

Transmission of H9N2 Influenza Viruses

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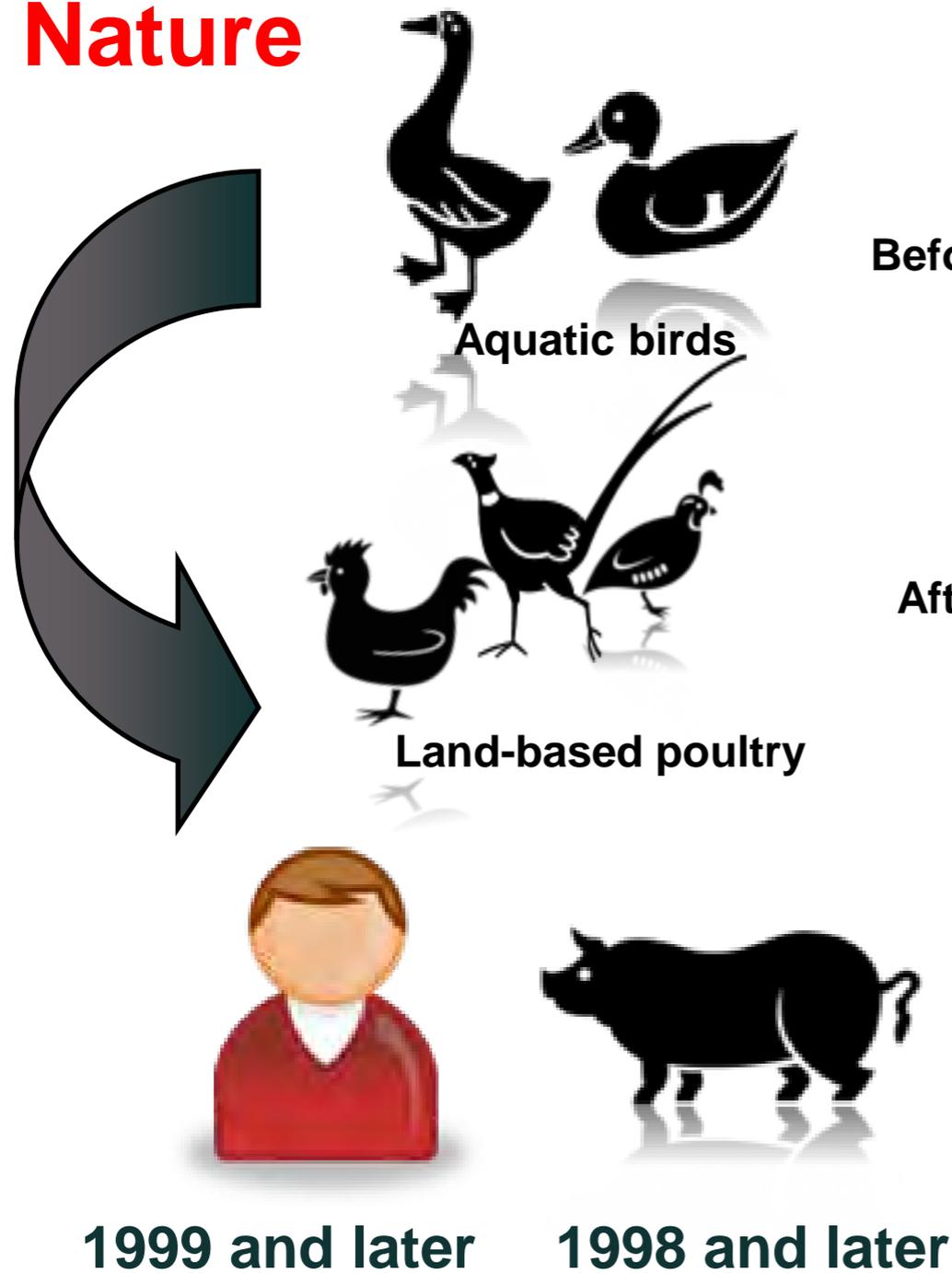


UNIVERSITY OF
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What we know

Overview of H9N2 avian influenza viruses

Nature



Before 1988

Aquatic birds

After 1988-1994

Land-based poultry

1999 and later

1998 and later

Laboratory

Early isolates do not or replicate poorly (1997 and before)



Recent field isolates replicate well (1998 and later)

Bind preferentially to human-like sialic acid receptors

H9N2 avian influenza viruses of the G1-like lineage show the largest geographic spread

From Middle East to Far East

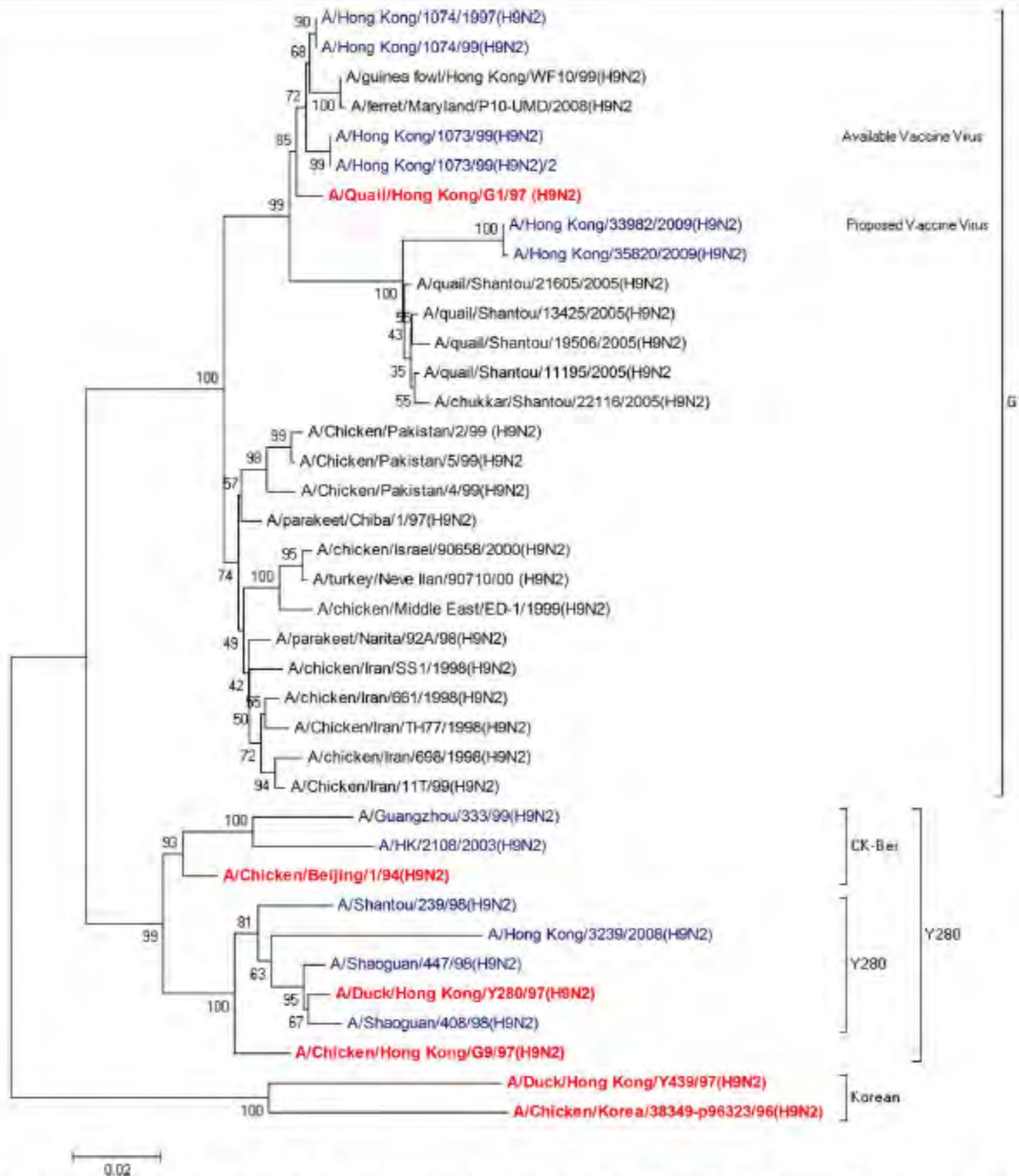
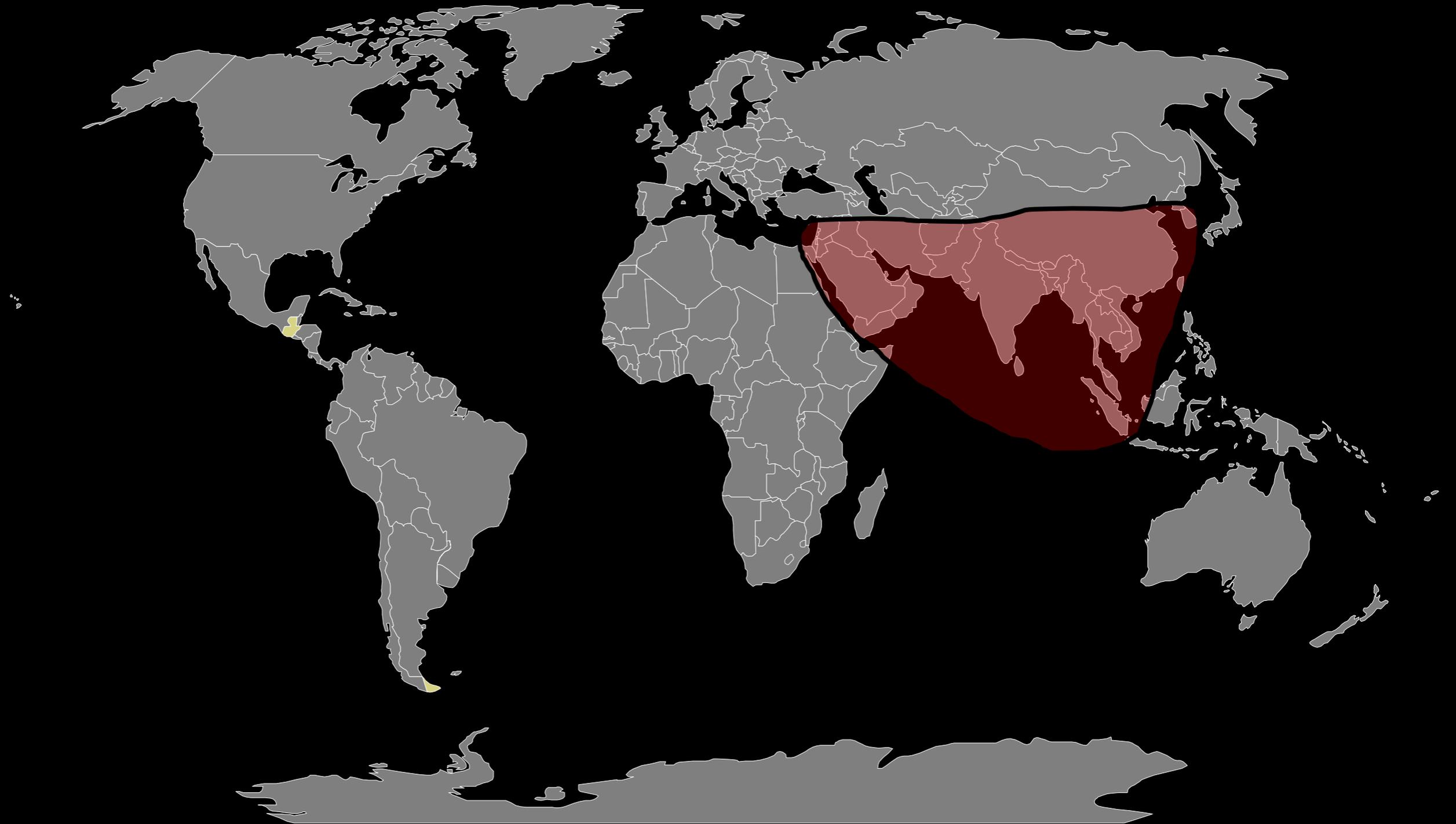


Figure 1 Phylogenetic relationships of HA genes in H9N2 influenza viruses isolated from humans between 1997 and 2009.

A phylogenetic tree was generated using minimum evolution analysis with maximum composite likelihood using the Tamura-Nei model with MEGA software version 4.0.2. Numbers below branches indicate bootstrap value percentages from 1000 replicates. The scale bar represents the distance unit between sequence pairs. Representative prototype viruses for different Eurasian lineages are indicated as red. The sequences of H9N2 influenza viruses isolated from the human population are indicated as blue.

H9N2 geographic range



What we know

Land-based birds posses $\langle 2,3\text{-gal}$ and $\langle 2,6\text{-gal}$ receptors in the respiratory tract

	Species	Age (Week)	Trachea		Lung		Large intestine	
			2,3	2,6	2,3	2,6	2,3	2,6
Aquatic	Duck	1	+	-	+	+	+	-
		2	+	-	++	+	++	+
		4	+	-	++	+	++	+
	Goose	1	+	-	+	+	+	-
		2	+	-	+	+	++	+
		4	+	-	+	-	+	+
Land	Pheasant	1	++	+	+	+	+	+
		2	++	+	+	+	+	+
		4	++	+	+	+	+	+
	Turkey	1	++	+	+	+	+	-
		2	++	+	+	+	+	-
		4	++	++	++	++	+	-
	Guinea fowl	1	+	+	+	+	+	-
		2	+	+	+	+	+	-
		4	+	+	+	+	+	-
	Quail	1	+	+	+	+	+	-
		2	+	+	+	+	+	-
		4	+	+	+	+	+	-

Only a handful cases of H9N2 infections have been reported in humans. Is H9N2 causing asymptomatic infections in people?

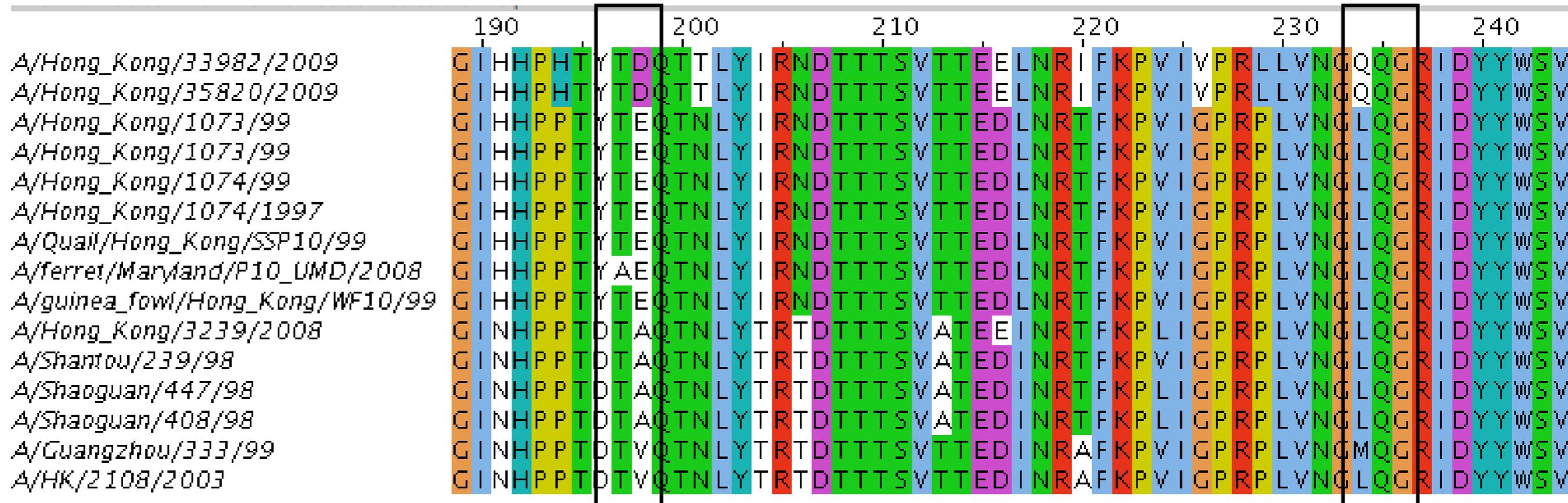


Table 1 GenBank accession numbers genes and proteins of avian influenza A (H9N2) viruses isolated from 1997 to 2009 from humans

Year	Strains	Accession Numbers			
		Hemagglutinin		Neuraminidase	
		Genes	Proteins	Genes	Proteins
1997	<i>A/Hong Kong/1074/97</i>	GU053179.1	ACZ48627	GU053180.1	ACZ45242
1998	<i>A/Shantou/239/98</i>	AY043015.1	AAL32476	AY043021.1	AAL32481
	<i>A/Shaoquan/408/98</i>	AY043017.1	AAL32477	AY043022.1	AAL32482
	<i>A/Shaoquan/447/98</i>	AY043018.1	AAL32478	AY043023.1	AAL32483
1999	<i>A/Hong Kong/1073/99^a</i>	AJ404626.1	CAB95856	AJ404629.1	CAB95859
	<i>A/Hong Kong/1074/99</i>	AJ404627.1	CAB95857	AJ404628.1	CAB95858
	<i>A/Guangzhou/333/99</i>	AY043019.1	AAL32479	AY043024.1	AAL32484
2003	<i>A/Hong Kong/2108/03</i>	DQ226106.1	ABB58945	DQ226128.1	ABB58956
2008	<i>A/Hong Kong/3239/08</i>	CY055156.1	ADC41863	CY055158.1	ADC41865
2009	<i>A/Hong Kong/33982009^b</i>	CY055140.1	ADC41843	CY055142.1	ADC41845
	<i>A/Hong Kong/35820/09</i>	CY055148.1	ADC41853	CY055150.1	ADC41855

^a A(H9N2) Available vaccine virus (G1 Clade; WHO report, 2010)

^b A(H9N2) Proposed vaccine virus (G1 Clade; WHO report, 2010)

Swine H9N2 infections have more flexibility at position 226 in RBS?

	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240																	
<i>A/swine/Korea/S452/2004</i>	W	G	K	N	I	L	F	M	W	G	I	H	H	P	P	T	D	T	E	Q	M	N	L	Y	K	K	A	D	T	T	T	S	I	T	T	E	D	I	N	R	T	F	K	P	V	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Korea/S81/2004</i>	W	G	K	N	I	L	F	M	W	G	I	H	H	P	P	T	D	T	E	Q	M	N	L	Y	K	K	A	D	T	T	T	S	I	T	T	E	D	I	N	R	T	F	K	P	V	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Korea/S190/2004</i>	W	G	K	N	I	L	F	M	W	G	I	H	H	P	P	T	D	T	E	Q	M	N	L	Y	K	K	A	D	T	T	T	S	I	T	T	E	D	I	N	R	T	F	K	P	V	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Korea/S83/2004</i>	W	G	K	N	I	L	F	M	W	G	I	H	H	P	P	T	D	T	E	Q	M	N	L	Y	K	K	A	D	T	T	T	S	I	T	T	E	D	I	N	R	T	F	K	P	V	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Korea/S109/2004</i>	W	G	K	N	I	L	F	M	W	G	I	H	H	P	P	T	D	T	E	Q	M	N	L	Y	K	K	A	D	T	T	T	S	I	T	T	E	D	I	N	R	T	F	K	P	V	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Taizhou/5/2008</i>	Q	E	K	N	I	L	F	M	W	G	I	N	H	P	P	T	D	T	A	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	E	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	L	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Yangzhou/1/2008</i>	Q	E	K	N	I	L	F	M	W	G	I	N	H	P	P	T	D	T	A	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	E	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	L	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Shandong/na/2003</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	G	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	T	T	E	D	I	T	R	T	F	K	P	M	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Shandong/nc/2005</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	A	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	T	T	E	D	I	T	R	T	F	K	P	M	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Guangxi/FS2/2005</i>	R	G	R	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	A	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	K	T	F	K	P	L	I	G	P	R	P	L	V	N	G	M	Q	G	R	I	D	Y	Y	W	S	I	L	K	F
<i>A/swine/Guangxi/58/2005</i>	R	G	R	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	T	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	I	L	K	F
<i>A/swine/Guangxi/S15/2005</i>	R	G	R	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	W	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	I	L	K	F
<i>A/swine/Guangxi/S11/2005</i>	R	G	R	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	T	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	I	L	K	F
<i>A/swine/Hong_Kong/9/98</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	W	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	T	T	E	D	I	N	R	T	F	K	P	V	I	G	P	R	P	L	V	N	G	L	H	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Hong_Kong/10/98</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	W	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	T	T	E	D	I	N	R	T	F	K	P	V	I	G	P	R	P	L	V	N	G	L	H	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Hong_Kong/10/98</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	W	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	T	T	E	D	I	N	R	T	F	K	P	V	I	G	P	R	P	L	V	N	G	L	H	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Hong_Kong/9/98</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	W	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	T	T	E	D	I	N	R	T	F	K	P	V	I	G	P	R	P	L	V	N	G	L	H	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Shandong/FHZ/2003</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	T	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/sw/ShanDong/1/2003</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	A	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	L	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Shandong/w4/2003</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	A	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	L	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Shandong/3/2003</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	A	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	L	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Shandong/FN/2003</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	A	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	L	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Shandong/FNY/2003</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	A	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	L	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Shandong/8/2003</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	A	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	L	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Shandong/FLS/2003</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	A	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	L	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Shandong/FIC/2003</i>	R	G	K	S	I	L	F	M	W	G	I	N	H	P	P	T	D	T	A	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	L	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Hebei/012/2008</i>	R	G	K	N	I	L	F	M	W	G	I	N	H	P	P	T	D	T	W	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	I	L	K	F
<i>A/swine/Guangxi/8/2007</i>	R	G	K	N	I	L	F	M	W	G	I	N	H	P	P	T	D	T	T	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	N	P	L	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Guangxi/10/2007</i>	R	G	K	N	I	H	F	M	W	G	I	N	H	P	P	T	D	T	T	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Guangxi/9/2007</i>	R	G	K	N	I	L	F	M	W	G	I	N	H	P	P	T	D	T	T	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	Y	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Guangxi/7/2007</i>	R	G	K	N	I	L	F	M	W	G	I	N	H	P	P	T	D	T	T	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N	R	T	F	K	P	L	I	G	P	R	P	L	V	N	G	Q	Q	G	R	I	D	Y	Y	W	S	V	L	K	F
<i>A/swine/Hong_Kong/3297/98</i>	R	G	K	N	I	L	F	M	W	G	I	N	H	P	P	T	D	T	A	Q	T	N	L	Y	T	R	T	D	T	T	T	S	V	A	T	E	D	I	N																													

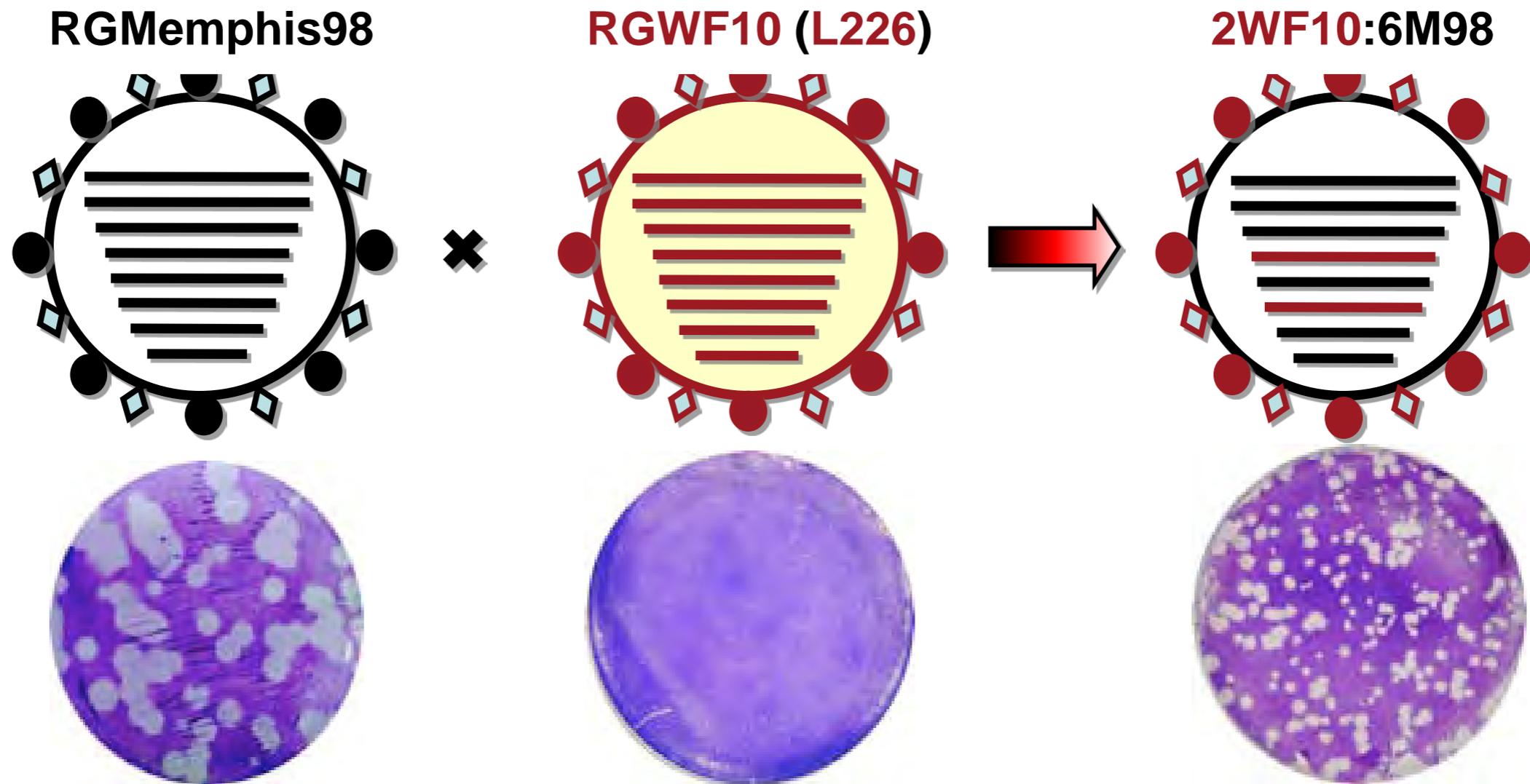
Amino acid 226 in RBS of HA modulates the glycan binding profile of H9N2 viruses

Contemporary H9N2 avian influenza viruses with human-like receptor specificity (L226**) infect mostly nonciliated cells in HAE cultures.**

L226 in the HA H9N2 viruses favors infection of nonciliated cells in HAE cultures with less cytopathic effect than a typical H3N2 human influenza virus

What we do

Would an avian/human H9N2 reassortant virus show improved replication and transmission in ferrets?



The **2WF10:6M98 (H9N2) shows increased virulence in ferrets compared to the wt WF10 but still cannot transmit by respiratory droplets**

Is it possible to generate a H9N2 virus that transmits by respiratory droplets in ferrets? - Implications for pandemic preparedness

2WF10:6M98

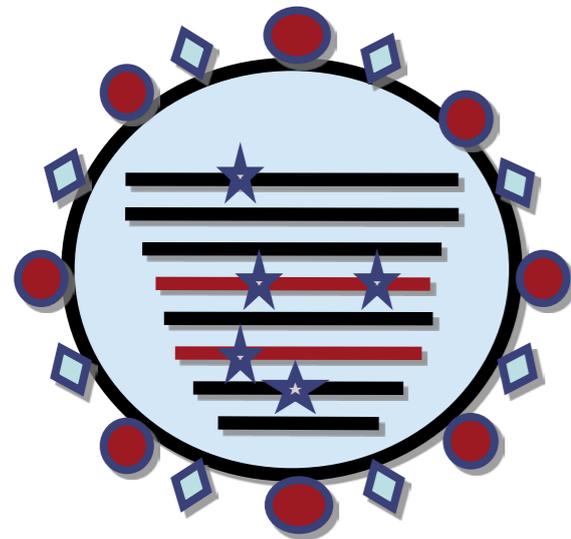
Passage 1



Nasal washes used to infect new set of ferrets

P10-2WF10:6M98

Passage 10



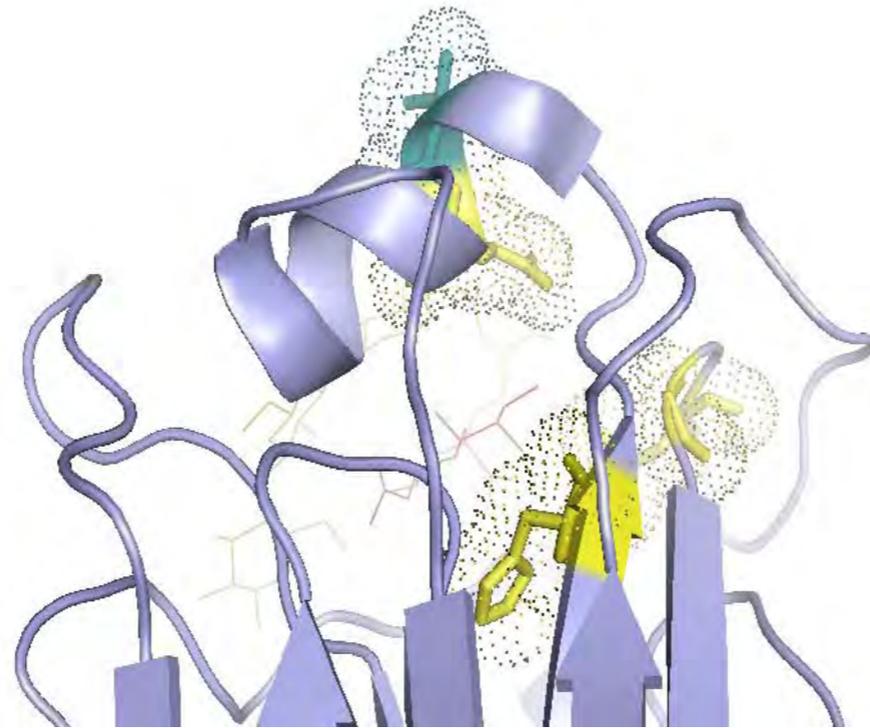
Five amino acid changes occur during adaptation of the **2WF10:6M98** virus in ferrets

Gene	Origin	Amino Acid Position	Parent	P8	P9	P10	RCP10	RCP10 ₂
PB2	Human	374	L	I	I	I	I	I
PB1	Human	No changes ^a		nd ^b	nd			
PA	Human	No changes		nd	nd			
HA	Avian	HA1 189	T	T	T	T	A	A
		HA2 192	G	G	<i>G/R^c</i>	<i>G/R^c</i>	R	R
NP	Human	No changes		nd	nd			
NA	Avian	28	I	V	V	V	V	V
M1	Human	110	H	<i>H/Y^c</i>	Y	Y	Y	Y
M2	Human	No changes		nd	nd			
NS1	Human	No changes		nd	nd			
NEP	Human	No changes		nd	nd			

^a No amino acid changes detected between the parent and either the RCP10 or the RCP10₂ viruses. ^b nd, sequencing not done. ^c Bold and italicized letter denotes more prominent residue at particular amino acid position based on electropherograms of sequencing profiles.

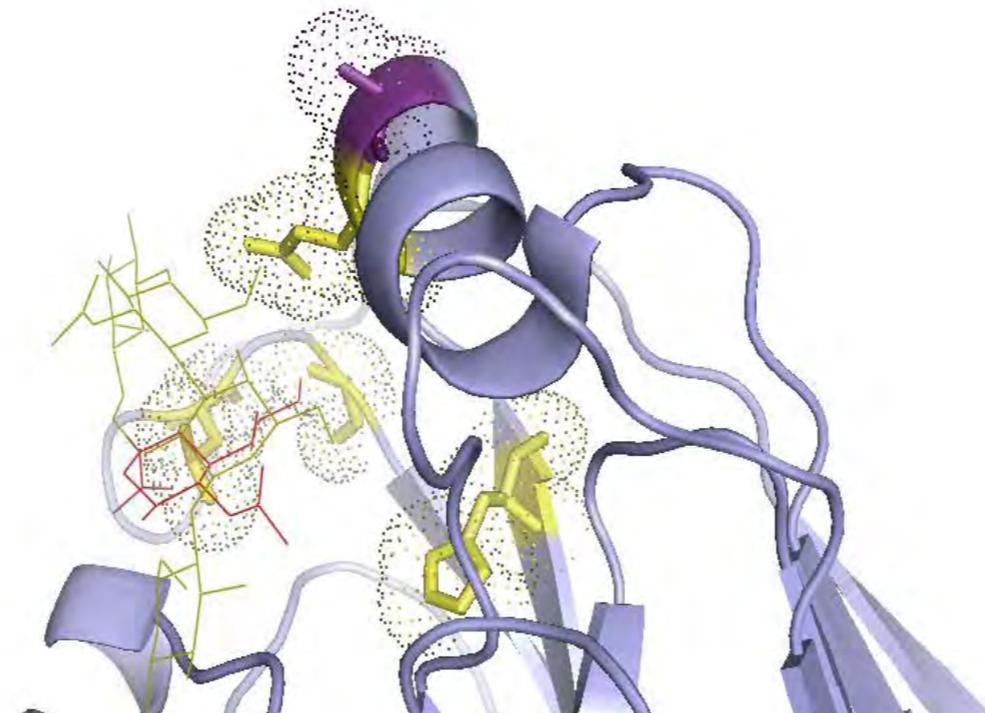
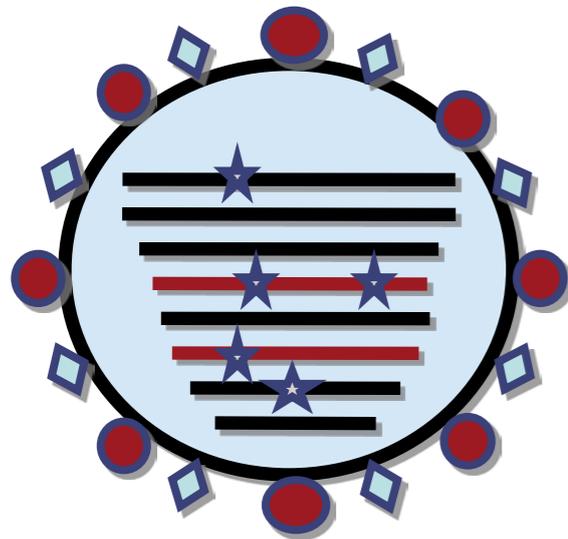
Position T189A: RBS and antigenic profile

2WF10:6M98



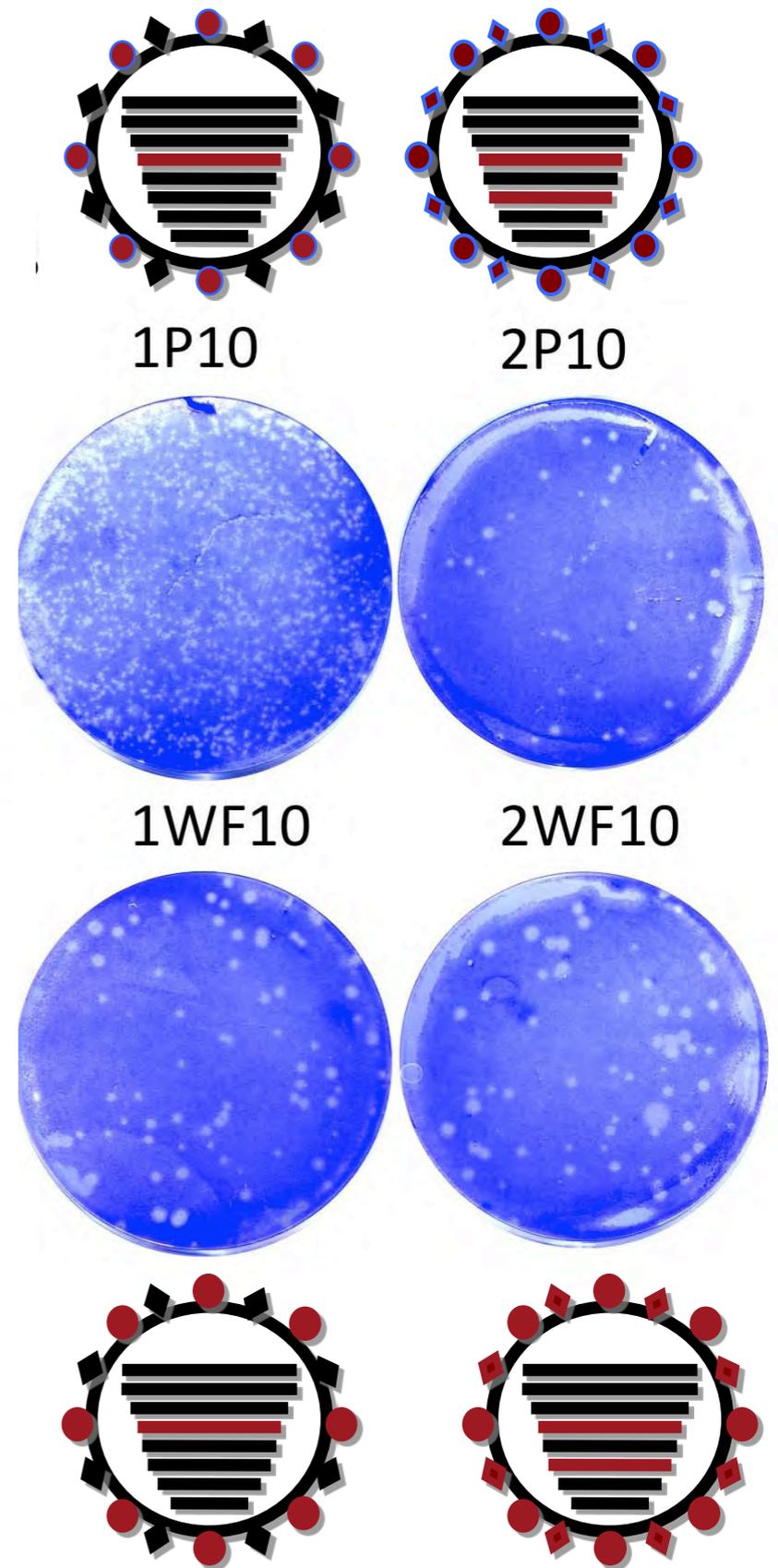
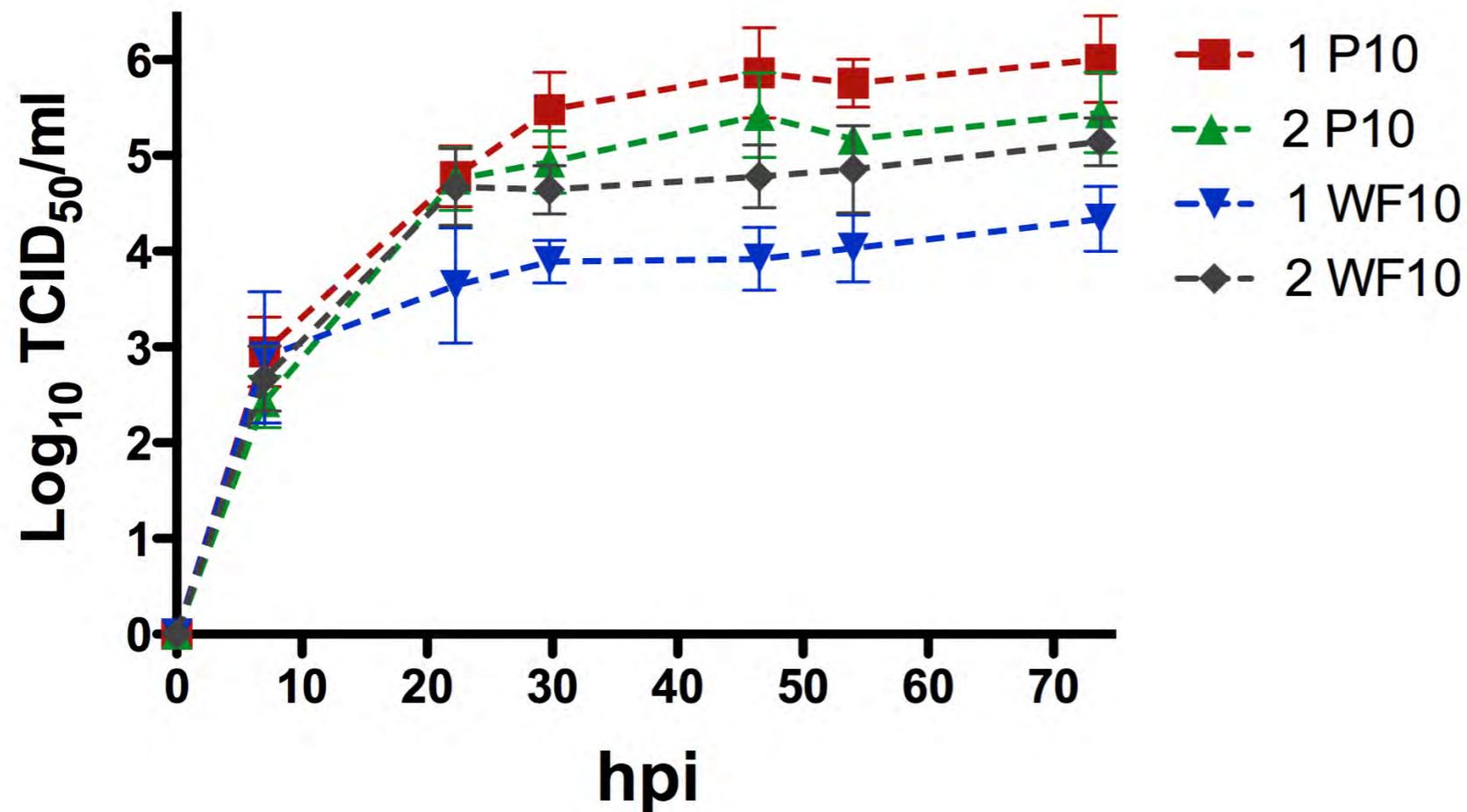
← Threonine

P10-2WF10:6M98



← Alanine

What are the chances of respiratory transmission of an H9 virus in the context of H1N1pdm genes?



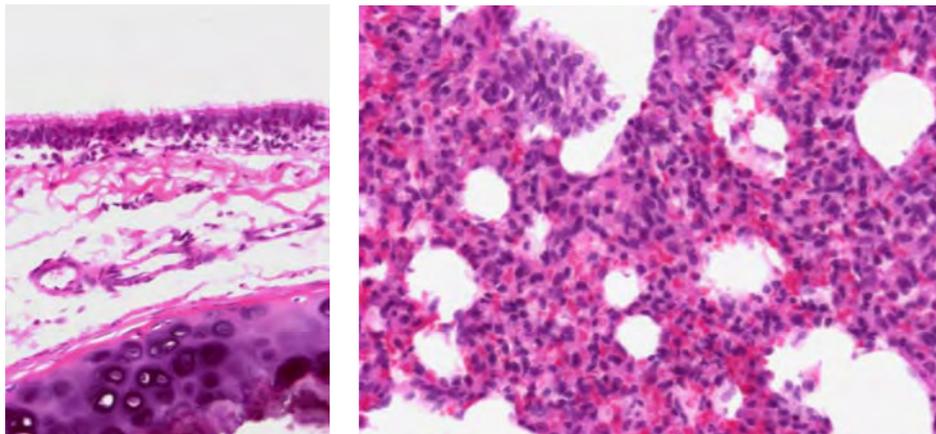
Ferrets infected with H9:pdm reassortants show signs of disease similar to infections with seasonal influenza

P10				2P10		
Ferret	% wt loss	St Dev		Ferret	% wt loss	St Dev
Infected	5.976	±0.592		Infected	8.279	±0.902
DC	5.877	±2.654		DC	2.947	±0.336
RC	6.041	±1.652		RC	6.497	±2.523
WF10				2WF10		
Infected	3.708	±0.479		Infected	4.298	±2.388
DC	6.340	±6.788		DC	5.406	±0.856
RC	--*	--*		RC	4.647	±2.796

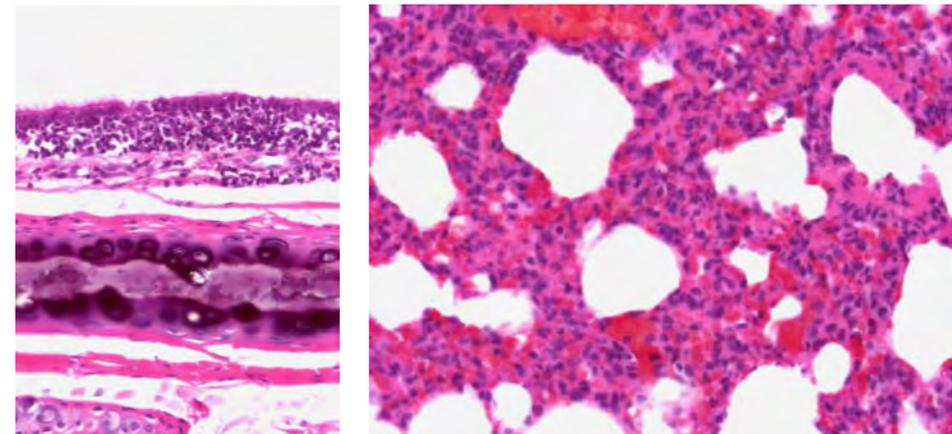
* indicates no weight loss seen.

Ferrets infected with H9:pdm reassortants show signs of disease similar to infections with seasonal influenza

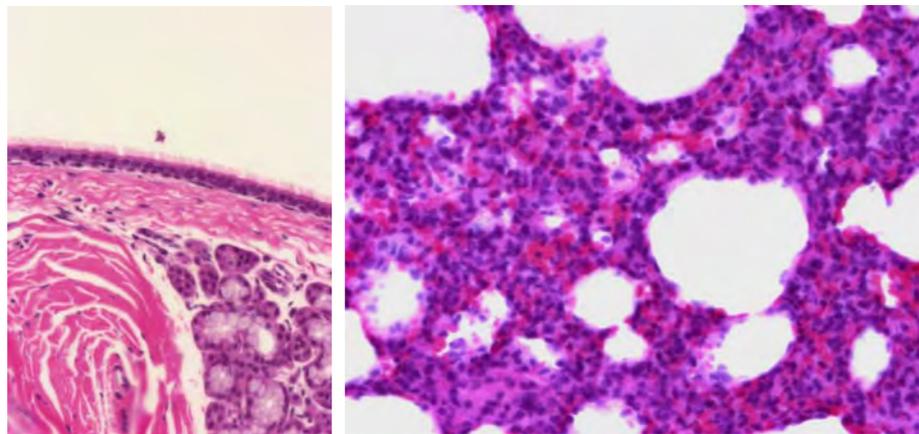
P10



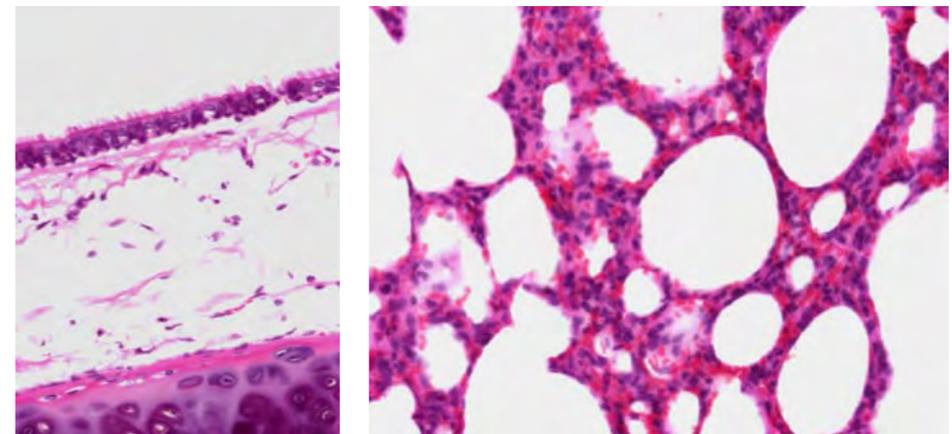
2P10



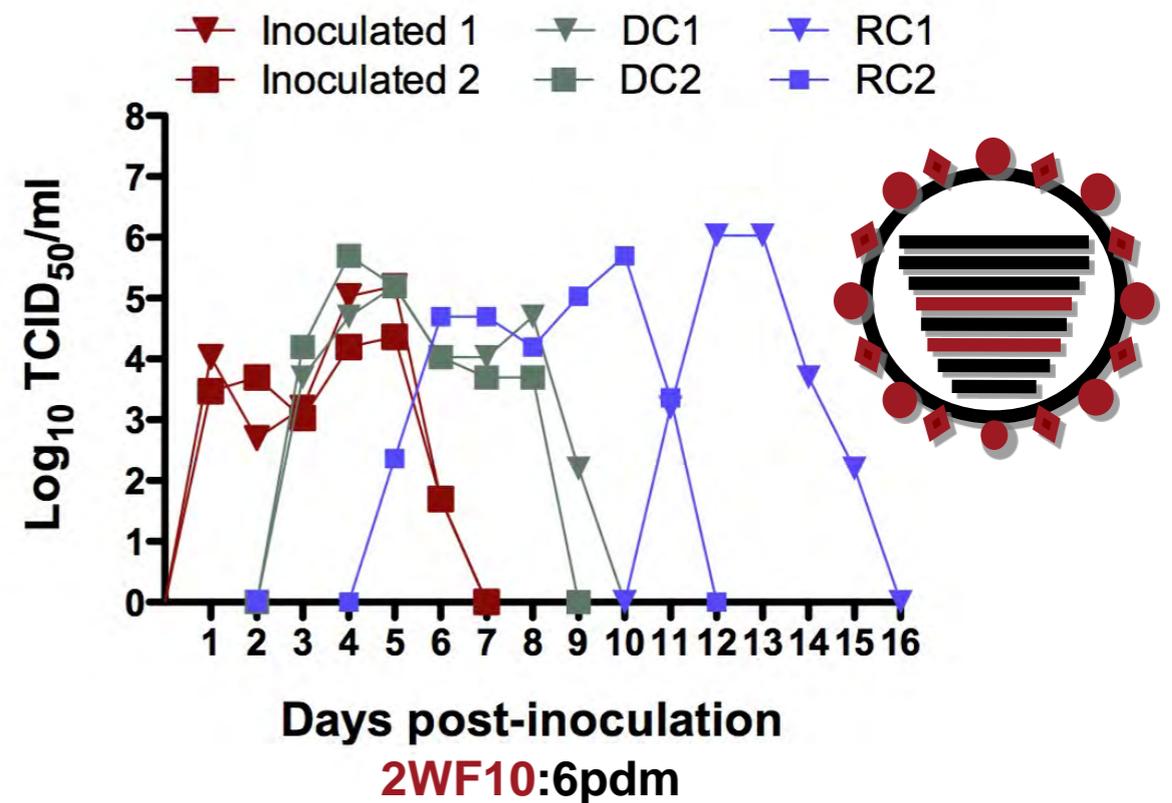
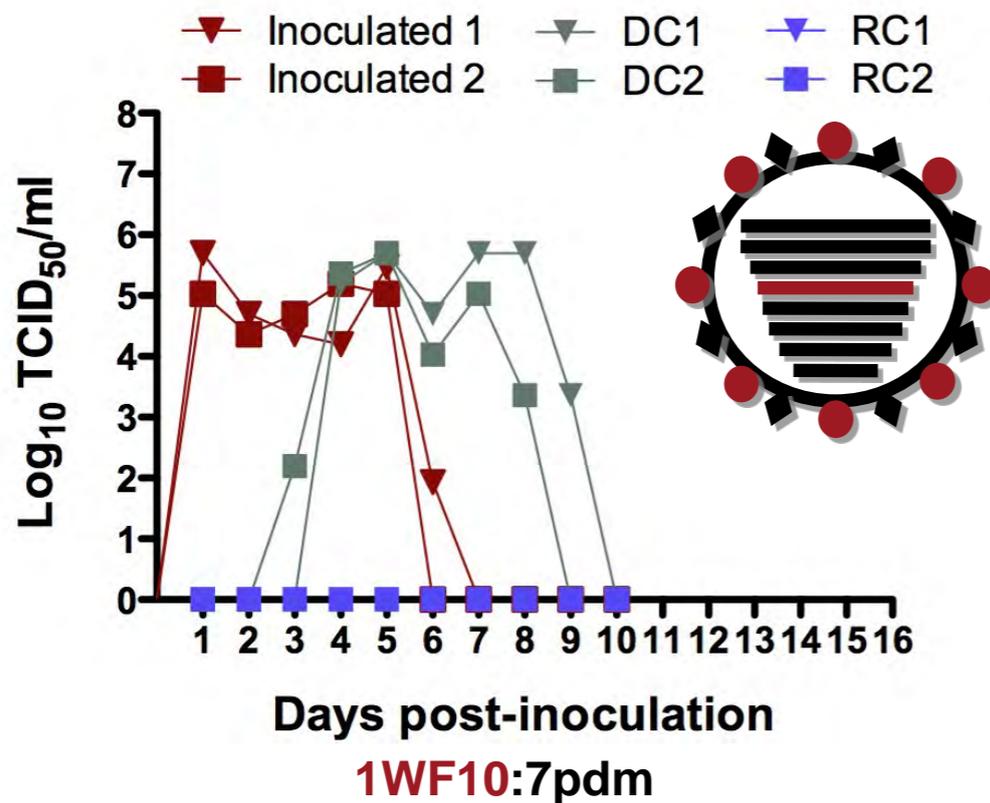
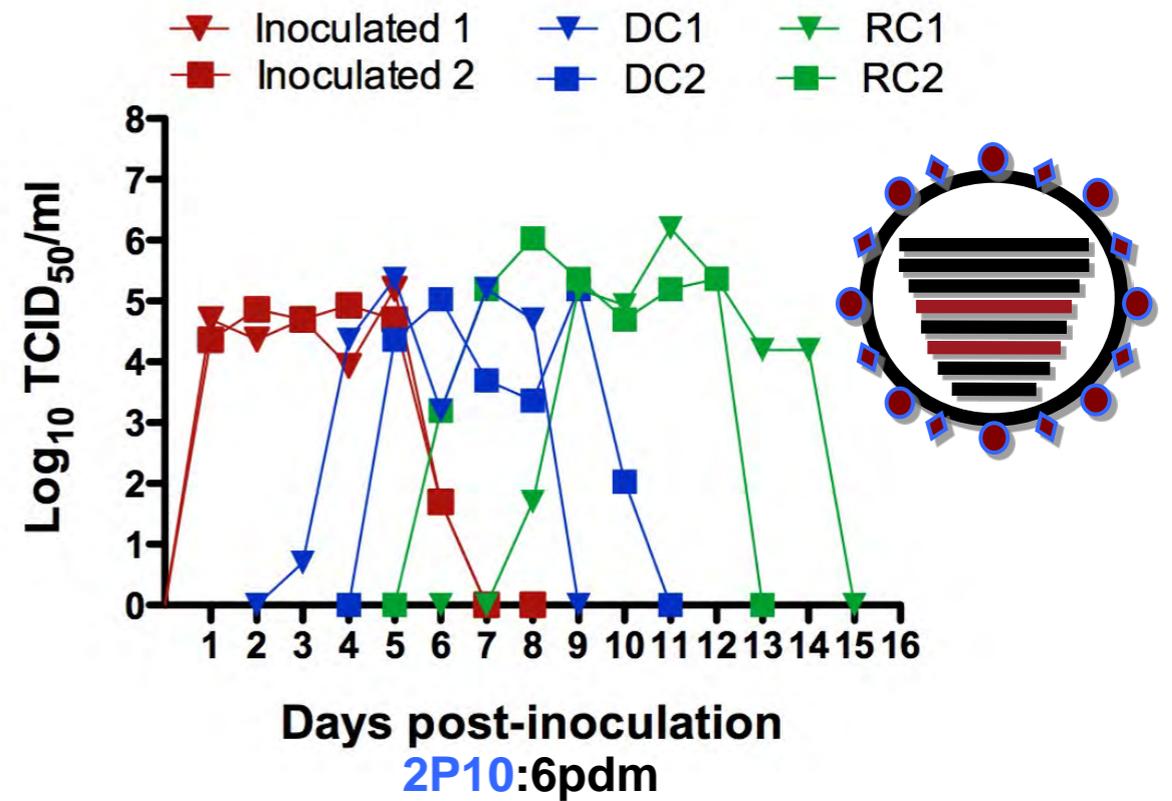
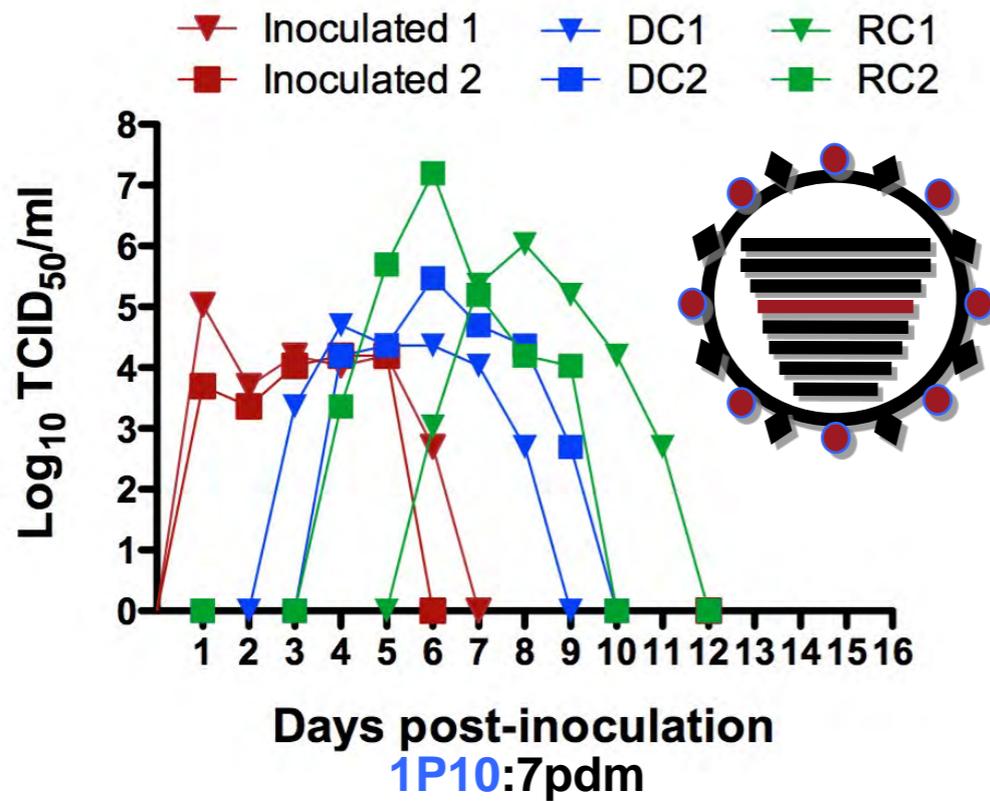
WF10



2WF10



H9N2:H1N1pdm reassortants can be transmitted by respiratory droplets in ferrets



SUMMARY

- Contemporary H9N2 avian influenza viruses with human-like receptor specificity infect mostly nonciliated cells in cultures of human airway epithelium (HAE).
- **L226** in the HA of H9N2 viruses favors infection of nonciliated cells in HAE cultures with less cytopathic effect than a typical H3N2 human influenza virus.
- **L226** containing H9N2 viruses show efficient direct - but not respiratory droplet - transmission in ferrets. No signs of disease.
- An avian/human H9N2 reassortant shows improved replication in ferrets with no detectable respiratory droplet transmission.
- Respiratory droplet transmission in ferrets of an avian/human H9N2 virus requires few amino acid changes with major implications in replication, virulence and antibody recognition profiling.
- H9 HA in the context of N2 or N1 NA is compatible with respiratory transmission when the internal genes are derived from the H1N1pdm virus

Discussion and Future Directions

- The transmission potential H9N2 viruses (in humans) and its mechanism remain largely under studied
- Giving the characteristics of receptor binding and the mild presentation of the H9N2 infections in humans (and animals), it is tempting to speculate that H9N2 human infections could be more frequent than what the epidemiological data suggests
- The replication and transmission characteristics of H9N2 strains needs to be further explored
- The epidemiological impact of H9N2 infections in humans needs further analysis

Immediate future steps

- Reassortment studies ongoing between various H9N2 strains and seasonal and H1N1pdm strains.
- Develop reagents (mAbs) to improve H9N2 detection in avian and mammalian species.

mAbs against HA of H9

Assay/clone	AIV-H9-HA-3G8 (IgG2a)	AIV-H9-HA-1E4 (IgG2a)	AIV-H9-HA-1E2 (IgG1)
IFA	+	+	+
Western blot	+	+	+
HI	+	+	+

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