Intrinsic and extrinsic factors for the emergence of zoonotic viruses at the animal-human interface

6th Eurosurveillance scientific seminar

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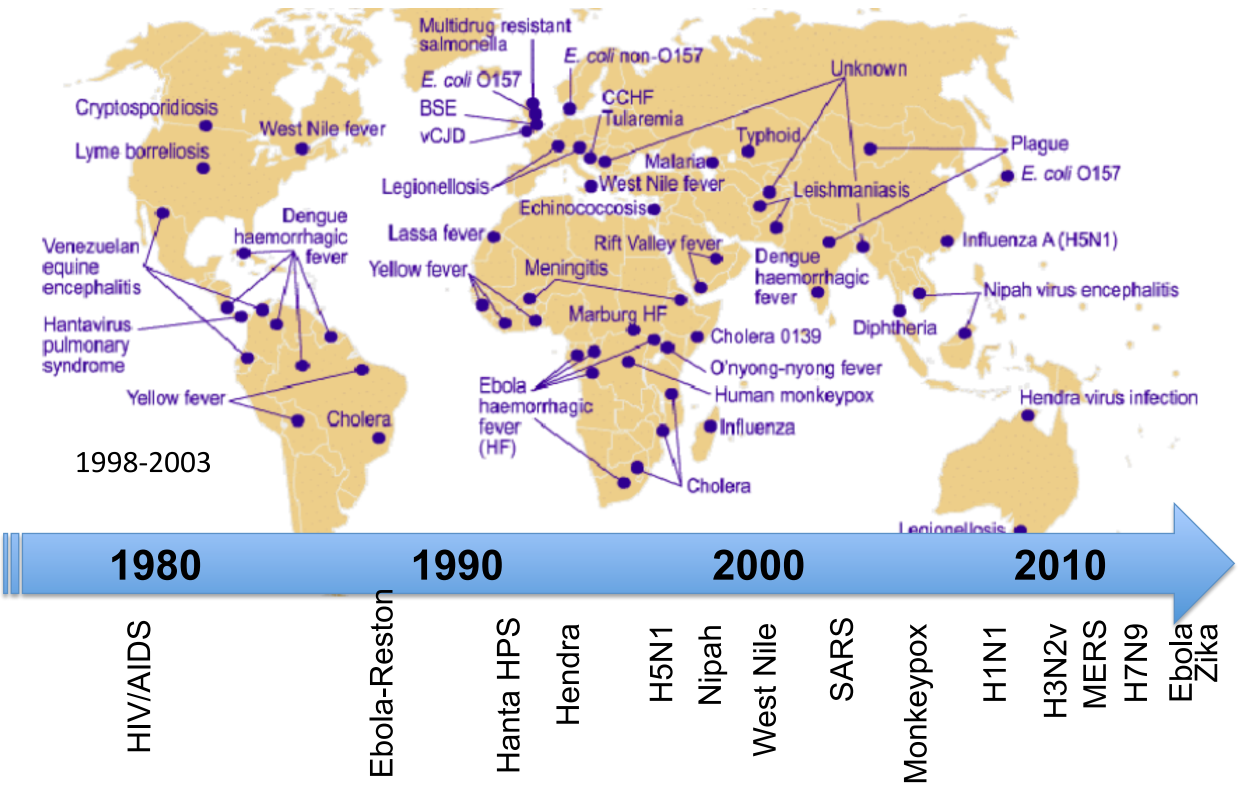
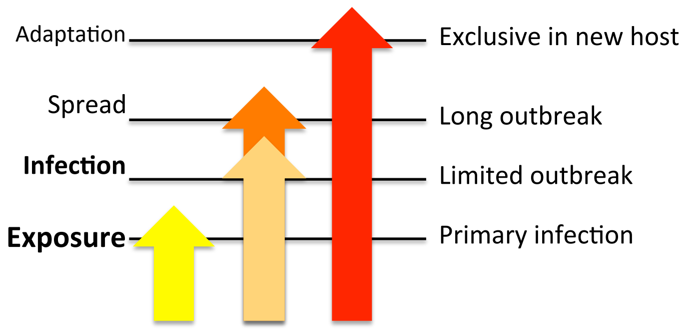
USPC Université Sorbonne Paris Cité
CNRS
Institut Pasteur | Virology1

Emergence at the animal-human interface

Dominated by RNA viruses

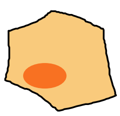


Stages of emergence and barriers



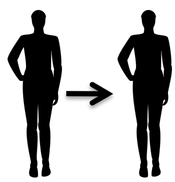
Stages of emergence and barriers

Barriers



Cell
entry
expression/replication
exit

intra-host
access to target organ
diffusion (amplification)
escape host responses

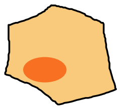


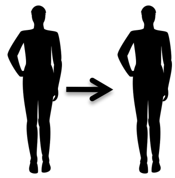
between hosts
transmission
survival
escape immunity

Stages of emergence and barriers

Animal → **Human** → **Human**

Barriers





Cell
entry
expression/replication
exit

intra-host
access to target organ
diffusion (amplification)
escape host responses

between hosts
transmission
survival
escape immunity

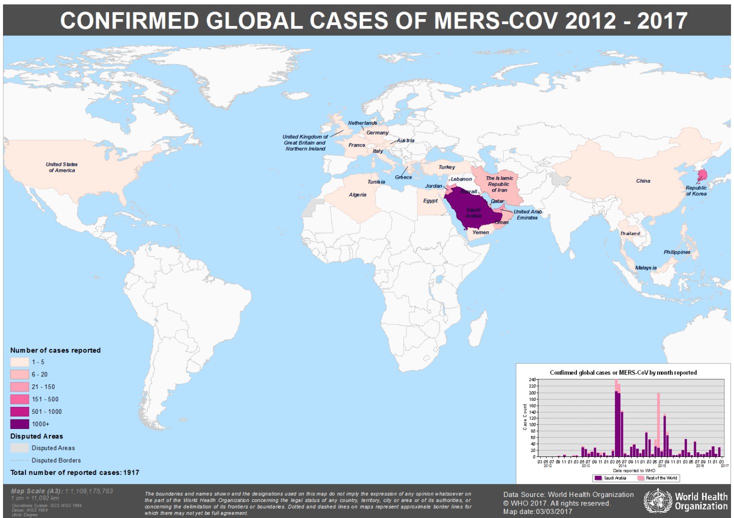
Determinants and factors

intrinsic viral determinants

host determinants

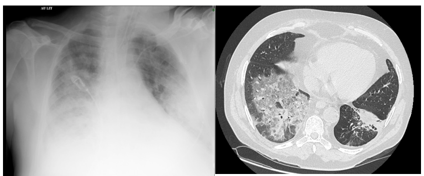
extrinsic factors

MERS epidemiology

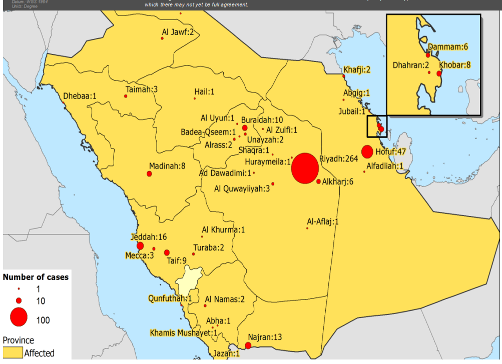


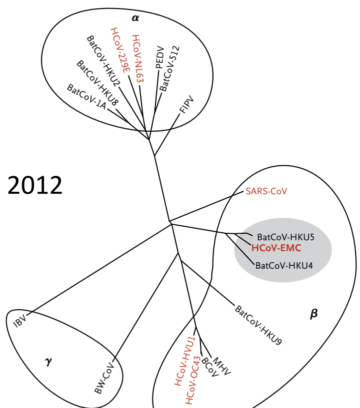
10 March 2017 (WHO)
1917 confirmed cases 684 deaths (CFR ≈40%)

27 countries
Saudi Arabia >1480 cases (>550 deaths)
clusters



Guery et al. Lancet2013

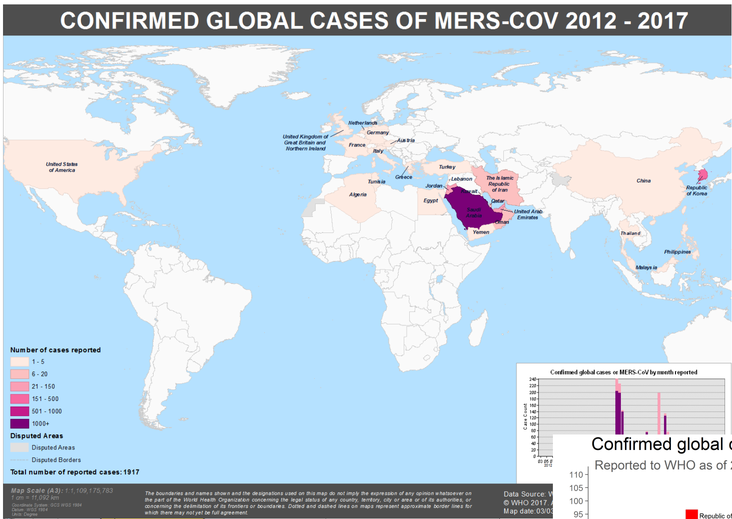


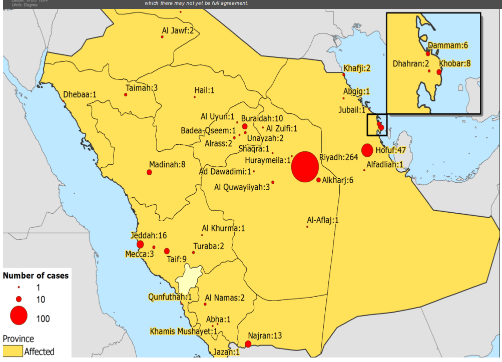


virus first identified in June 2012
at Erasmus MC (NL)

Zaki et al. 2012 NEJM

MERS epidemiology





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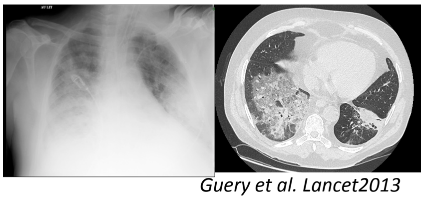
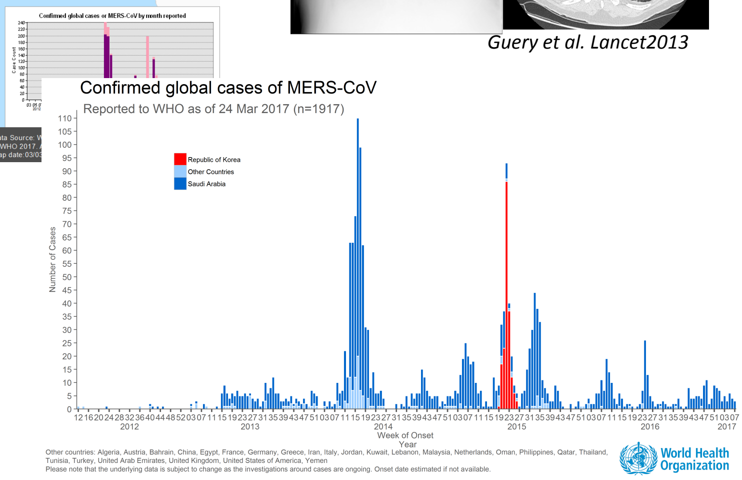
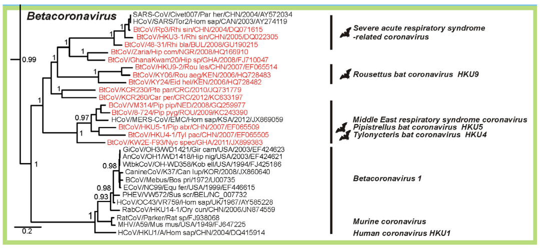


Guery et al. Lancet2013



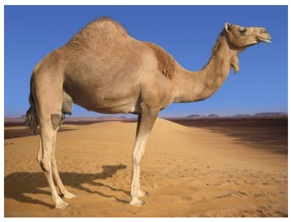
Origin of the virus and source of infection



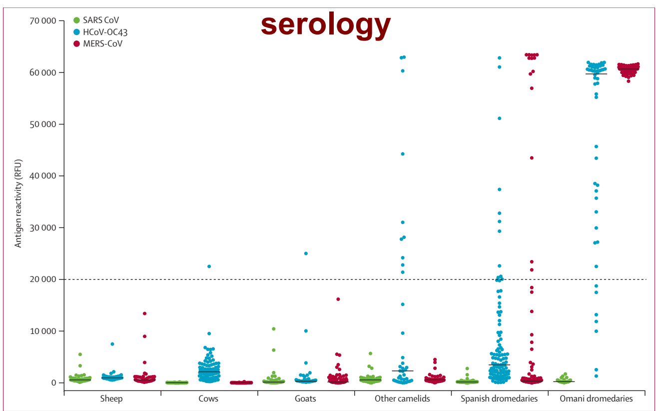
Ithete et al. EID 2013

bats

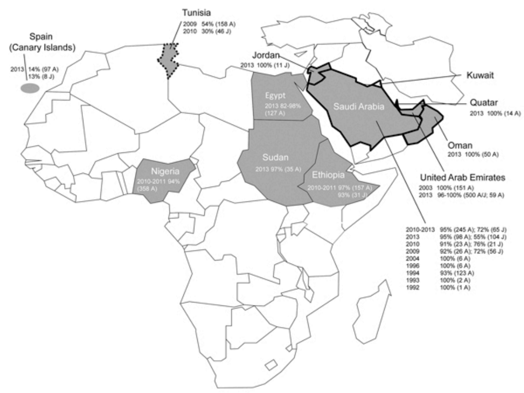
Dromadary camels



serology

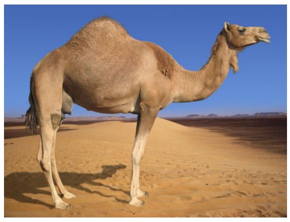


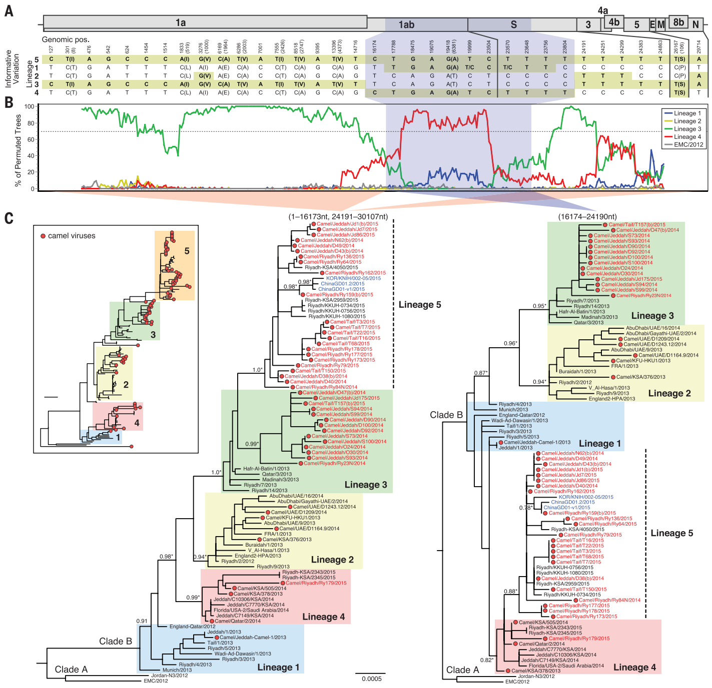
- Positive detections date back to 1992
- Large geographic distribution



Pereira et al. Eurosurveillance 2013 *Reusken et al. Lancet 2013*

MERS-CoV evolution in camels

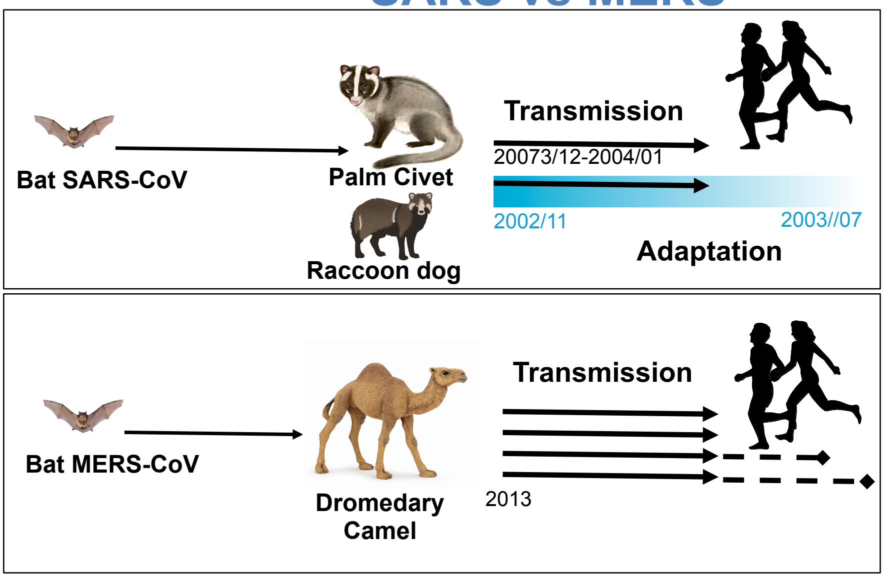




- MERS-CoV related to human viruses detected and isolated from dromedary camels
- Circulation of different lineages
- Evidence for recombination
- Identity among quasispecies
- Experimental infections in dromadary camels (mild disease; high levels of viral shedding)

Nowotny et al. Eurosurveillance 2014 *Briese et al. mBio 2014* *Sabir et al. Science 2016*

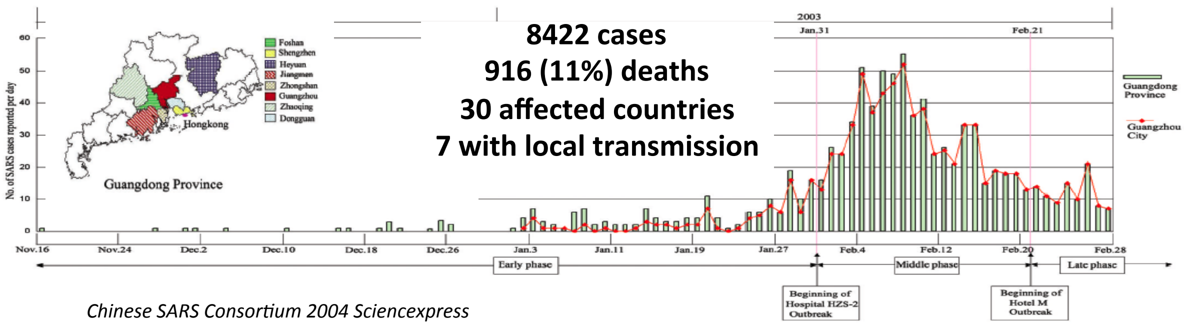
SARS vs MERS



Lee N. *et al.* NEJM 2003

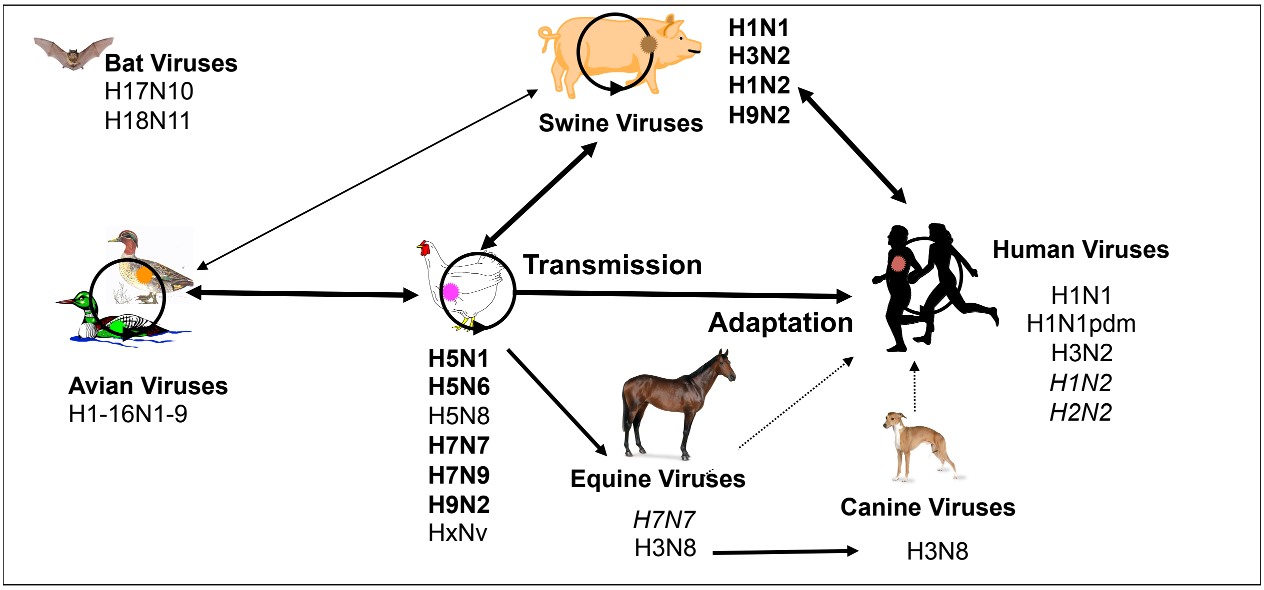
Similar pathology
pneumonia
ARDS

LRT
≠ shedding patterns

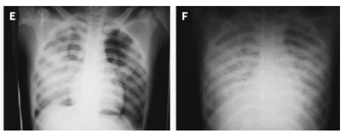


Chinese SARS Consortium 2004 Sciencexpress

Influenza A viruses



H5N1 exposure



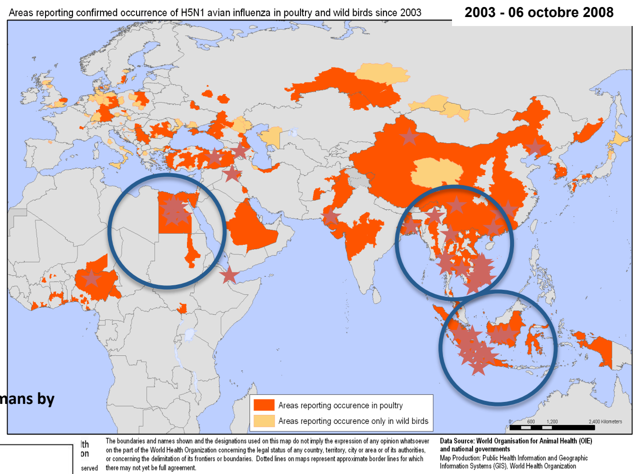
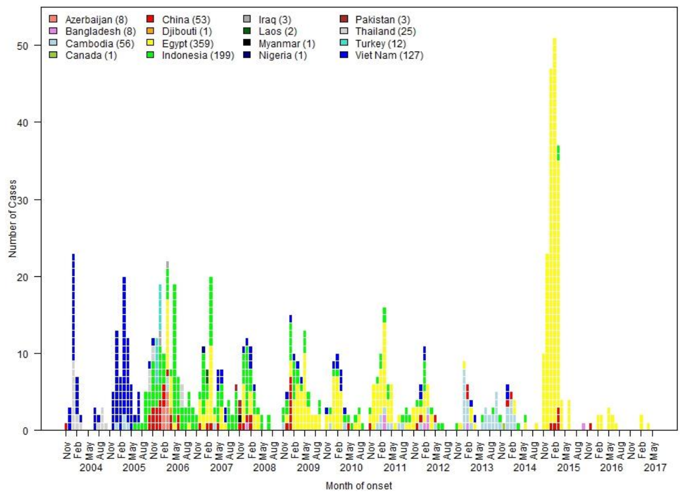


Figure 1: Epidemiological curve of lab-confirmed avian influenza A(H5N1) cases in humans by month of onset, 2003-2017



- (RE)-emergence in 2003
- Endemic in SE-Asia, Egypt, Indonesia
- Highly pathogenic for poultry

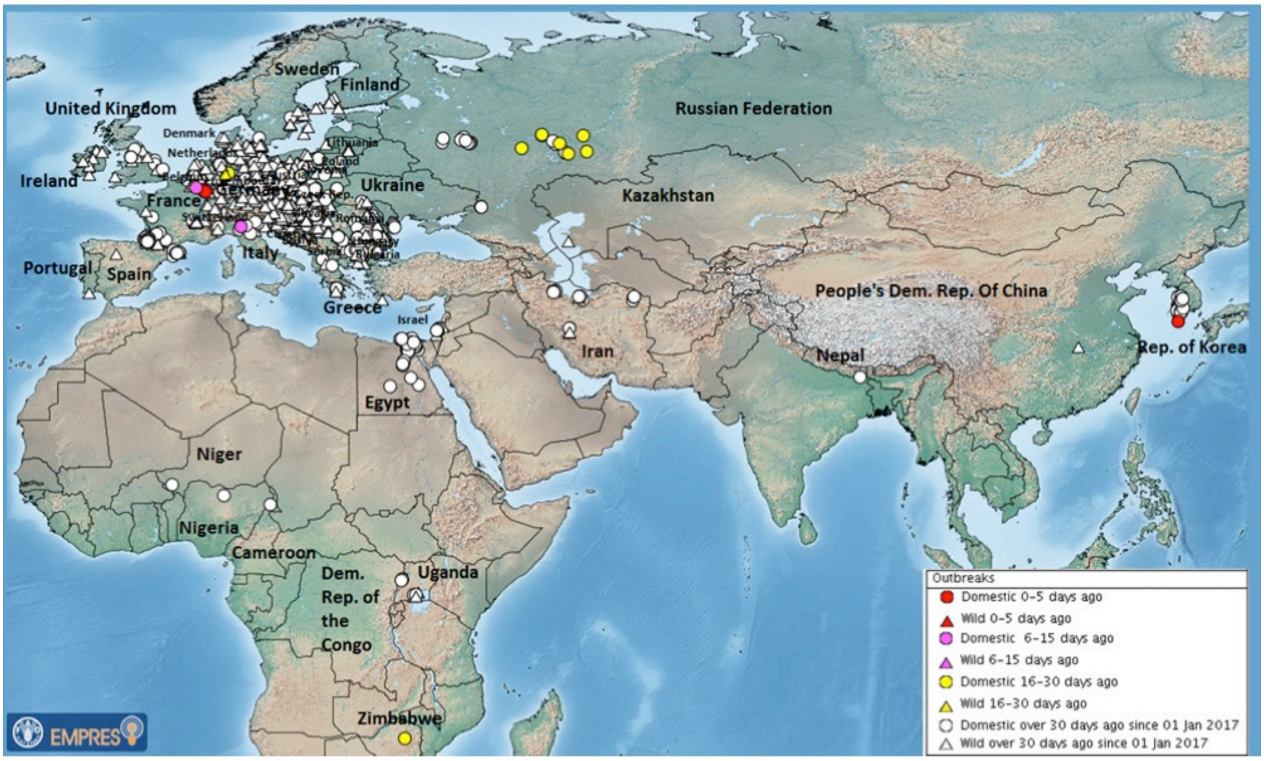
Humans

- Source poultry : limited human-to-human transmission documented
- Severity of infections total 870 cases 458 deaths (WHO – 29/09/2016)

Extensive genetic diversification
Reassortment w/ other circulating viruses

→ H5N8, H5N6 (clade 2.3.4.4)

H5N8 exposure

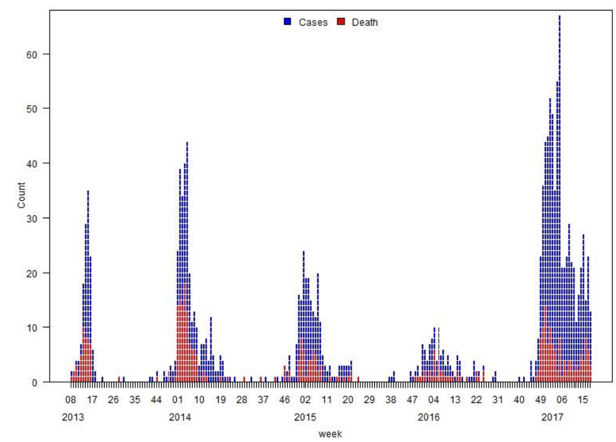


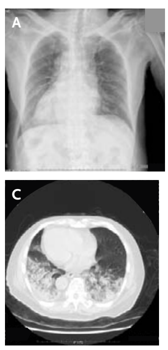
Widespread in Europe in wild birds and poultry - **no human cases** detected

H7N9 exposure

- **31 March 2013 1st detections of human cases in China (2 in Shanghai ; 1 in Anhui)**
- **Source : poultry (markets)**
- **urban settings initially; recent spread to rural areas**
- **Low pathogenicity for birds**
- **Evolution to highly pathogenic form in 2017**

Figure 2: Epidemiological curve of avian influenza A(H7N9) cases in humans by week of onset, 2013-2017



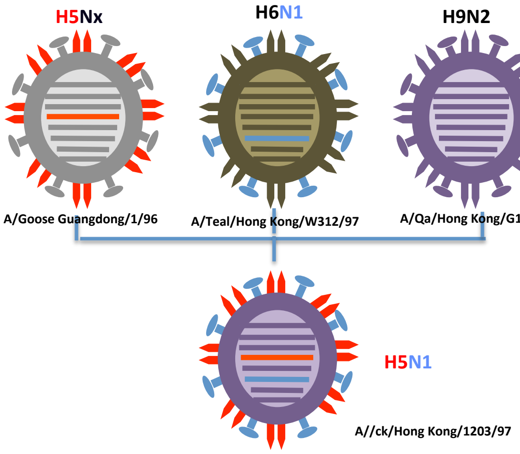


Humans

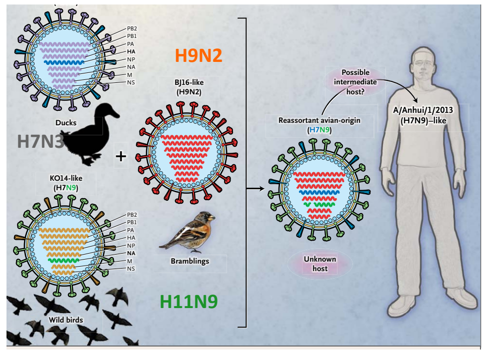
- **Source poultry :**
- **limited human-to-human transmission documented**
- **Severity of infections total 798 cases 320 deaths (WHO – 29/09/2016)**

Genome origin

H5N1

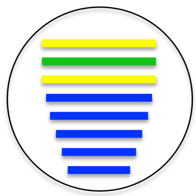


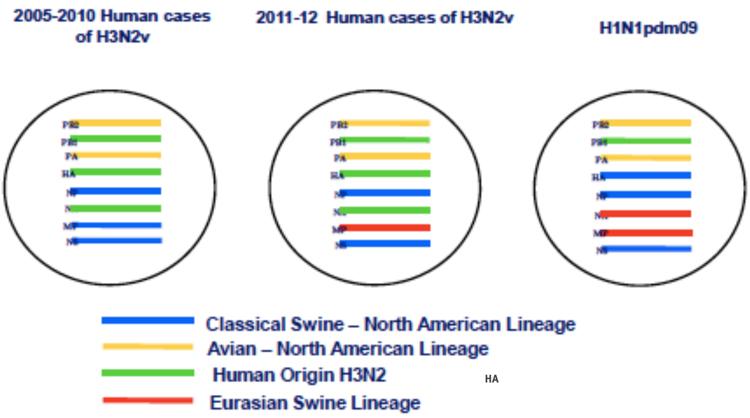
H7N9



H3N2v swine influenza viruses

H1N1v triple reassortant



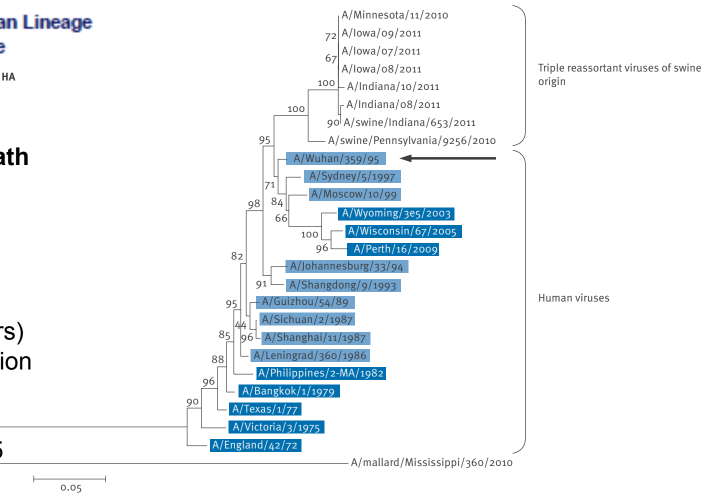


- **H3N2v 422 cases - 24 hospitalized; 1 death**
- **H1N1v : 20 cases**
- **H1N2v: 11 cases**

(29/09/2017 – CDC)

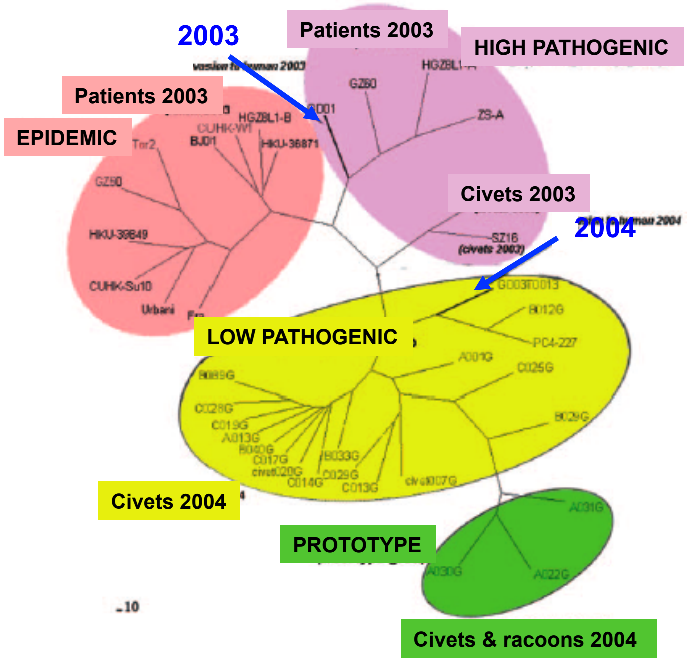
- **Mostly mild disease (ILI)**
- Source : exposure to swine (agricultural fairs)
- Some cases of human-to-human transmission documented – estimated R0 ≈ 0.5

- H3N2v viruses antigenically related to 1995 human viruses



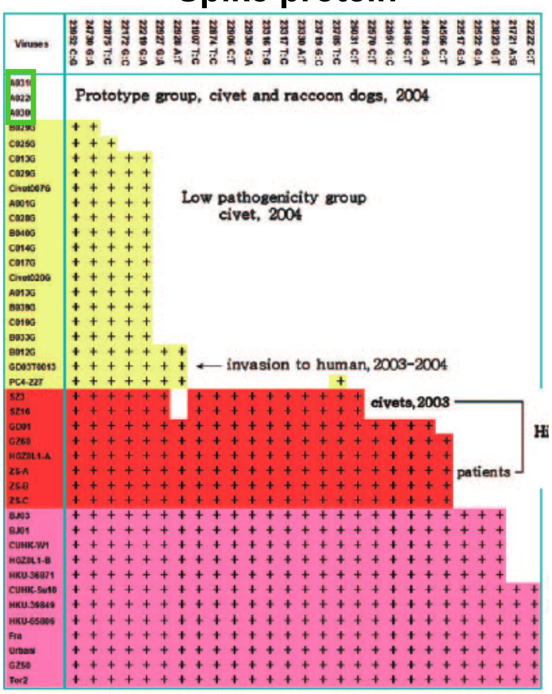
Intrinsic viral determinants
determinants of cell and host infection

SARS-CoV Evolution



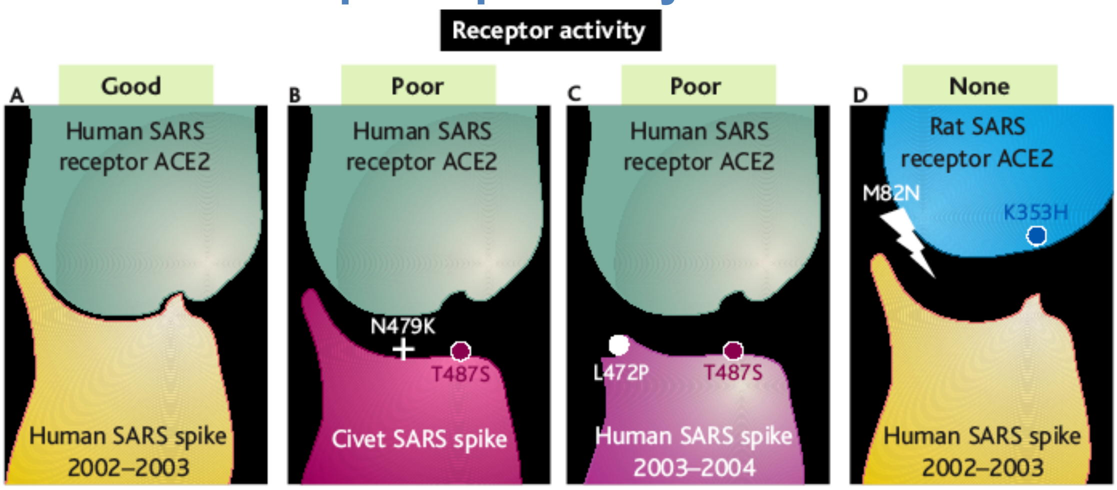
S gene maximum likelihood PAUP

Spike protein



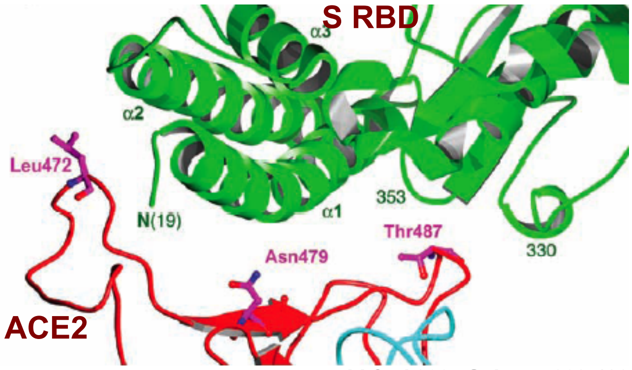
Kan et al. 2005 JV 79:11892

SARS-CoV Receptor specificity



Holmes 2005 Science 309:1822

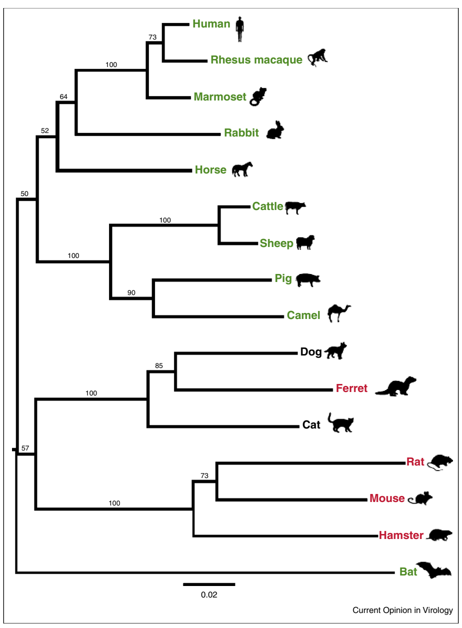
Adaptation to humans involves strong interaction with hACE2

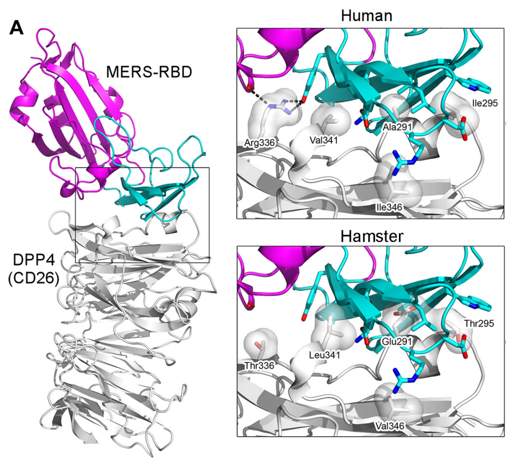


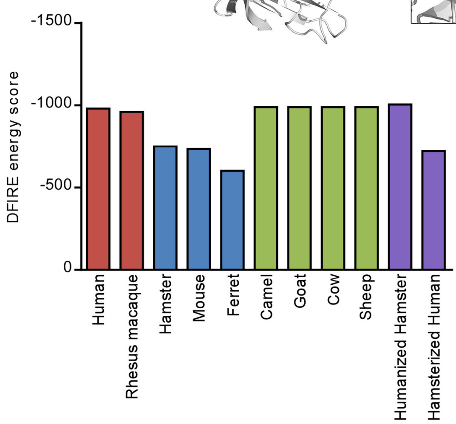
Li & al.2005 Science 309:1824

MERS-CoV Receptor specificity

DPP4 phylogeny





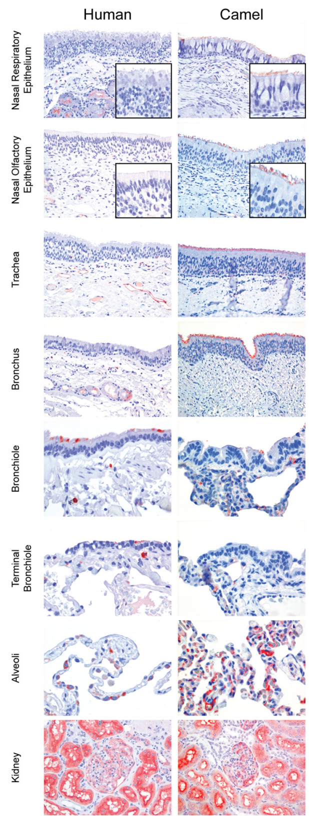


Reusken et al.2016 Curr Opin Virol

van Doremalen et al.2014 JVI

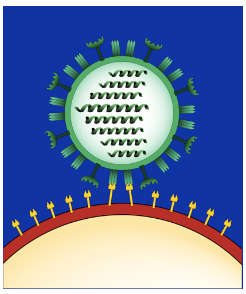
MERS-CoV Receptor expression

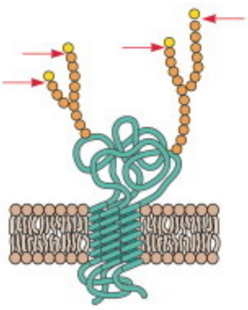
DPP4 expression

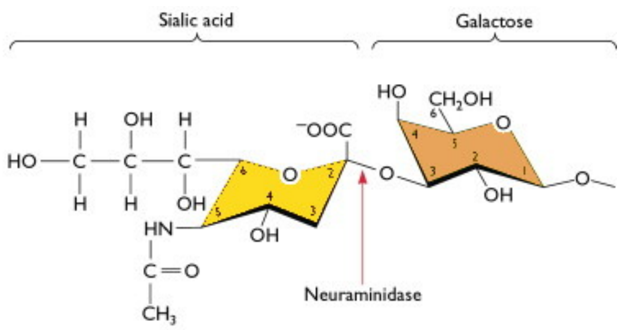


Widagdo et al.2016 JVI

Binding to sialic acid receptors

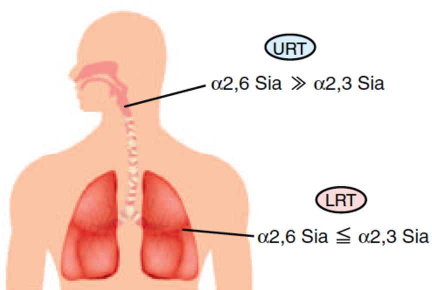


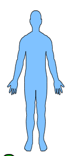




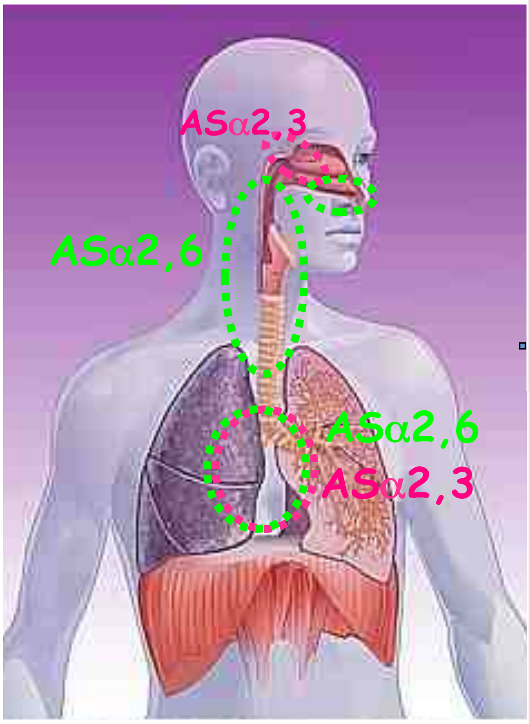
Sialic acid: N-acetylneuraminic acid (NANA)

SA linked to Gal by an α2,3 linkage (shown) or α2,6 linkage

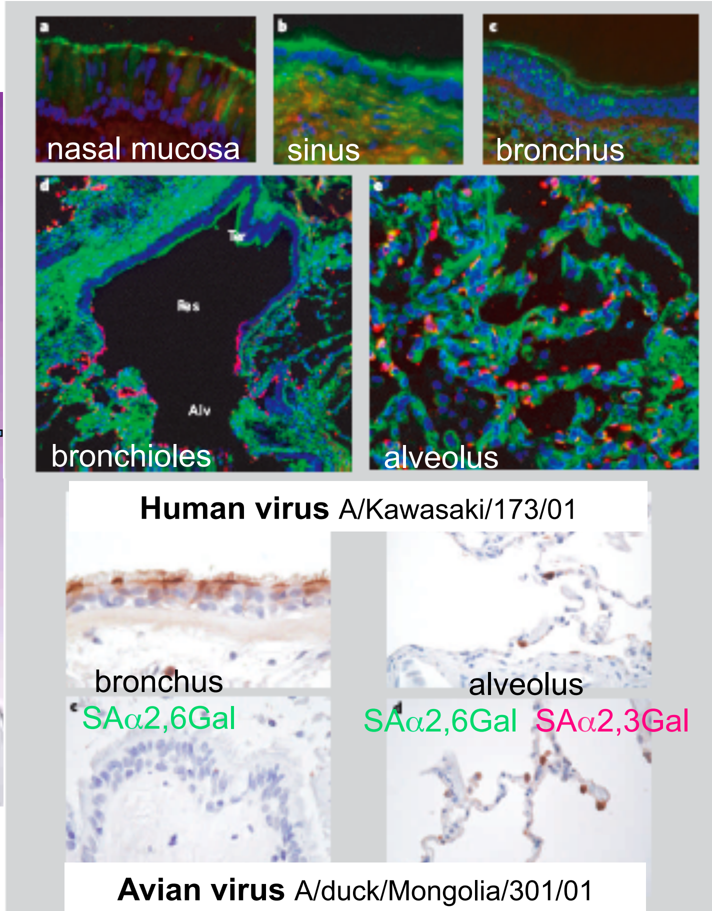


	HA	Specificity
	226	
	Leu	
	Gln	

Distribution of HA receptors In respiratory tract

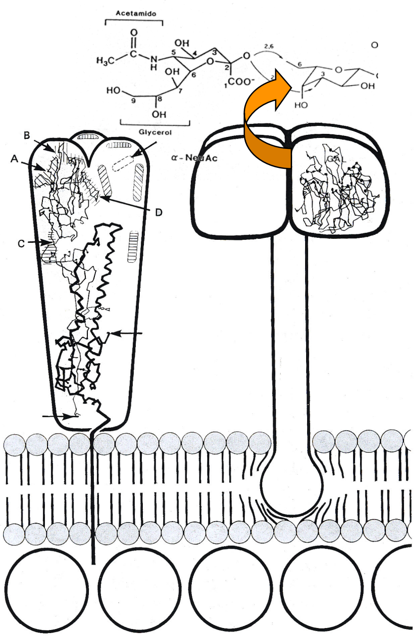


Shynia et al. Nature 2006
Van Riel et al. Science 2006



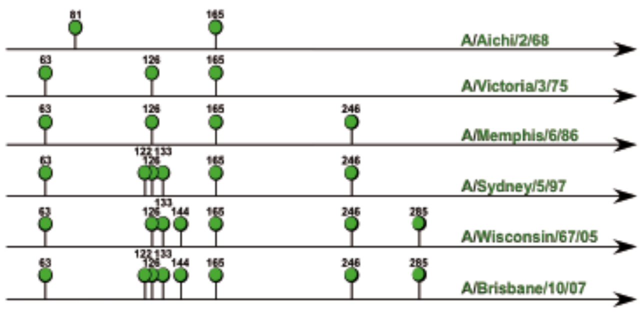
HA and NA glycosylation

Sialic acid



HA / NA Balance

Evolution of glycosylation of human H3
beween 1968 and 2007

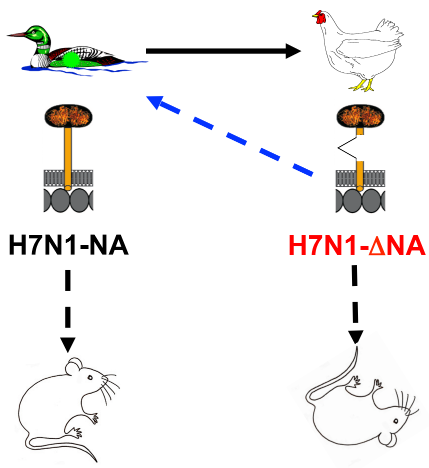


Variations according to species

Evolution upon adaptation to new species

Impact on HA – NA balance

NA and interspecies transmission



Munier et al., J Virol 2010

Hoffmann et al., J Virol 2011

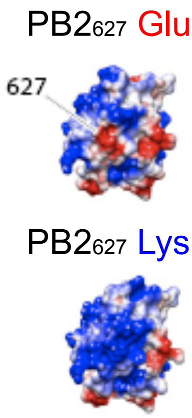
Matsuoka et al., J Virol 2009

Virus	MLD_{50} ($\log_{10}$)[a]		
	Short NA	Long NA	Difference (long NA − short NA)
486-none	4.82[c]	9.2	4.38
486-131	6.2	7.32	1.12
486-158	7.41	7.2	−0.21
486-169	5.53	>7.0[d]	>1.47
486-131/169	6.2	>7.7[d]	>1.5
486-158/169	6.7	7.12	0.42
486-131/158/169	8.37	>9.0[d]	>0.63

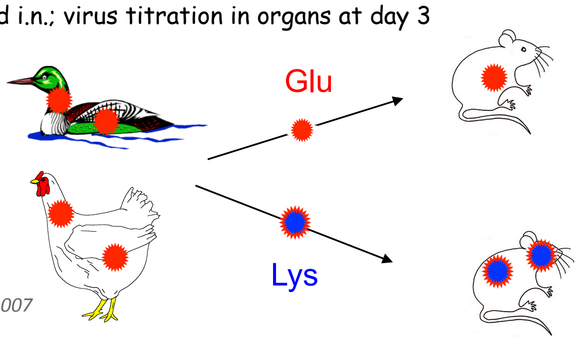
Effect of PB2 residue 627 on multiplication of avian viruses in the upper respiratory tract of mice

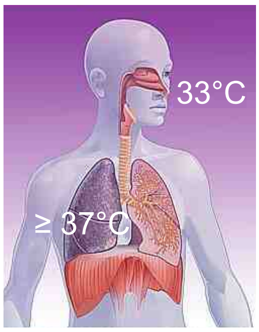
Virus	PB2627	Virus titer (log PFU)			
		lungs	NT	spleen	heart
VN1203/04	Lys	7.2	$\boxed{4.6}$	4.9	2.1
VN1204/04	Glu	5.7	2.3	<	3.4
VN1203PB2-627E	Glu	3.3	<	2.1	<
VN1204PB2-627K	Lys	8.2	$\boxed{6.6}$	7.3	6.8
Mal/NY	Glu	6.0	<	nt	nt
Mal/NYPB2-627K	Lys	6.8	$\boxed{5.5}$	nt	nt
VD5	Glu	3.1	<	nt	nt
VD5PB2-627K	Lys	5.8	$\boxed{5.3}$	nt	nt

Balb/c mice infected i.n.; virus titration in organs at day 3



PB2627 Glu





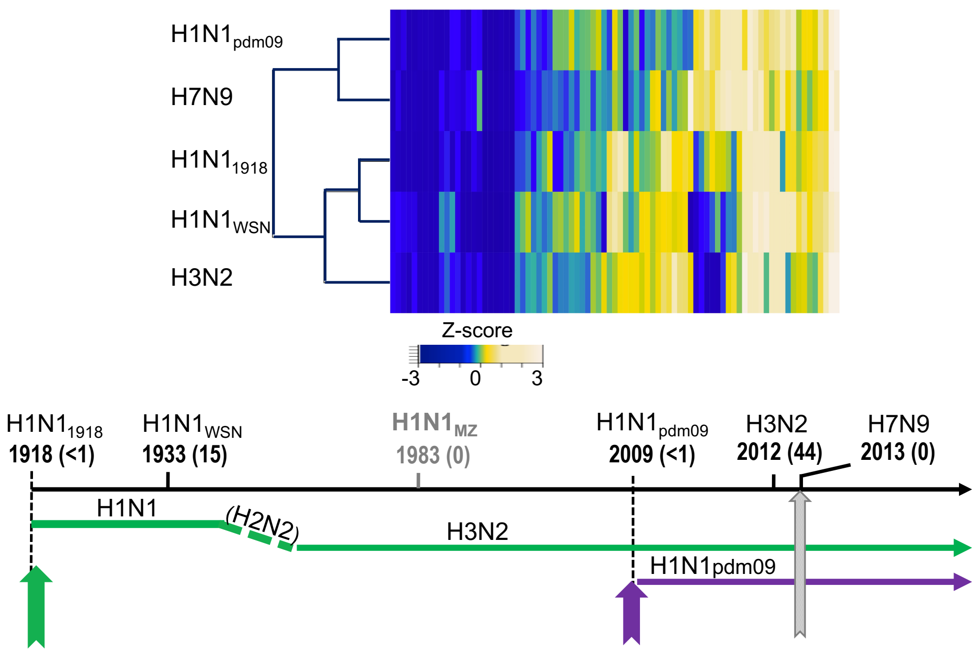
Hatta et al. PLOS Pathogen 2007

Pathogenicity determinants in the polymerase

PA	F35L	> polymerase activity and virulence in mice. Serially passaged pdm in mice	Seyer et al., 2012
	A36T	> polymerase activity and growth in human and porcine cells. Mouse adapted pdm	Zhu et al., 2012
	T85I	> polymerase activity and virus replication in human cells. pdm	Bussey et al., 2011
	T97I	> polymerase activity and virulence. Serially passaged H5N2 in mice	Song et al., 2009
	P224S	> polymerase activity and virulence (combined with A70V mutation). pdm in mice	Sun et al., 2014
	L295P	> polymerase activity and replication. Mouse adapted pdm	Ilyushina et al., 2010
	L336M	> polymerase activity and virulence. pdm in mice	Bussey et al., 2011
	N409S	> polymerase activity, viral growth and pathogenesis. H7N9 in mice	Yamayoshi et al., 2014
	I550L	> polymerase activity, RNAPII degradation and pathogenicity. H1N1 & pdm in mice	Llompart et al., 2014; Rolling et al., 2009
	T552S	> polymerase activity, virus replication and pathogenicity. Avian & human viruses	Mehle et al., 2012
	G631S	> virulence. H5N1 in mice	Hiromoto et al., 2000
PB1	H99Y	> polymerase activity and airborne transmission. H5N1 in ferrets	Linster et al., 2014
	L473V	> polymerase activity and viral growth. H5N1 & pdm in mice	Xu et al., 2012
	L598P	> polymerase activity and viral growth. H5N1 & pdm in mice	Xu et al., 2012
PB2	D9N	Mitochondrial localization. < IFN expression. > pathogenicity. H1N1 & H5N1 in mice	Graef et al., 2010; Kim et al., 2010
	M147L	> viral replication and virulence (combined with E627K mutation). H9N2 in mice	Wang et al., 2012
	E158G/A	> polymerase activity and replication. Mouse adapted pdm	Ilyushina et al., 2010
	D256G	> polymerase activity in mammalian cells and replication in pigs. H5N1 in pigs	Manzoor et al., 2009
	H357N	> polymerase activity and pathogenicity. Mouse adapted pdm	Zhu et al., 2012
	I504V	> polymerase activity, RNAPII degradation and pathogenicity. H1N1 & pdm in mice	Llompart et al., 2014; Rolling et al., 2009
	T588I	> polymerase activity, replication and virulence.> binding and inhibition to MAVS. pdm in mice	Zhao et al., 2014
	G590S	> polymerase activity and viral replication. Pdm	Mehle and Doudna, 2009
	Q591R	> polymerase activity, viral replication and pathogenicity. pdm & H5N1 in mice.	Mehle and Doudna, 2009; Yamada et al., 2010
	E627K	> polymerase activity, viral protein expression, viral growth in mammalian cells, brain invasiveness and virulence. Confers avian viruses efficient growth in mammalian upper and lower respiratory tracts. H5N1, H7N7, H9N2, H7N9 in mice and ferrets	de Jong et al., 2013; Hatta et al., 2001; Hatta et al., 2007; Li et al., 2012; Mok et al., 2014; Munster et al., 2007; Shinya et al., 2004; Shinya et al., 2007; Subbarao et al., 1993; Zhang et al., 2014
	D701N	> polymerase activity, cap-1 RNA binding activity, viral growth, transmission and virulence. Mouse adapted H7N7, H5N1, H3N2, H7N9 in mice	Czudai-Matwich et al., 2014; Gabriel et al., 2005; Li et al., 2005; Mok et al., 2014; Ping et al., 2010; Zhang et al., 2012
	S714R	> polymerase activity, cap-1 RNA binding activity and virulence. Synergistic effect with 701N. H5N1 & H7N7 in mice	Czudai-Matwich et al., 2014; Gabriel et al., Zhang et al., 2012

Rodriguez-Frandsen et al. 2015 Virus Res

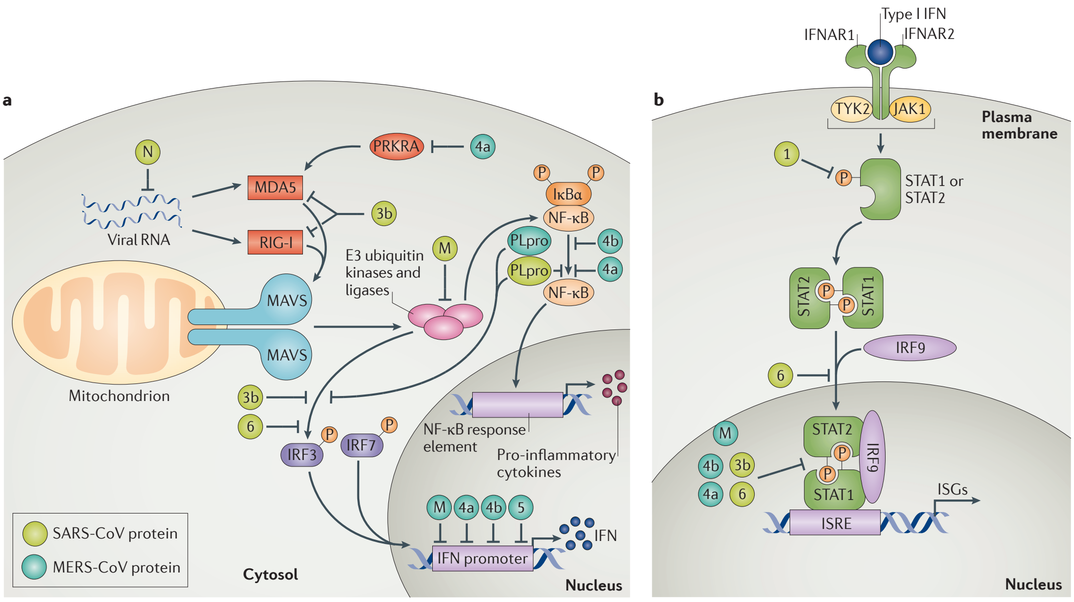
Comparative interactomics of PB2 with the UPS



correlation w/ origin and duration of circulation of PB2 in humans

Biquand et al. mSphere, accepted

SARS-CoV and MERS-CoV evasion of innate responses

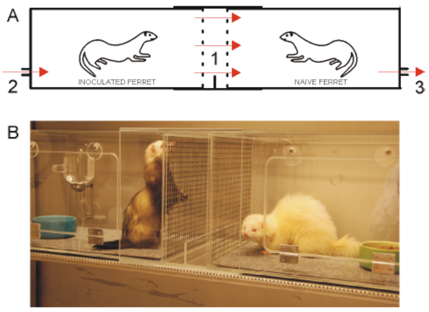


de Wit et al.2016 Nature Rev Microbiol

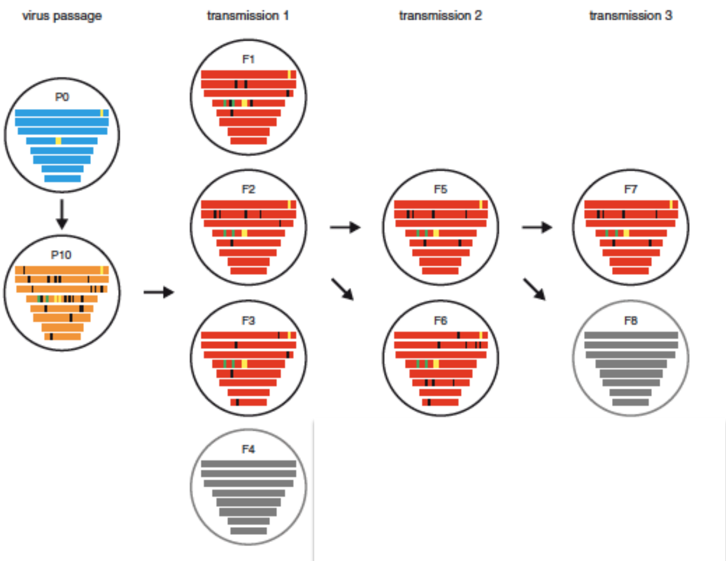
Intrinsic viral determinants

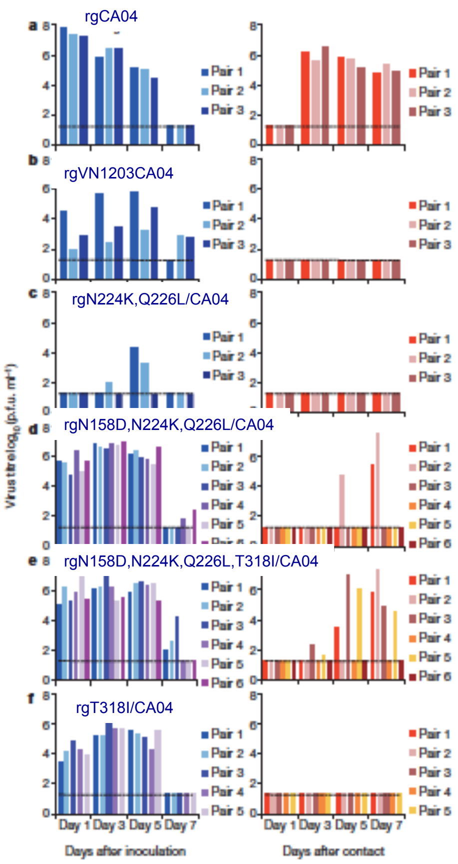
determinants of transmission

Airborne transmission in ferrets

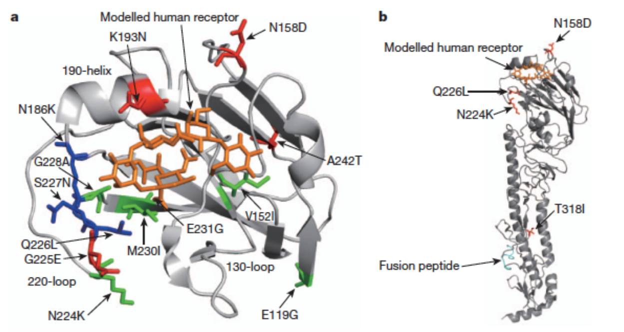


Herfst et al Science 2012





Airborne transmissible



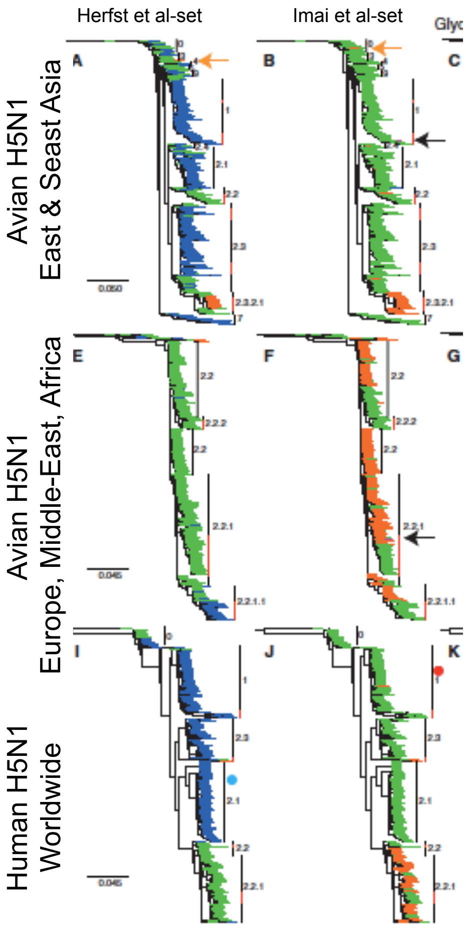
Substitutions confering airborne transmissibility to H5N1 (reassortant) viruses

HA N224K, Q226L (receptor specificity)
HA N158D (glycosylation sequon)
HA T318I (trimer interface)

HA Q222L, G224S (receptor specificity)
HA T156A (glycosylation sequon)
HA H103Y (trimer interface)
PB2 E627K

Imai et al Nature 2012

Herfst et al Nature 2012



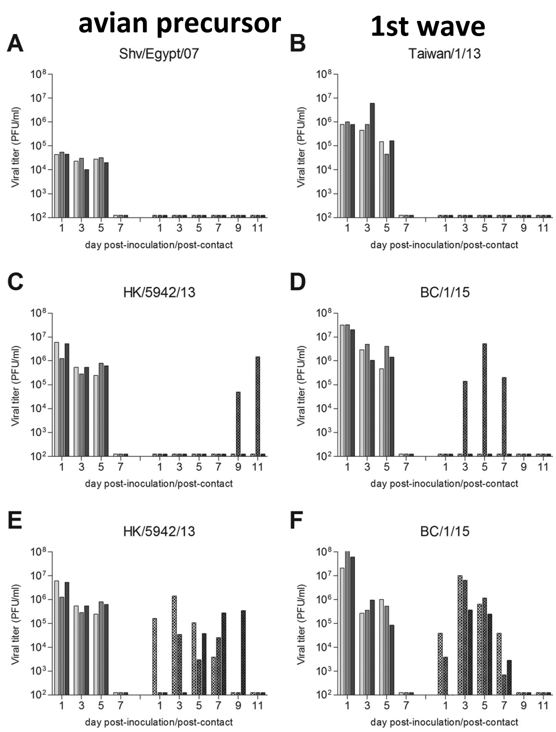
Markers of Airborne transmissibility in H5N1 in nature

Minimum mutations required in HA according to:
Herfst et al (left column)
Imai et al. (right column)

Blue = 5 nt changes
Green= 4 nt changes
Orange = 3 nt changes

Russel et al Science 2012

H7N9 transmissibility in ferrets



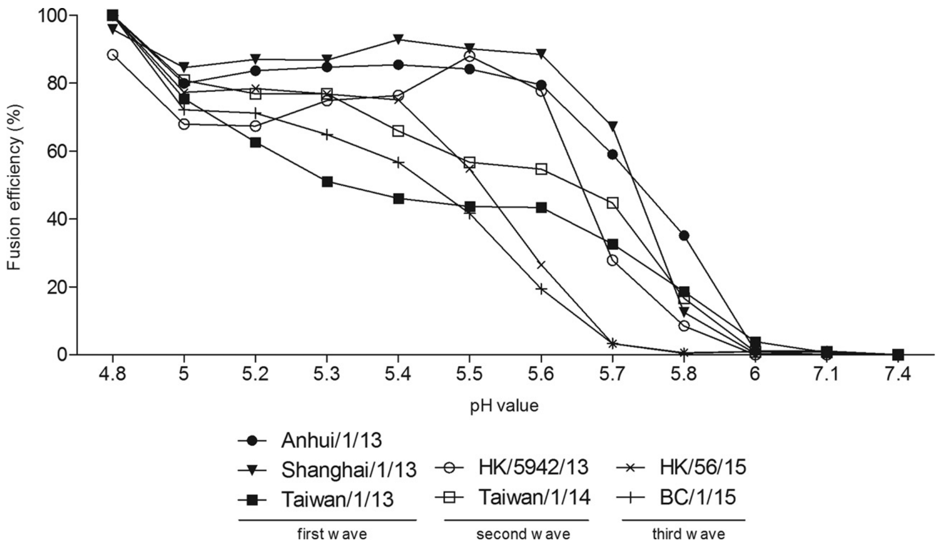
2nd wave

3rd wave

Belser et al 2016 JVI

→ Increased respiratory droplet transmission for 3rd wave viruses

H7N9 fusion pH threshold

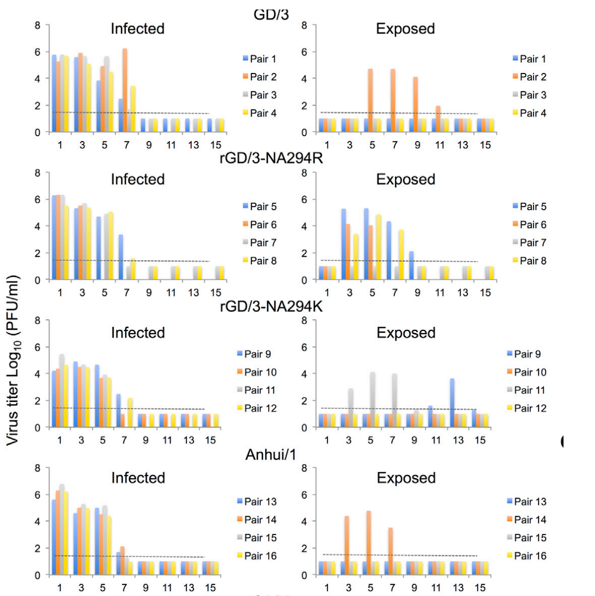


→ Lower fusion pH threshold for 3rd wave H7N9 viruses

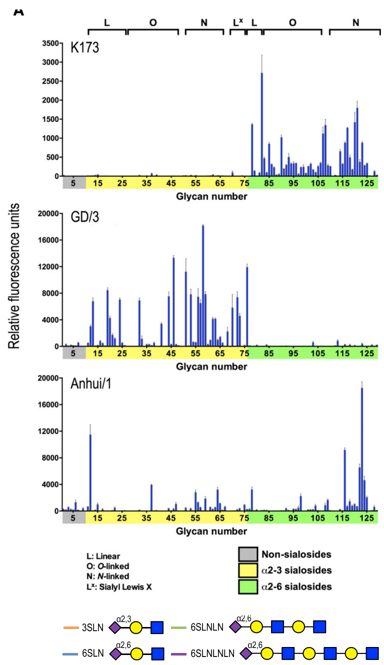
Belser et al 2016 JVI

HP-H7N9 transmissibility

Respiratory droplets transmission in ferrets



Receptor specificity



Imai et al 2017 Cell Host & Microbes

Transmission
other intrinsic factors to consider

Survival in the environment

- **H5N1: in dejections (7d at 20°C, 35d at 4°C);water (105d)**
- **MERS-CoV: stability >> 72h in camel milk**

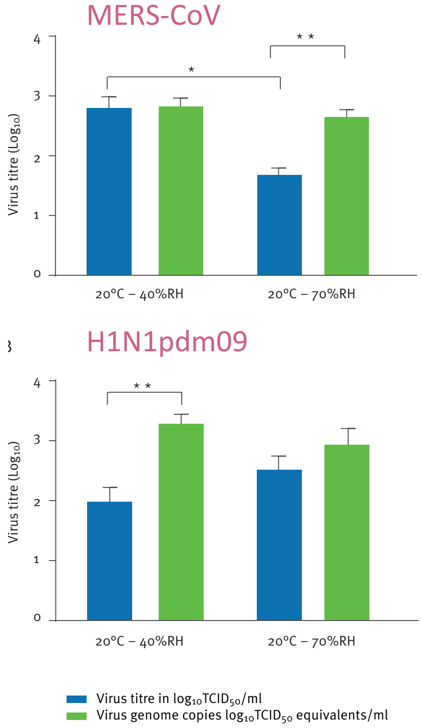
Ability to infect/disseminate w/in host (replication site, virus yield, ..)

Efficiency of replication

Fitness and competitiveness

Escape host response

Virus population diversity



van Doremalen et al, 2015 Eurosurveillance

Transmission
other intrinsic factors to consider

Survival in the environment

- H5N1: in dejections (7d at 20°C, 35d at 4°C);water (105d)
- MERS

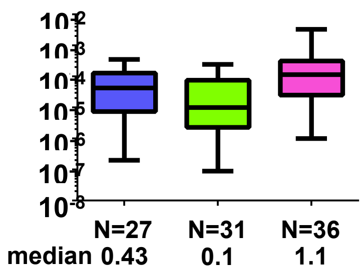
Ability to infect/disseminate w/in host (replication site, virus yield, ..)

Efficiency of replication

Fitness and competitiveness

Escape host response

Virus population diversity (quasispecies)



sH1N1 H3N2 pH1N1

higher mutation frequency for pH1N1

Barbezange et al, unpublished

H7N9 Pathogenicity determinants

Gene		Mutation		Human isolates	Environmental isolates
HA	Receptor binding site	G186V	V	81	2
		Q226L/I	L	80	2
			I	1	0
NA	Related to drug resistance	A246T	A	80	2
			T	1	0
		R292K	R	79	2
			K	2	0
PB2	Increased virulence in mice	E627K	E	20	2
			K	59	0
			V	2	0
	Enhanced transmission in guinea pigs	D701N	D	71	2
			N	10	0
	species-associated signature positions	K702R	K	65	1
			R	16	1
PB1	Increased transmission in Ferret	I368V	I	5	0
			V	76	2
PB1-F2	Increased pathogenicity in mice	87–90 amino acids in length	11AA	3	1
			34AA	10	0
			76AA	3	0
			87AA	1	0
			90AA	62	1
			101AA	2	0
	altered virulence and antivirus response in mice	N66S	N	75	2
			S	6	0
PA	species-associated signature positions	V100A	V	68	2
			A	13	0
	increase the polymerase activity in mice	L336M	L	81	2
	species-associated signature positions	K356R	R	81	2
		S409N	N	81	2
NS1	altered virulence in mice	D92E	D	81	2
	altered antiviral response in host	N205S	S	81	2
		G210R	G	81	2

Conclusions

- Evaluation of zoonotic potential
  - based on known determinants
  (CDC inventory http://www.cdc.gov/flu/pdf/avianflu/h5n1-inventory.pdf)
  - importance of genetic context
  - importance of gene constellations

→ consider knowledge about genetic lineage

- Challenges and uncertainties
  - level of preexisting immunity
  - level of of asymptomatic infections
  - potential for reassortment
  - genetic susceptibility of the population initially exposed

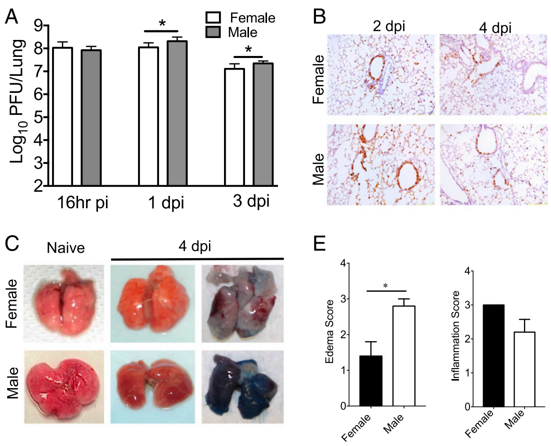
- Multiple complementary sources of information required

Extrinsic Factors
Host and Environment

Host determinants

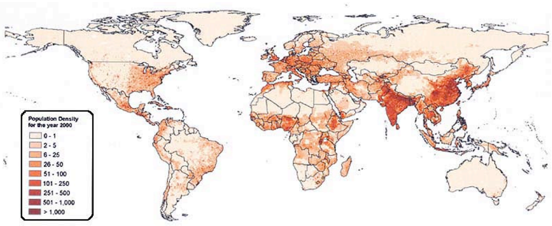
- **Pre-existing immunity**
- **Genetic susceptibility** (e.g. IFITM3)
- **Age** (e.g. ≠ H5N1, H7N9)
- **Sex**
- **Co-morbidities**

Male mice are more susceptible to SARS-CoV



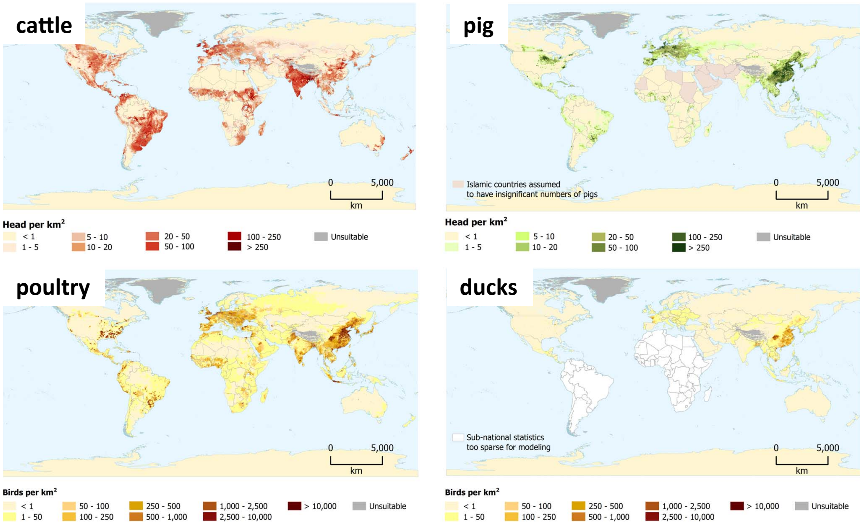
***Channappanavar** et al 2017 JVI*

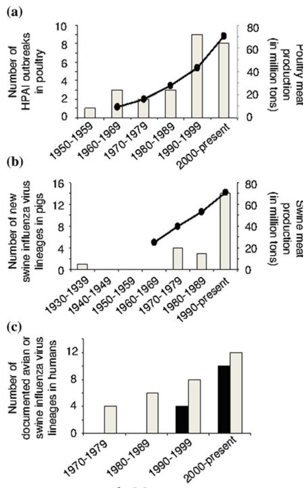
Population densities



Robinson et al, 2014 PLoS One

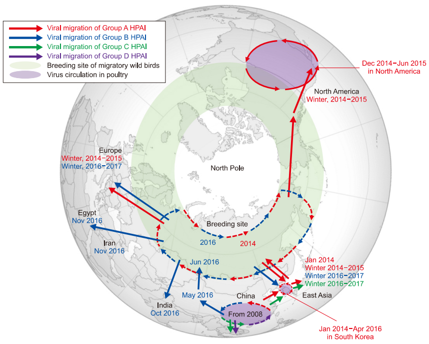
Source: *Source:* CIESIN; CIAT)



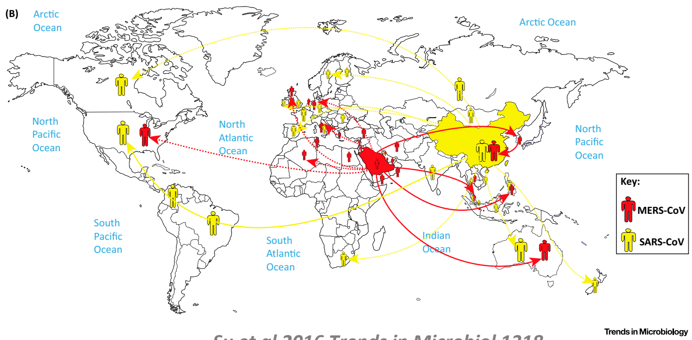


Reperant et al, 2014
Curr Top Microbiol Immunol

Geographical extension



Lee et al 2017 J Vet Sci

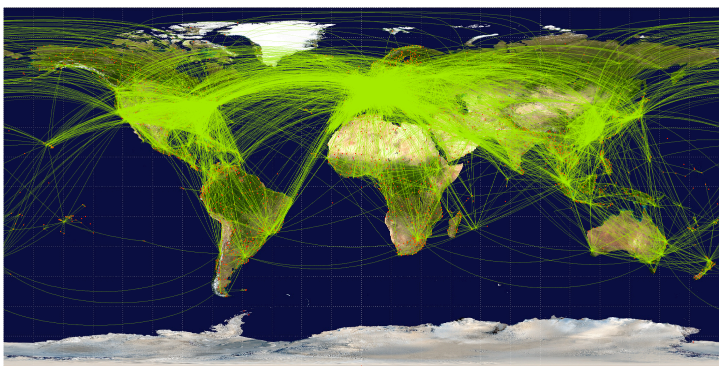


Su et al 2016 Trends in Microbiol 1318

Animal movement:
natural
trade (legal or illegal)

Human movement

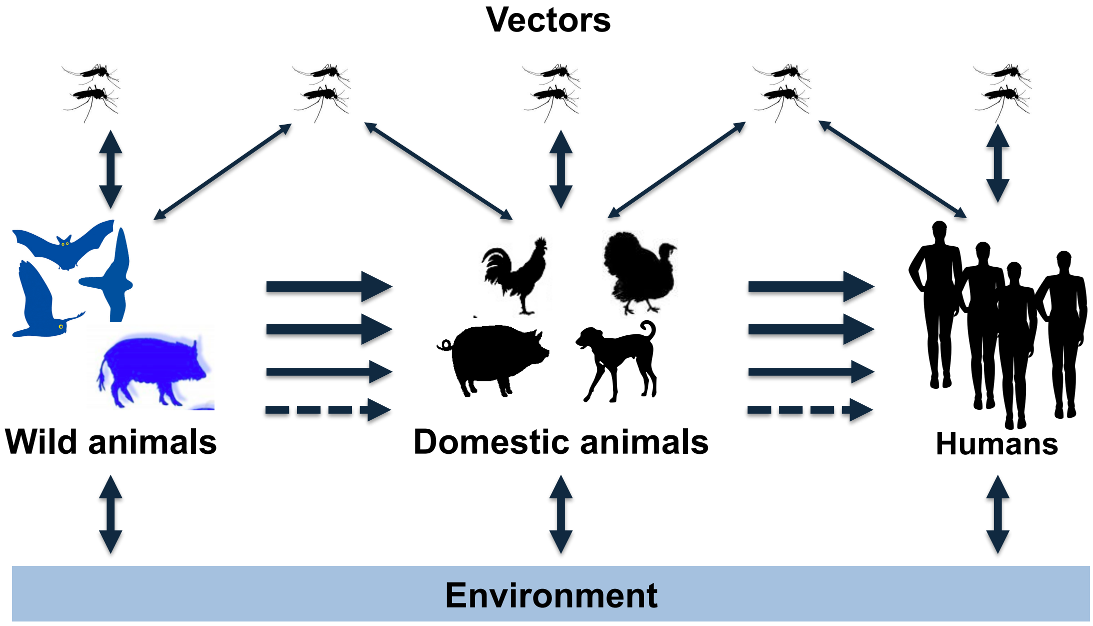
Transport (air, ship, etc..)



Social and Occupational Factors

- Number and type of contacts
- Hospital settings
- Family settings
- Slaughterhouses
- Breeders, animal care takers
- Food-borne transmission respiratory viruses.

Potential for control of Emergence?



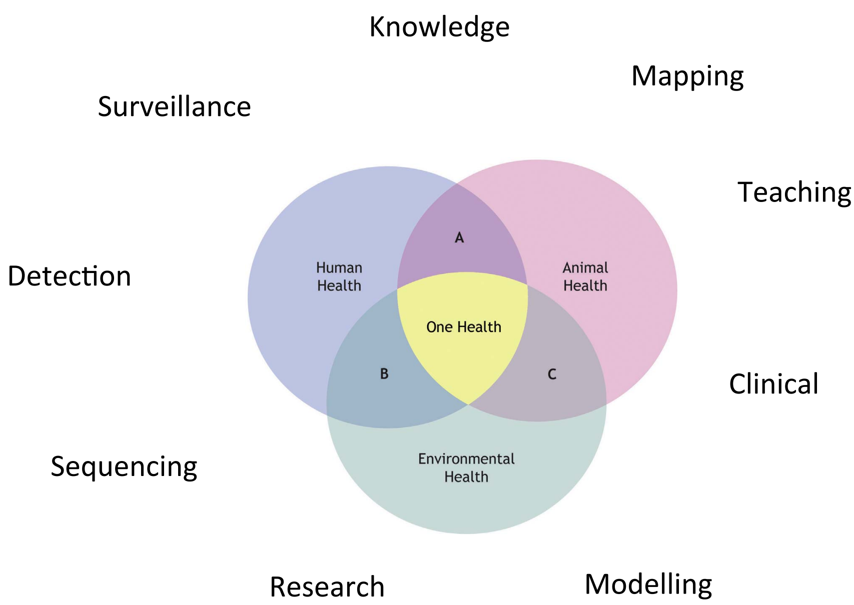
Pandemic risk assessment

- Exposure
- Potential for infection in humans – level of severity
- Potential for human-to-human transmission
- Potential for spread in the human population

Tools: IRAT (CDC); TIPRA(WHO)

Determinants of Pandemic risk

- Viral factors
- Host factors
- Social factors



MULTIDICIPLINARITY

Davis et al. 2017 One Health 4

Thank you for your attention