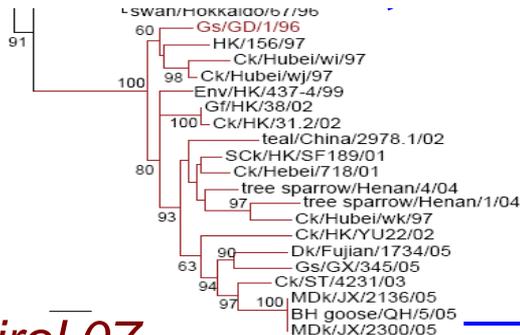


LPAI (1976-2005): Antigenically homogenous
HPAI (2002-5): Antigenically heterogenous

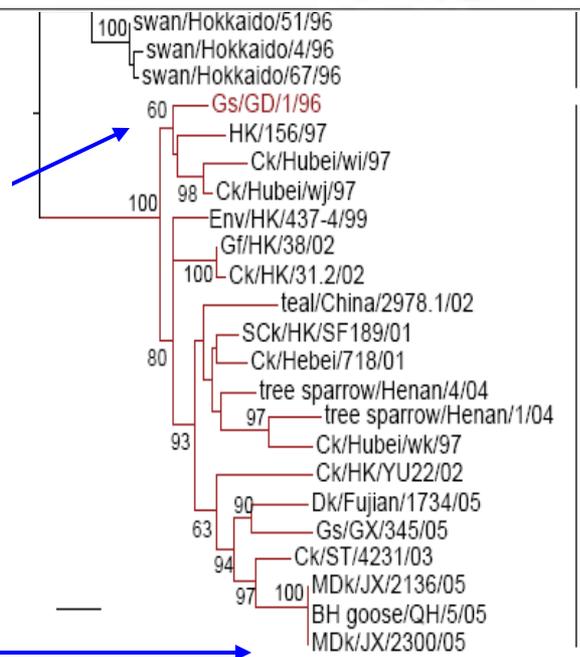


What is driving antigenic diversity of HPAI H5N1?

H5 HA

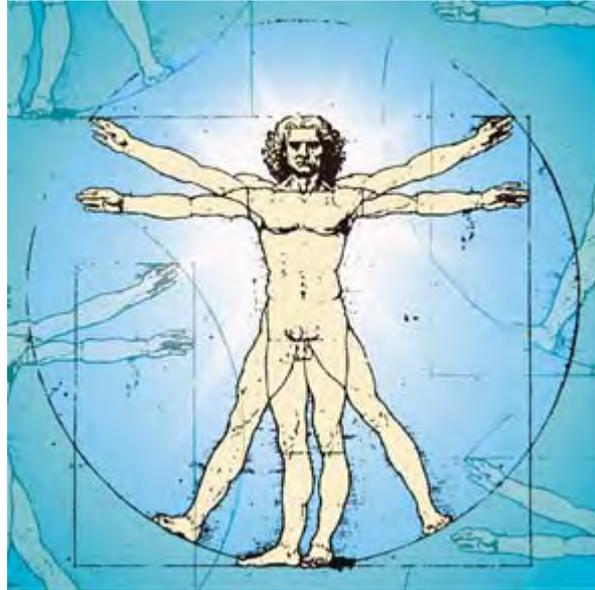


Gs/GD



**HPAI
H5N1
Outbreak
2003-7**

H5N1: crossing species barriers



Genetic markers of virulence
and transmissibility?

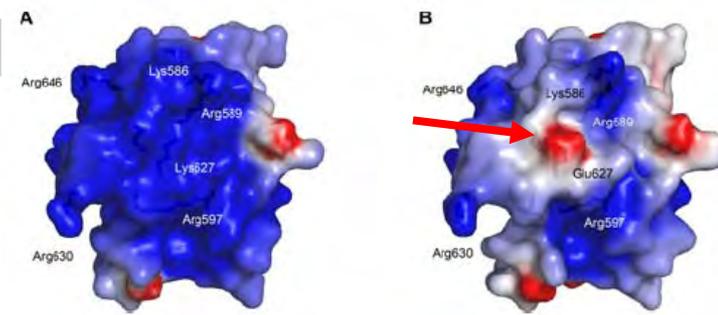
Haemagglutinin

- Connecting peptide -RRKKR-
 - Cleavability by ubiquitous proteases → dissemination
 - Associate with virulence in mice (*Hatta et al 2001*)
- Receptor binding: $\alpha 2-3 \rightarrow \alpha 2-6$
 - Human HK 2003 viruses: HA 227: ↓2-3, weak 2-6. (*Gambarayan et al 2006*)
 - Increased 2-6 binding: Asn182Lys; Gln192Arg (Seen in some human isolates from Vietnam, Azerbaijan, Iraq). (*Yamada et al 2006*)
 - HA A134T affects receptor binding and virulence in ferrets (Imai et al 2010)

Antigenic variation:

- Need to update human vaccine candidates
- Challenge for animal vaccines as well?

PB2



627Glu disrupts basic surface patch on PB2

■ Glu627Lys:

- associated with mammalian adaptation and virulence (*Subbarao et al 1993; Hatta et al 2001*)
- Replication at lower temp in respiratory tract epithelium (*Massin et al 2001*)
- In avian H5N1: Not typically seen, except for clade 2.2
- In human H5N1:
 - Clade 2.2 (with 627Lys): no increase in virulence, in fact apparently lower mortality rates (e.g. Egypt)?
 - Seen in some human isolates of non-clade 2.2 viruses (*De Jong et al 2006, Le et al 2010*)
- Other mammals (e.g. Tigers) (*Amonsin et al 2006*)

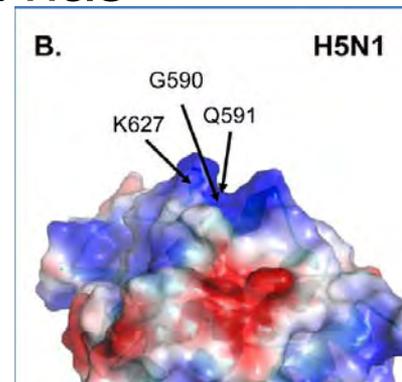
PB2

■ Asp701Asn:

- Seen in some human strains, An alternative adaptation to 627Lys (*De Jong et al 2006, Le et al 2010*)
- Increased mouse virulence (*Li et al 2005*)

■ 591Lys or Arg:

- Compensates for lack of 627Lys in mammalian adaptation (*Yamada et al 2010*)
- 591: Structural proximity to 627
- (Pandemic H1N1 does not have 627Lys but has 591Arg)



NS1

- Interferon antagonist, interacts with RIG-I, inhibits TRIM-25 mediated RIG-I CARD ubiquitination
- Asp92Glu (Seo et al 2002):
 - increases H5N1 resistance to IFN
 - Increased virulence for pigs
- C terminal PDZ ligand domain X-S/T-X-V
 - Associated with mouse virulence, independent of IFN antagonism (Obenauer et al 2006; Jackson et al 2008).
 - 15nt deletion (263-277) affects pathogenicity for mice and chicken (Long et al 2008). Found in two virus isolates from pigs (Zhu et al 2008)

PB1-F2

- Localises to mitochondria and causes apoptosis.
 - PB1-F2 66Ser associated with high pathogenicity in mice. Conenello et al 2007

Transmissibility in ferrets



H5N1 virus or reassortants of H5N1xH3N2

- No evidence of aerosol Tx
 - Maines et al 2006
 - Yen et al 2007
 - Jackson et al 2009

Reassortment between avian H5N1 and human seasonal influenza: retain mouse virulence

Parental origin of genes in reassortant viruses								Virus	LD ₅₀	MID ₅₀	Weight Loss (%)	MST	Virus titer				
HA	NA	PB2	PB1	PA	NP	M	NS						Lung	Spleen	Brain	Nasal turbinate	
								TH04 WT	1.8	1.5	19.8	6	7.1 ± 0.8	1.9 ± 1.2	3.3 ± 0.5	5.5 ± 0.8	
								r6(H5N2)	2.2	1.4	≥ 25.0	6	6.7 ± 1.1	2.3 ± 1.5	1.5 ± 0.3	4.3 ± 0.4	
Virulence Group A1																	
								r1/3/5/7/8	2.5	1	17.2	7	6.5 ± 0.1	—	1.1 ± 0.4	5.5 ± 0.7	Avian H.N,PB1
								r1/3/7	2.7	1.5	21.8	7	6.9 ± 0.3	1.1 ± 0.4	1.3 ± 0.6	5.2 ± 0.1	
								r1/7	2.8	1.5	19.4	6.6	7.7 ± 0.4	2.7 ± 0.2	1.6 ± 0.3	3.9 ± 0.4	
Virulence Group A2																	
								r1/2/3/7	3.4	1.5	≥ 25.0	6	6.0 ± 1.2	1.0 ± 0.3	1.5 ± 0.8	4.4 ± 0.6	
								WY03 WT	> 6.0	6.0	0	>14	—	—	—	—	

63 reassortants rescued by reverse genetics
 Some with avian H5 and N1 have high lethality in mice

Reassortment by co-infection of H5N1 x pH1N1

- M2 defective viruses grown in MDCK cells stably expressing M2
- 33 different “genotypes”
- 15% parent H5N1; 85% reassortants
- Some reassortants have better growth kinetics than parental viruses in human lung epithelial cell lines
- Compatibility in gene segments between the two viruses

Octaviani et al 2010

H5N1 infection in swine

- Low sero-prevalance in swine (clade 1) (*Choi et al 2004*)
- Experimental infection: leads to infection but not transmission (clade 1) (*Choi et al 2004, Lipatov 2008*)
- Swine H5N1 from China (Zhu et al 2008).
- Indonesia: (*Nidom et al EID 2010*)
 - isolation rates: ranged from 0-61% in different farms and years.
 - NT serology done in one year, though very low titres (4-16).
 - Swine viruses from diverse geographical regions seem very closely related. (e.g. E Java, N Sumatra, Banten in 2006). Swine viruses are being shipped across country in swine?
 - Most swine viruses bind α 2-3SA, but one isolate have dual binding to both α 2-3 and α 2-6SA and associated with change HA Ala134Ser. (NB Ser134 is never seen in avian viruses)

- Evidence of pdmH1N1 infecting pigs and reassortment with other swine viruses e.g, *Vijaykrishna et al 2010*

Questions

- Virus evolution and antigenic change
 - Drivers of genetic reassortment, virus fitness, antigenic change
- Control the panzootic in poultry
 - Epidemiological drivers of its maintenance in poultry
 - Critical control points
 - Vaccines / vaccine escape
 - Food safety
 - Wild birds: victim or vector (e.g. clade 2.2; clade 2.3.2)
- Transmissibility in humans:
 - What are virus genetic and host determinants of transmissibility?
 - **Paradox:** high exposure / low infection rate, but family clusters / high disease severity: Host-genetics? Host resistance?
 - Better sero-epidemiology tools
- Risk-behaviors and perceptions, KAP studies, changing behavior
 - (*Ly et al 2007; Fielding et al 2007*)
- Environmental virus contamination / stability
 - (*Vong et al 2008; Indirani et al 2010*)
- Pathogenesis / disease severity in humans?
 - Role of virus and host response
 - Will human transmissibility be associated with loss of virulence? How complete?
 - Animal models need to mimic human pathology (neuro-tropism vs lung pathology)