UNIVERSITEIT GENT

FACULTY OF **VETERINARY MEDICINE**
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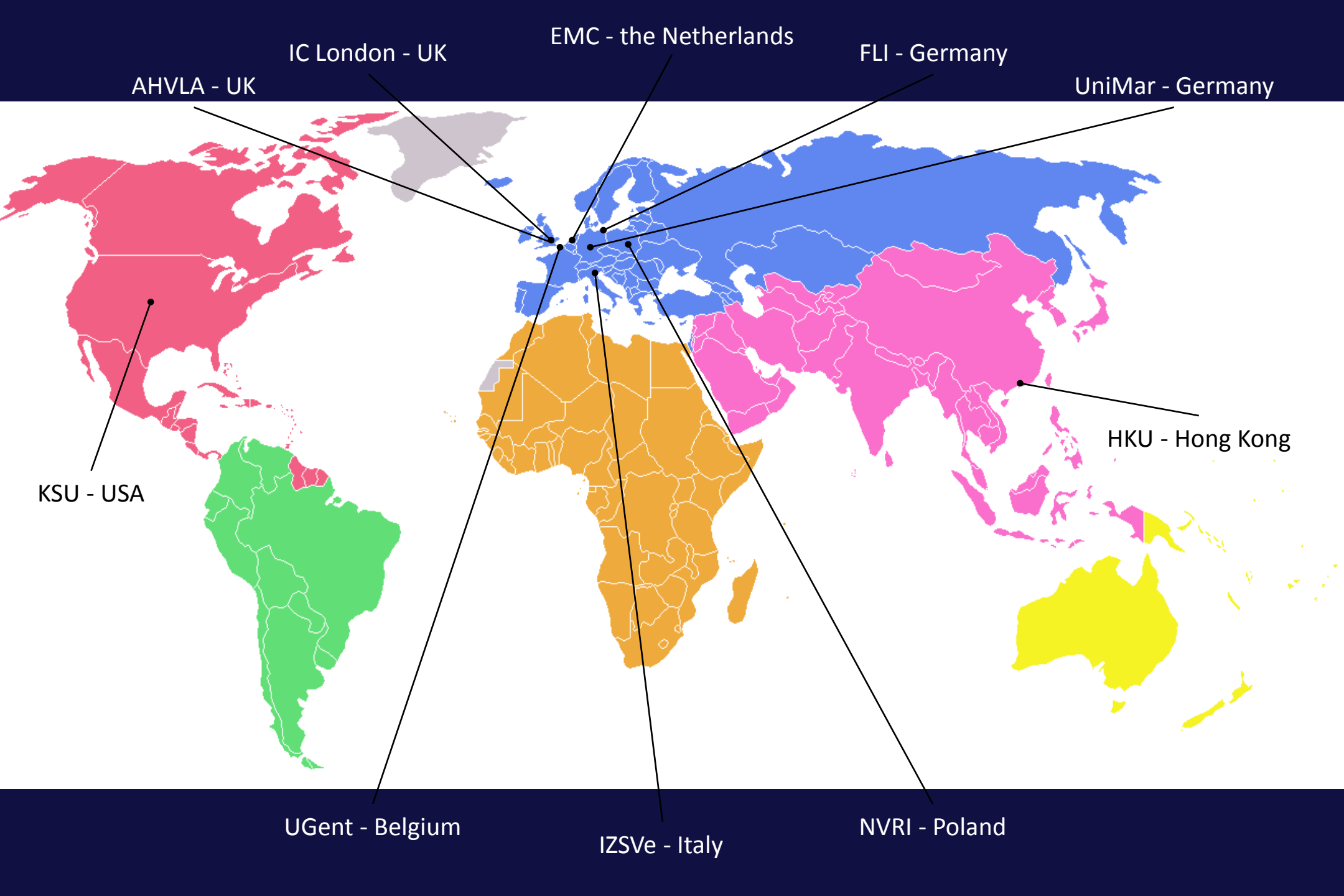
Swine influenza research in Europe: an introduction to “FLUPIG”

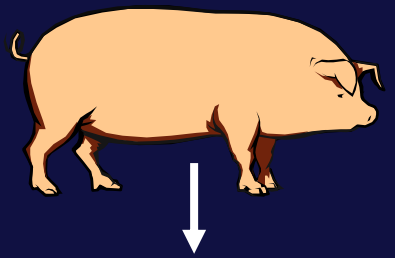
Prof. Kristien Van Reeth, Ghent University, Belgium

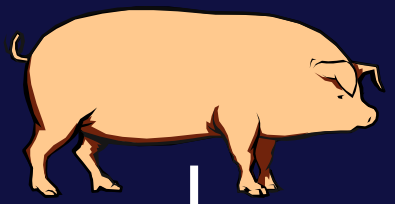
www.flupig.ugent.be

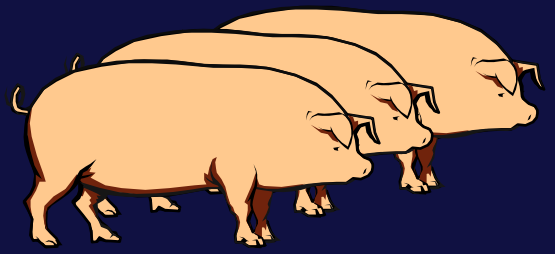
Pathogenesis and transmission of influenza in pigs

- Framework Program (FP) 7 project funded by the European Commission (approx. 5 million euros)
- 1st July 2010 - 31st December 2014
- 10 international partners


AHVLA - UK
IC London - UK
EMC - the Netherlands
FLI - Germany
UniMar - Germany
KSU - USA
HKU - Hong Kong
UGent - Belgium
IZSVe - Italy
NVRI - Poland







Swine-adapted viruses only occasionally infect people:

- Incidence unknown
- Mostly dead-end

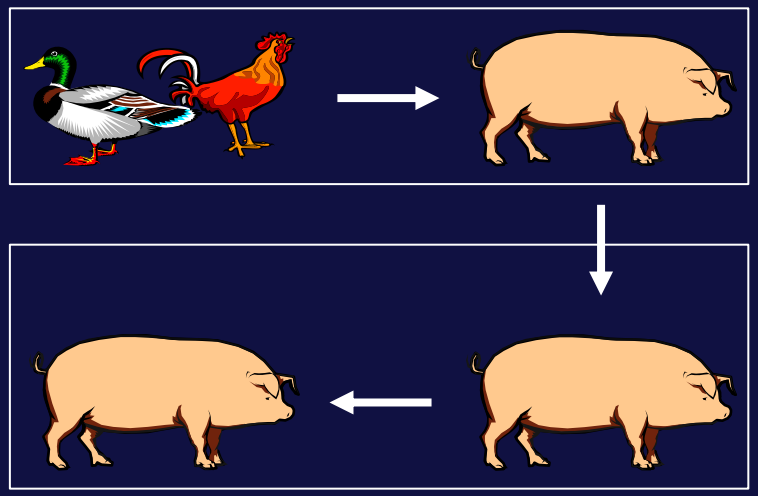
2009 pandemic H1N1 virus is an exception:

- Human-to-human transmission
- Underlying mechanisms largely unknown

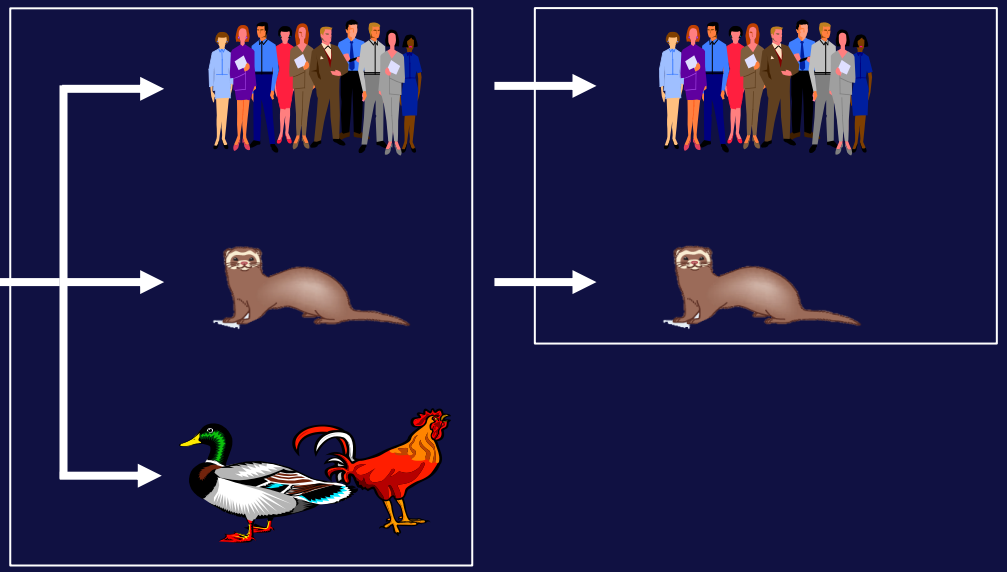
AIM OF FLUPIG

Gain insights into the role of pigs in the overall influenza ecology and specifically in the generation of human pandemic viruses

Q1. What makes avian influenza virus adapted to pigs?



Q2. between pigs?



What determines transmissibility?

Q3. from pigs to other relevant species?

Q4. between humans (ferret as a model)?

Viruses used in FLUPIG

avian H9N2

quail/Hong Kong/G1/97

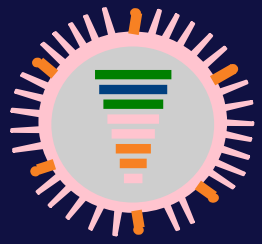
may potentially adapt to swine and/or humans (?)

avian H1N1

duck/Bavaria/77

precursor of European H1N1 SIV lineage (avian-like H1N1)

2009 pH1N1

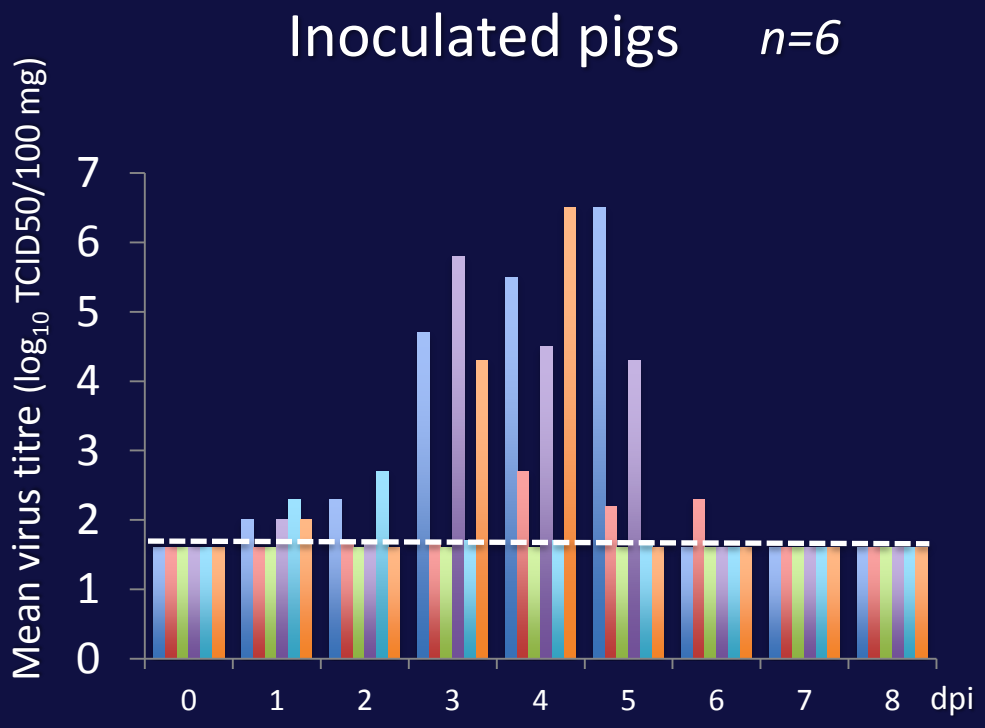


A/Regensburg/09

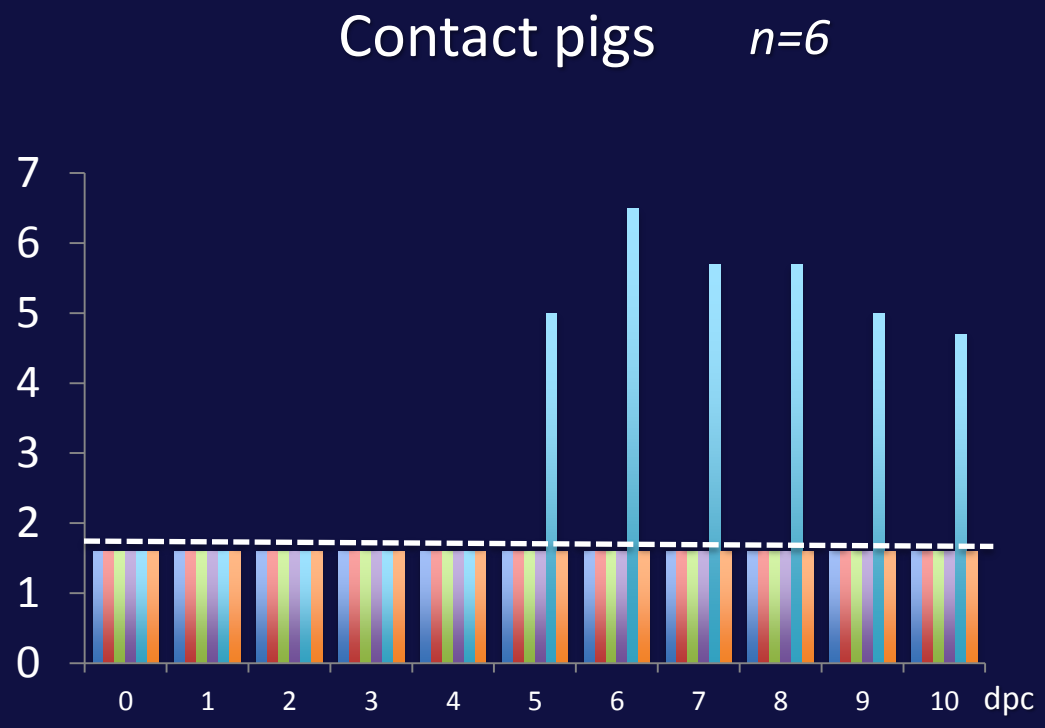
swine-origin pandemic influenza virus

H9N2* nasal excretion in pigs

*quail/Hong Kong/G1/97



5/6 pigs with seroconversion

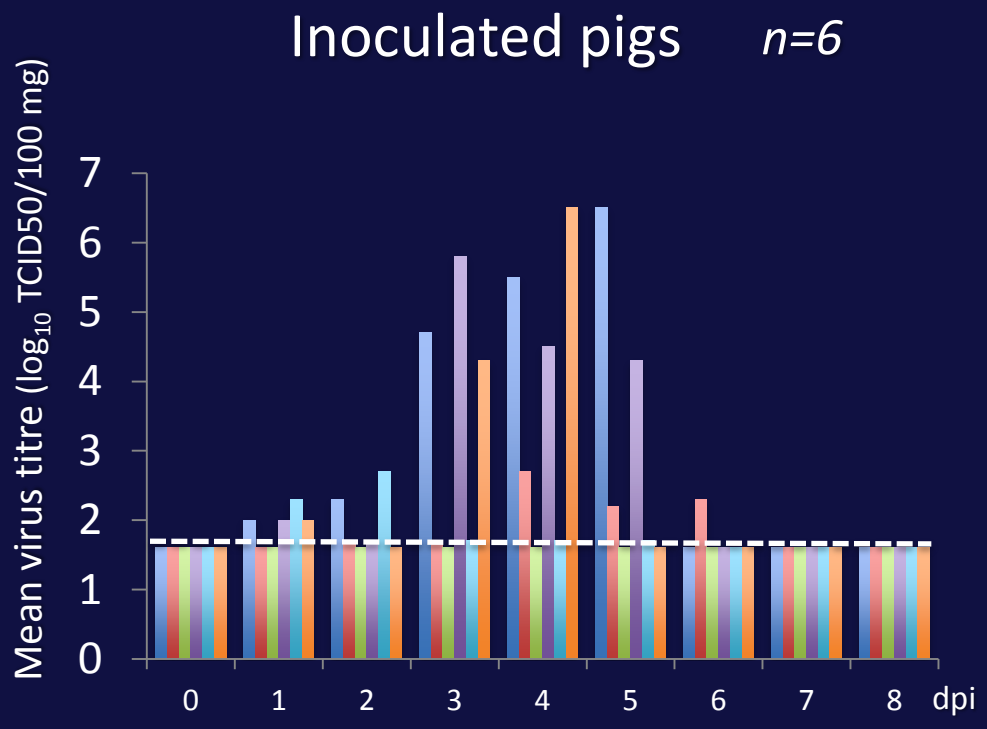


4/6 pigs with seroconversion

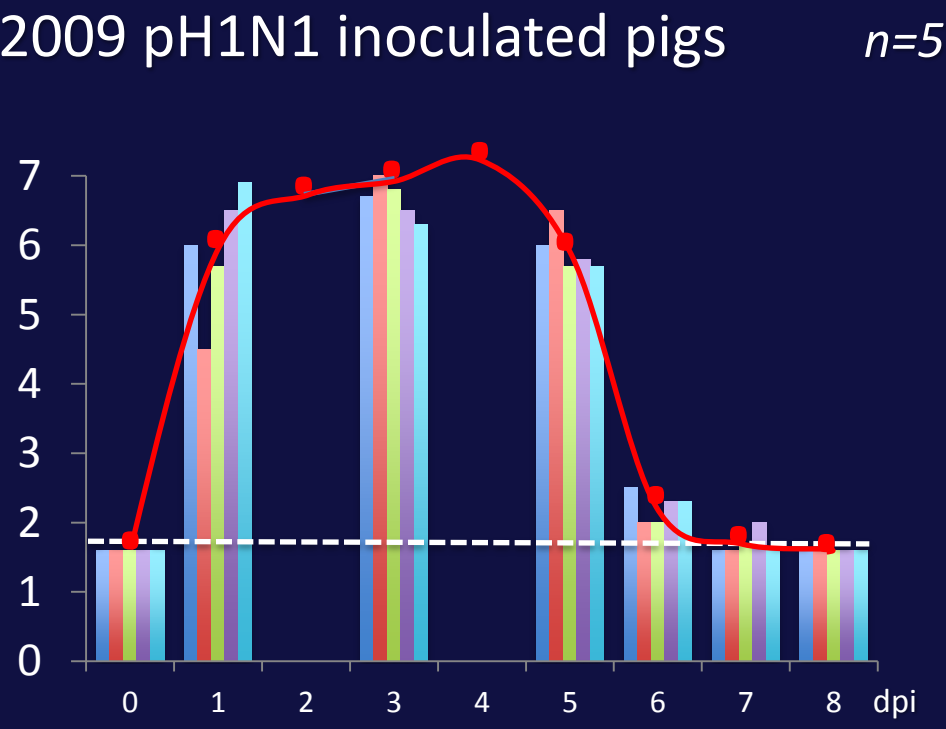
- ✓ Most directly inoculated pigs shed virus, variable duration and titers
- ✓ Transmission to contact pigs is inefficient

Van Reeth et al., unpublished data

H9N2 nasal excretion in pigs



5/6 pigs with seroconversion



- ✓ Most directly inoculated pigs shed virus, variable duration and titers
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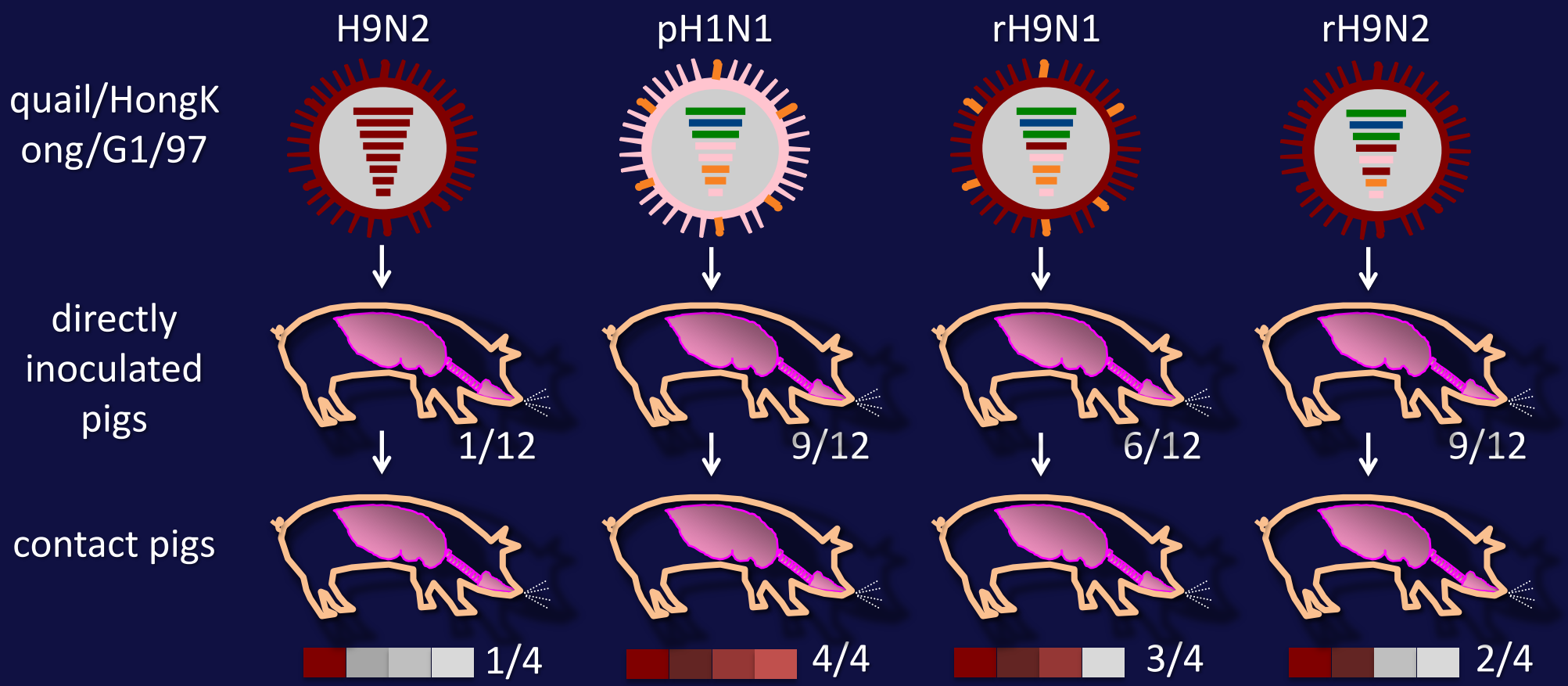
Van Reeth et al., unpublished data

Reassortants H9 x 2009 pH1N1

Qiao, Ma et al. JGVi 2012

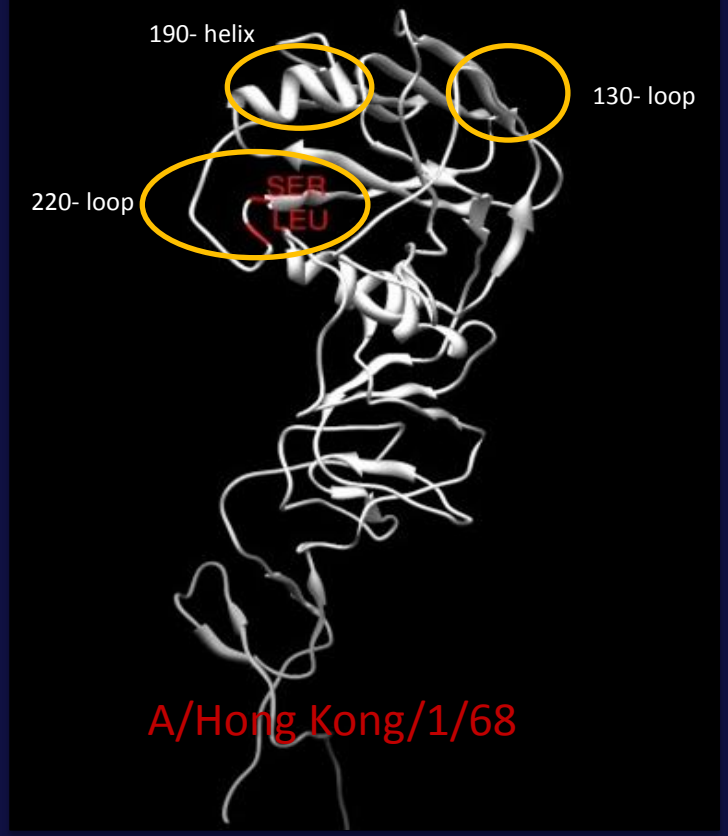


Reassortant H9 viruses tend to show enhanced replication and transmissibility

A/Hong Kong/ 1/68 (H3N2) and mutants

Mikhail Matrosovich – Philipps University Marburg, Sjouke Van Poucke - UGent

R1-HK



A/Hong Kong/1/68

R2-HK

2 avian-like mutations in RBS:
Q226L, G228S

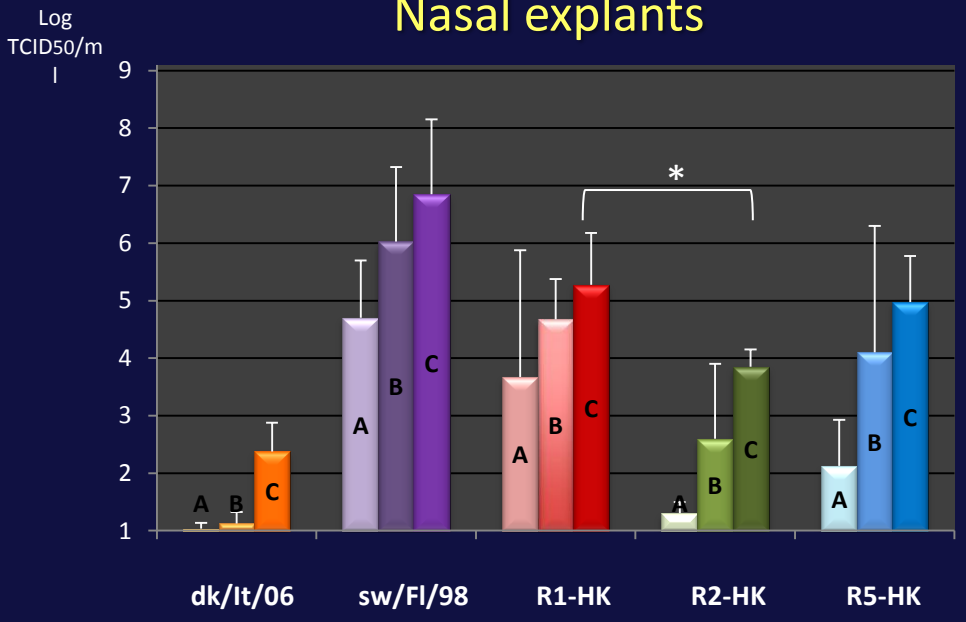
R5-HK

5 additional mutations

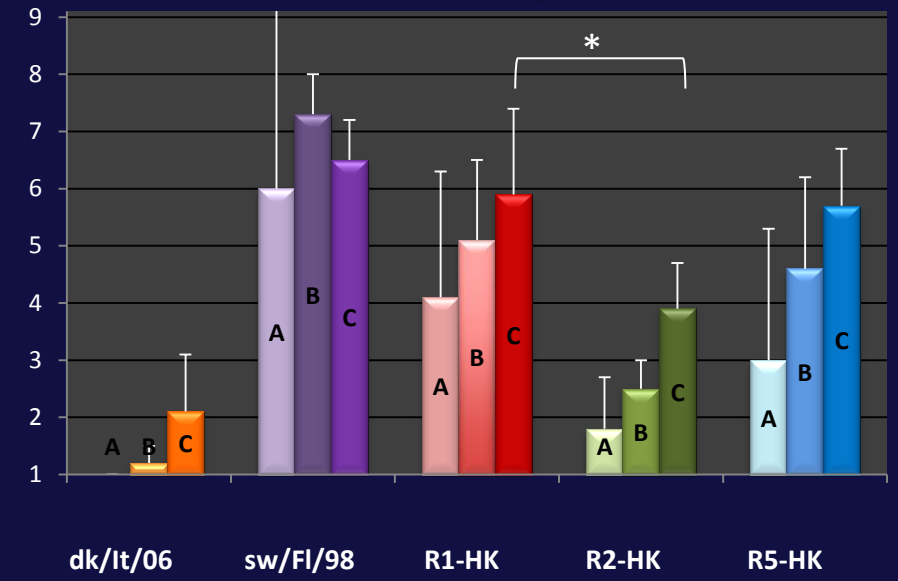
Will mutations affect: (1) replication in porcine respiratory explants;
(2) replication and organ tropism in pigs; (3) pig-to-pig transmission

Virus replication in porcine respiratory explants 48 hpi

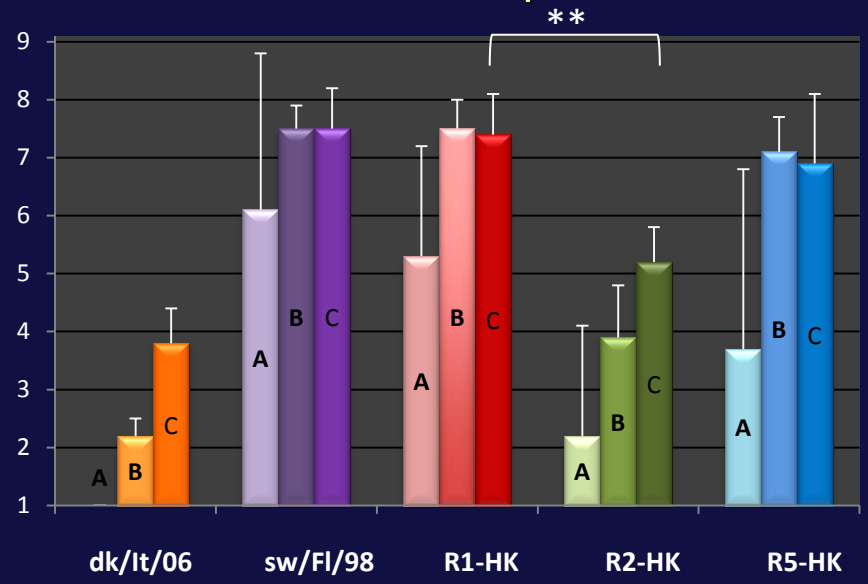
Nasal explants



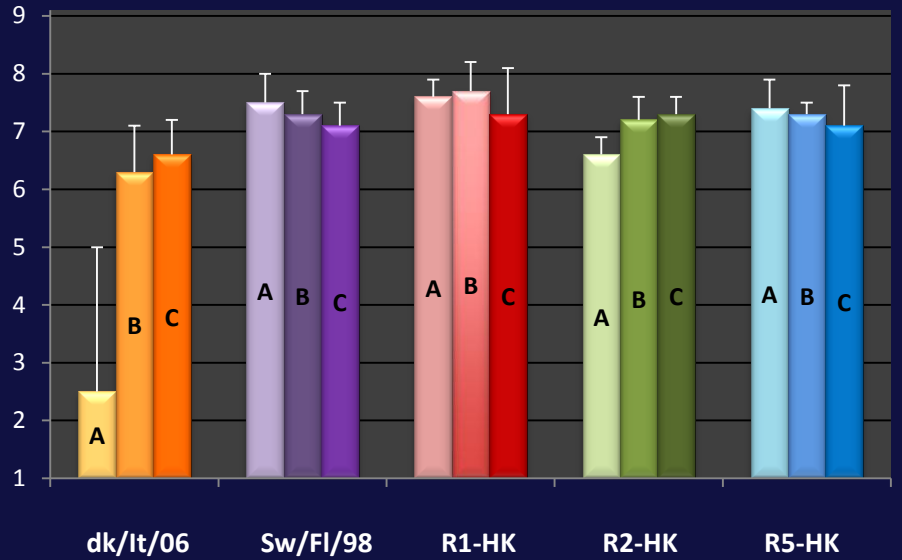
Tracheal explants



Bronchial explants

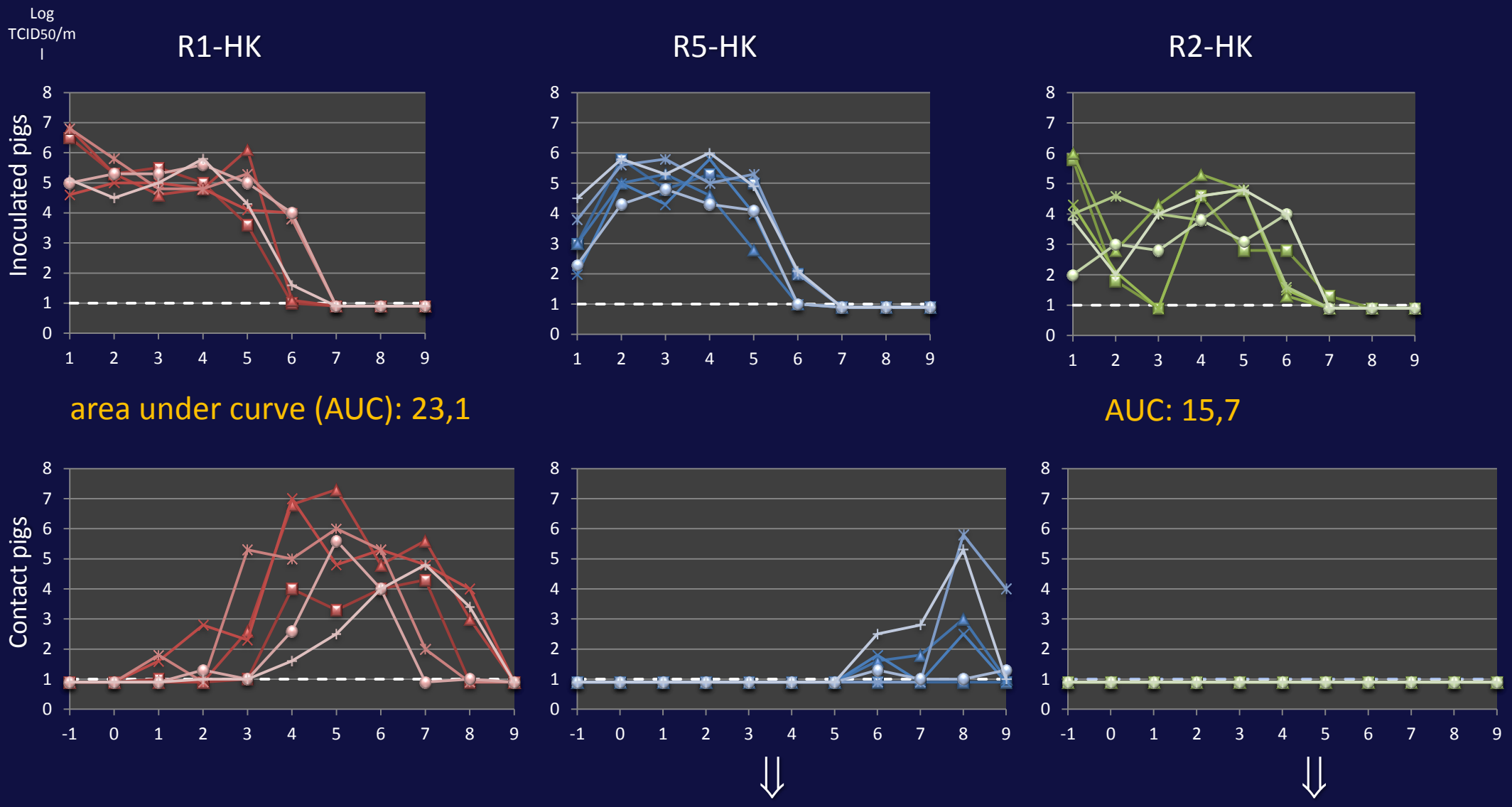


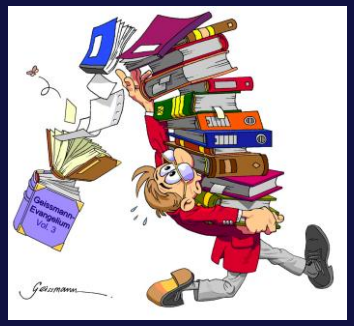
Lung explants



A: 104PFU
B: 105 PFU
C: 106PFU

Transmission: nasal virus shedding

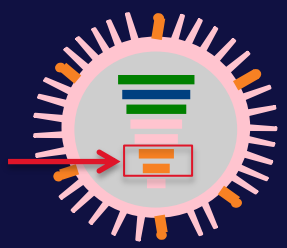
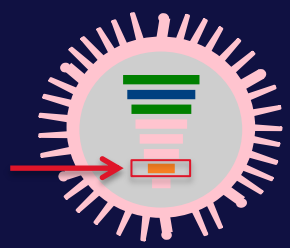
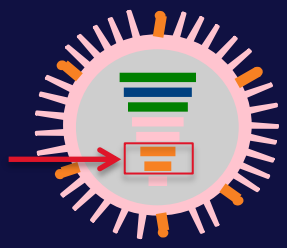
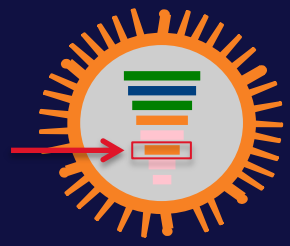




Summary

- Both Sia a2-3 and a2-6 receptors found in porcine respiratory tract, but a2-3 mainly in lungs (cf humans)
- Wholly avian viruses replicate poorly in nasal or tracheal explants, but acceptably in lung explants
- Human-like receptor binding tropism is required for virus transmission between pigs, but not sufficient
- Hong Kong/68 and mutant with avian-like RBS have a similar cell tropism in porcine respiratory tract, but avianized mutant replicates to lower titers

Studies in *ex vivo* cultures of the human respiratory tract: endemic SIVs do not replicate in URT

	2009 pH1N1	Reassortant SIV, Eurasian M	⇒ Rg virus 2009 pNA	Reassortant SIV, 2009pNA
				
Nasopharynx	+	+	Not tested	-
Bronchus	++	++	++	-
Lung	++	-	+	+
				
	3/3	1/5	3/3	0/3

Chan et al. J Vi 2011, Yen et al. PNAS 2011

AIMS OF FLUPIG

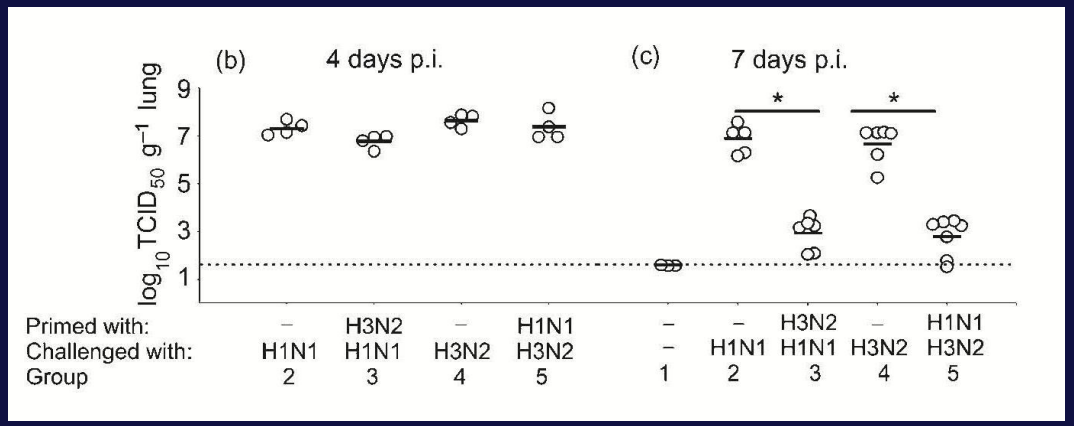
Gain insights into the role of pigs in the overall influenza ecology and specifically in the generation of human pandemic viruses

Cross-protection between influenza viruses and its effect on the emergence and severity of pandemics:

- Extent of heterovariant/heterosubtypic protection in pigs and in other animal models
- Immune mechanisms involved

Cross-protective immunity against influenza pH1N1 2009 viruses induced by seasonal influenza A (H3N2) viruses is mediated by virus-specific T cells

M. Hillaire, …, A. Osterhaus, G. Rimmelzwaan
J Gen Virol 2011

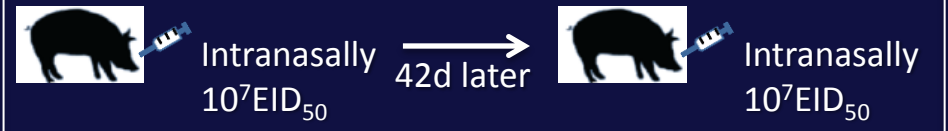


Cross-protection between 2009 pandemic H1N1 and European H1 SIVs

	Gene origin		Relationship to pH1N1	
	HA	NA	% of aa identity in HAI	% of aa Identity in NA
H1N1 (sw/Gent/28/10)			72	92
rH1N1 (sw/CA/46/08)			70	91
H1N2 (sw/Gent/26/12)			68	42

aa: amino acid

Experimental design



Group	Priming virus	Challenge virus
1	pH1N1	pH1N1
2	pH1N1	H1N1
3	-	H1N1
4	pH1N1	rH1N1
5	-	rH1N1
6	pH1N1	H1N2
7	-	H1N2

Nasal excretion of challenge virus

N. positive pigs	Duration (days)
0	0
0	0
5	5-6
3	1
5	5-6
4	1-3
5	5-6

Complete or partial protection in pigs pre-inoculated with pH1N1

t's likely impossible to accurately predict which animal influenza
iruses will cause a pandemic BUT we can gain better insights -
Thanks to the European Commission