UNIVERSITEIT GENT

FACULTY OF VETERINARY MEDICINE
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# Swine influenza research in Europe in 2015: an update

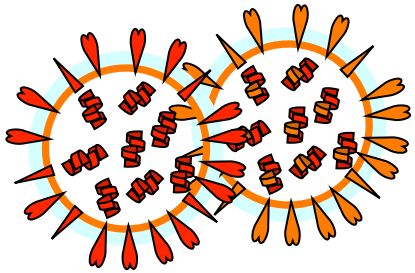
Prof. Kristien Van Reeth, Ghent University, Belgium
Jose Carlos Mancera Gracia, Ghent University, Belgium

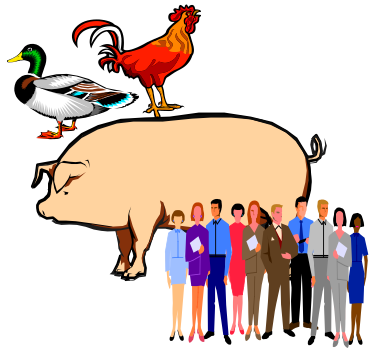
# Pathogenesis and transmission of influenza in pigs

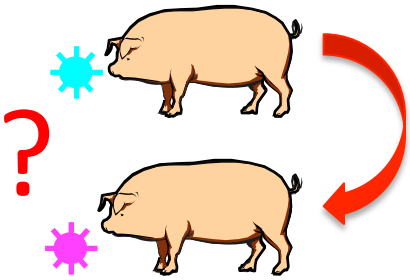

*www.flupig.ugent.be*

- Framework Program (FP) 7 project funded by the European Commission (5 million euros)
- 1st July 2010 - 31st December 2014
- 10 international partners: UGent (Belgium), IZSVe (Italy), IC London (UK), AHVLA (UK), UniMar (Germany), EMC (the Netherlands), NVRI (Poland), HKU (Hong Kong), KSU (USA), FLI (Germany)

# FLUPIG aims








<u>Aim 1</u>: Role of pigs in the generation of pandemic influenza viruses for humans – What makes avian influenza viruses adapted to pigs?

<u>Aim 2</u>:

Extent of cross-protection between different influenza virus subtypes and lineages, underlying immune mechanisms

Broadly protective vaccines

# Aim 2: cross-immunity and protection

Qiu *et al. Veterinary Research* (2015) 46:105
DOI 10.1186/s13567-015-0236-6

VETERINARY RESEARCH

RESEARCH ARTICLE Open Access

## Cross-protection against European swine influenza viruses in the context of infection immunity against the 2009 pandemic H1N1 virus: studies in the pig model of influenza

Yu Qiu, Karl De hert and Kristien Van Reeth*

# Aim 2: cross-immunity and protection

*Journal of General Virology* (2014), 95, 948–959 DOI 10.1099/vir.0.059253-0

## Immunization of pigs with an attenuated pseudorabies virus recombinant expressing the haemagglutinin of pandemic swine origin H1N1 influenza A virus

Katharina Klingbeil,[1] Elke Lange,[2] Jens P. Teifke,[2] Thomas C. Mettenleiter[1] and Walter Fuchs[1]

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Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres


## Protection of pigs against pandemic swine origin H1N1 influenza A virus infection by hemagglutinin- or neuraminidase-expressing attenuated pseudorabies virus recombinants

Katharina Klingbeil[a], Elke Lange[b], Ulrike Blohm[c], Jens P. Teifke[b], Thomas C. Mettenleiter[a], Walter Fuchs[a,*]

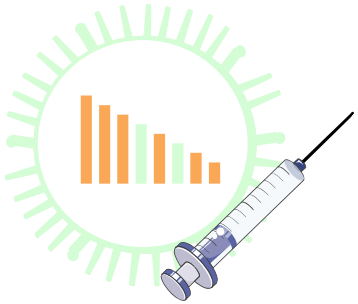
# Aim 2: cross-immunity and protection

## Heterologous prime-bost vaccination with inactivated H3N2 SIVs

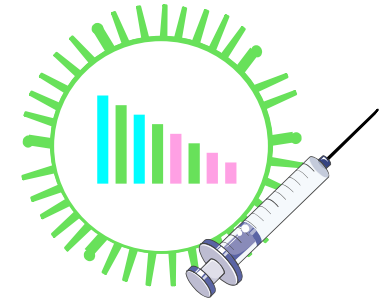
***Van Reeth et al. unpublished***

Sw/Gent/172/08 (Eu)

Sw/Pennsylv/10 (US)



only 80.2% amino acid identity in HA1



1st vaccination

– 4 wk interval –

2nd vaccination

- ✓ Homologous prime-boost fails to induce antibodies and protection against other lineage
- ✓ Heterologous prime-boost induces high serum antibody titers (HI, VN, NI) against both lineages and protection against challenge

# Aim 1: Adaptation of avian (potentially pandemic) viruses to pigs - H3N2

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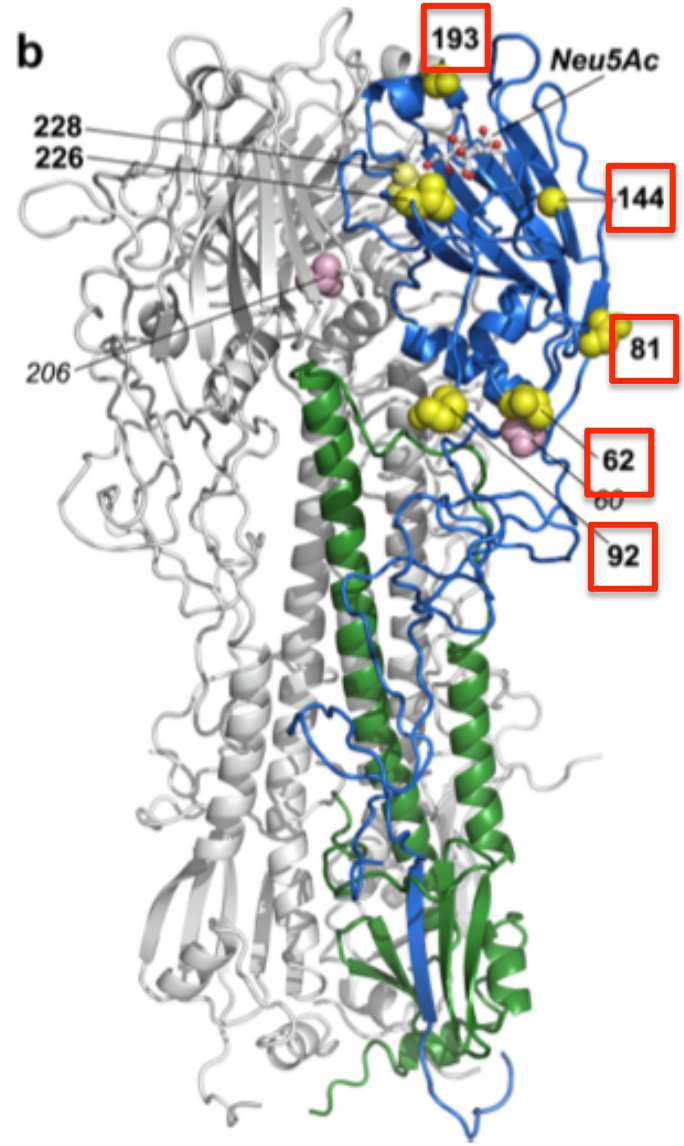
## Role of Substitutions in the Hemagglutinin in the Emergence of the 1968 Pandemic Influenza Virus

Sjouke Van Poucke,[a*] Jennifer Doedt,[b*] Jan Baumann,[b] Yu Qiu,[a] Tatyana Matrosovich,[b] Hans-Dieter Klenk,[b] Kristien Van Reeth,[a] Mikhail Matrosovich[b]

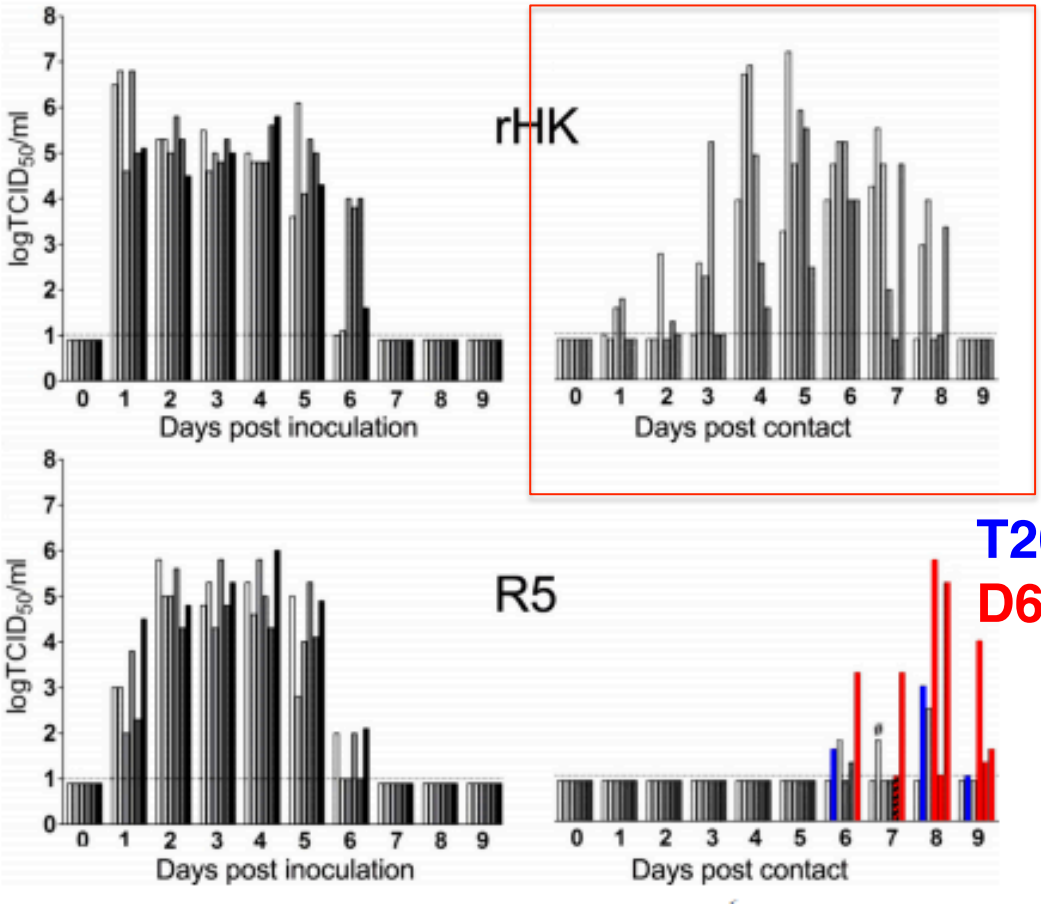
Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium[a]; Institute of Virology, Philipps University, Marburg, Germany[b]

# 1968 pandemic (H3N2) HA1 differs from avian precursor in 2 aa in RBS (226, 228) and 5 other aa

| | Amino acid in HA | | | | | | |
|---|---|---|---|---|---|---|---|
| | 62 | 81 | 92 | 144 | 193 | 226 | 228 |
| 1968 pandemic virus | I | N | K | G | S | L | S |
| Supposed avian precursor | R | D | N | A | N | Q | G |
| rHK | - | - | - | - | - | - | - |
| R5 | R | D | N | A | N | - | - |



# Nasal shedding of directly inoculated and contact pigs



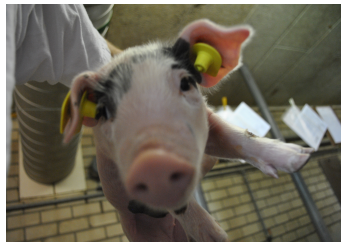

- Inefficient transmission of R5 mutant
- 5 out of 6 contact pigs had one additional mutation in HA1
- Lower R5 yields in human tracheobronchial epithelial cells

At least some of the 5 aa mutations (62, 81, 92, 144 and 193 of HA1) could have played a role in bird-to-human adaptation, next to Q226L and G228S

# Aim 1: Adaptation of avian (potentially pandemic) viruses to pigs – H9N2

- Endemic in Eurasian land-based poultry since mid 90's *(Zhou et al. 2012)*

- Sporadic isolation from pigs and humans in Asia since late 90's:
  - ✓ no sustained transmission
  - ✓ G1-like and Y280 lineages possess human-like receptor specificity (Q226L mutation in HA)



*(Guo et al. 1999; Peiris et al. 1999, 2001; Matrosovich et al. 2001; Xu et al. 2004; Butt et al. 2010; Cong et al. 2007; Wan et al. 2007; Shi et al. 2008; Yu et al. 2008; Cheng et al. 2011)*

# H9N2 virus: transmission experiments in mammals

- H9N2 isolates containing L226 replicated in pigs and ferrets but transmission was not efficient

  *(Wan et al. 2008; Qiao et al. 2012; SJCEIRS group 2013)*

- Reassortant viruses containing H9N2 surface proteins and mammalian adapted internal genes showed higher replication and transmission in pigs and ferrets,
  but transmission remains less efficient than with mammalian-adapted viruses

  *(Sorrell et al. 2009; Kimble et al. 2011; Quiao et al. 2012; Obadan et al. 2015)*

# Adaptation of H9N2 to pigs: approach

1. Serial passages in swine
   - Wholly avian H9N2 (A/quail/Hong Kong/G1/1997 *G1-like*)
     (A/Qa/HK/P0)
   - Reassortant H9N2 x 2009 pH1N1 internal genes
     A/quail/HK/G1/97 HA and NA
     A/California/04/09 internal genes
     } (H9N2/CA09/P0)
2. Comparative transmission studies

   Parental viruses versus pig-passaged virus with highest replication efficiency

3. Genetic analysis

# Serial passages: experimental design

Intranasal Inoculation

Pig 1 Pig 2

Passage 1

0 1 2 3 4 DPI

Nasal swabs √ √ √ √ √

Respiratory tissues √

Intranasal inoculation with homogenate of nasal mucosa

Pig 1 Pig 2

Passage 2

0 1 2 3 4 DPI

Nasal swabs √ √ √ √ √

Respiratory tissues √

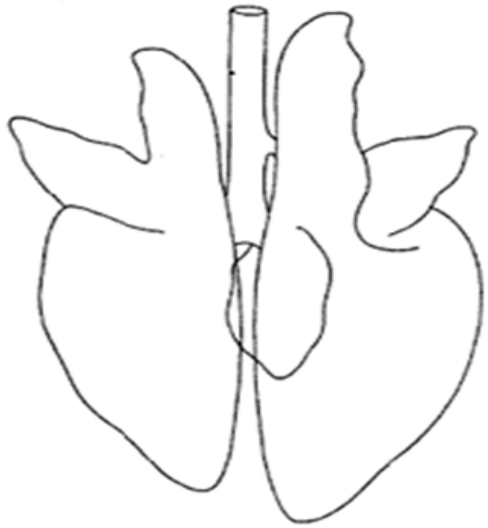
... (until passage 10)

# Serial passages: H9N2 replication efficiency

**Upper respiratory tract**

- Nasal mucosa (respiratory part)
- Nasal mucosa (olfactory part)

| Virus | % of positive samples |
|---|---|
| A/Qa/HK/P0 | 100 |
| A/Qa/HK/P4 | 100 |
| H9N2/Ca09/P0 | 100 |
| H9N2/Ca09/P7 | 100 |



**Lower respiratory tract**

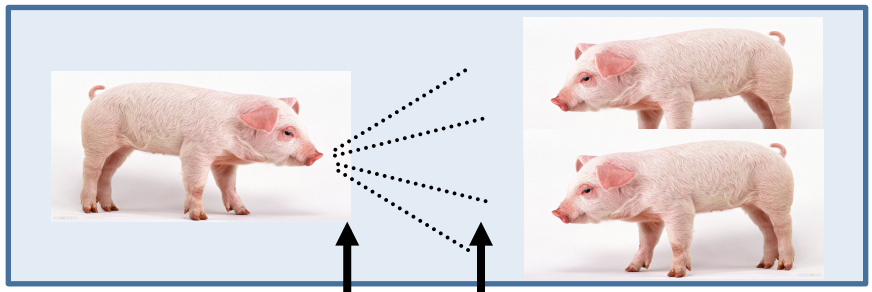
- Distal and proximal trachea
- Samples of all 7 lung lobes (apical and cardiac lobes were collected together)

| Virus | % of positive samples |
|---|---|
| A/Qa/HK/P0 | 50 |
| A/Qa/HK/P4 | 100 |
| H9N2/Ca09/P0 | 0 |
| H9N2/Ca09/P7 | 93 |

Starting material and passages with consistent replication in the complete respiratory tract were selected for transmission experiments

# Transmission studies: experimental design

**1 Directly inoculated pig** **2 Direct contact pigs (2dpi)**



**3x isolators** (n=9)

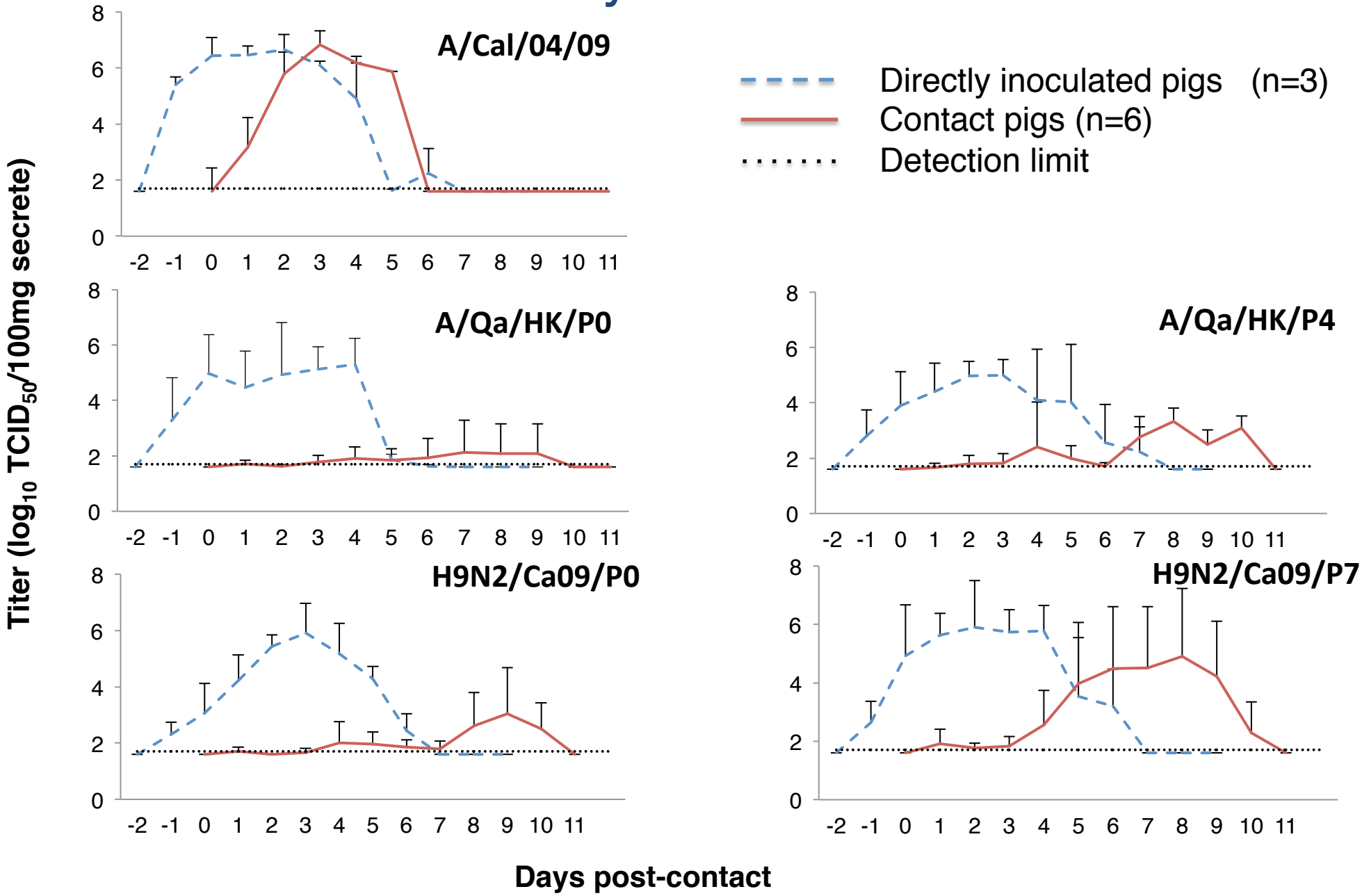
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | .... | 23 | 30 | DPI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Nasal swabs** | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | | | | | | | | | |
| **Serum** | √ | | | | | | | | | | | | | | | | √ | | √ | √ | |

| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | .... | 21 | 28 | DPC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Nasal swabs** | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | | | | | | | |
| **Serum** | √ | | | | | | | | | | | | | | √ | | √ | √ | |

**Viruses used in transmission experiments:**

- A/quail/Hong Kong/G1/97 (H9N2) parental: Original virus (A/Qa/HK/P0)
- A/quail/Hong Kong/G1/97 (H9N2) (P4): “pig-adapted” virus (A/Qa/HK/P4)
- Reassortant H9N2:2009 pH1N1: Original virus (H9N2/Ca09/P0)
- Reassortant H9N2:2009 pH1N1 (P7): “pig adapted”virus (H9N2/Ca09/P7)

# Transmissibilty of H9N2 viruses



# Transmissibility of H9N2 viruses

| Virus | Mean AUC of | | Nr of pigs with VN antibodies at week 4 (titer range) | |
|---|---|---|---|---|
| | Directly inoculated pigs | Contact pigs | Directly inoculated pigs | Contact pigs |
| A/Cal/04/09 | 26,6 | 19,7 | 3/3 (512-768) | 6/6 (512-1024) |
| A/Qa/HK/P0 | 18,4 | 3,0 | 3/3 (6-12) | 2/6 (<2-24) |
| A/Qa/HK/P4 | 19,1 | 6,5 | 3/3 (48-128) | 5/6 (<2-12) |
| H9N2/Ca09/P0 | 19,4 | 4,7 | 3/3 (32-192) | 6/6 (48->256) |
| H9N2/Ca09/P7 | 24,0 | 16,1 | 3/3 (48-128) | 6/6 (<2-192) |

Reassortant H9N2 virus containing 2009 pH1N1 internal genes: replication and transmission efficiency close to that of 2009 pH1N1

# Next generation sequencing of parental H9N2 virus and P4 virus

| Segment | aa change | Frequency of aa change (%) | | |
|---|---|---|---|---|
| | | A/Qa/HK/P0 | A/Qa/HK/P4 (nasal mucosa) | A/Qa/HK/P4 (lung) |
| PB1 | Glu172Gly | - | - | 42,34 |
| PA | Glu399Lys | - | 5,00 | - |
| | Lys488Glu | 10,25 | 11,21 | 7,84 |
| | Cys489Ser | 21,37 | 21,79 | 10,27 |
| HA | His52Arg | - | - | 23.37 |
| | Asp233Gly | - | 80.88 | 99.96 |
| | Phe303Leu | - | - | 44.19 |
| NP | Ala428Thr | - | - | 10.99 |
| M | Ala29Thr | - | 23.26 | 98.33 |
| NS | Thr49Ala | - | 7.29 | - |

Mutations with high frequency both in URT and LRT

# Next generation sequencing of parental H9N2 virus and P4 virus

- HA D233G and M A29T mutations were associated with partial adaptation of H9N2 virus to pigs

- HA D233G has been described to increase α 2,3 Sia binding activity *(Matrosovich et al, 2000; Liu et al. 2010; Iovine et al. 2015)*

- HA G472E appeared after seven passages in pigs, it was associated with the decrease in viral replication and the final loss of the virus

# Conclusions H9N2 adaptation to pigs

- Full adaptation is certainly a complex multi-step process involving mutations in multiple proteins and interactions between them (reassortment!)
- The internal gene cassette may have an essential role in the overcoming of host barrier
- H9N2 reassortant viruses pose a threat to pigs and humans

# European research published in 2014-2015

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## Cross-Species Infectivity of H3N8 Influenza Virus in an Experimental Infection in Swine

Alicia Solórzano,[a] Emanuela Foni,[b] Lorena Córdoba,[c] Massimiliano Baratelli,[c] Elisabetta Razzuoli,[d] Dania Bilato,[e] María Ángeles Martín del Burgo,[f] David S. Perlin,[a] Jorge Martínez,[c,g] Pamela Martínez-Orellana,[c] Lorenzo Fraile,[h] Chiara Chiapponi,[b] Massimo Amadori,[e] Gustavo del Real,[f] María Montoya[c,i]

## The respiratory DC/macrophage network at steady-state and upon influenza infection in the swine biomedical model

P Maisonnasse[1], E Bouguyon[1], G Piton[2,3], A Ezquerra[4], C Urien[1], C Deloizy[1], M Bourge[5], J-J Leplat[2,3], G Simon[6,7], C Chevalier[1], S Vincent-Naulleau[2,3], E Crisci[8], M Montoya[8,9], I Schwartz-Cornil[1] and N Bertho[1]

# European research published in 2014-2015

Pedersen *et al. Virology Journal* 2014, **11**:163
http://www.virologyj.com/content/11/1/163

VIROLOGY JOURNAL

**SHORT REPORT** **Open Access**

## Identification of swine influenza virus epitopes and analysis of multiple specificities expressed by cytotoxic T cell subsets

Lasse E Pedersen*, Solvej Ø Breum, Ulla Riber, Lars E Larsen and Gregers Jungersen

*Journal of General Virology* (2015), **96**, 1603–1612 DOI 10.1099/vir.0.000094

## New reassortant and enzootic European swine influenza viruses transmit efficiently through direct contact in the ferret model

Kristina Fobian,[1] Thomas P. Fabrizio,[2] Sun-Woo Yoon,[2]†
Mette Sif Hansen,[3] Richard J. Webby[2] and Lars E. Larsen[1]

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