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"In vitro cultures of the porcine respiratory tract and their susceptibility to influenza A viruses"

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- 1) Introduction
- 2) Optimalization of an in vitro system
- 3) Susceptibility to influenza A viruses
- 4) Receptor expression
- 5) Future plans
- 6) General conclusions

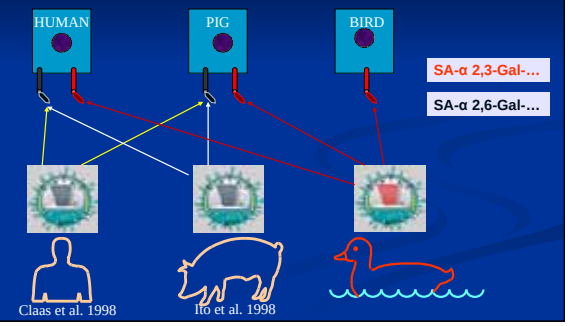
1. Introduction

- Avian IVs in pigs-"in vivo experiments"
  - Susceptible to most avian subtypes
  - Infection and replication less efficient
  - Large variations between pigs
  - No/inefficient pig to pig transmission

→ STRONG SPECIES BARRIER !

1. Introduction

→HYPOTHESIS: PIG=MIXING VESSEL



1. Introduction

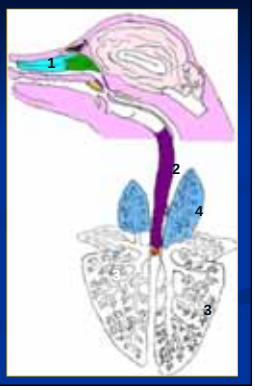
AIMS:

- Set up of the in vitro system→EXPLANTS
  - ENTIRE respiratory tract
  - Maximum similarity to in vivo situation
  - Follow up in time of virus yield
  - Well controlled conditions
- Susceptibility of explants to influenza A viruses
- Receptor expression and relationship with replication capacity

2. Optimalization of an in vitro system

2A) Materials and methods:

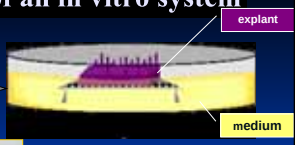
- Piglets 6 weeks old
- Conventional – swine influenza virus seronegative (H1N1, H3N2 and H1N2)
- Euthanasia by thiopental and exsanguination
- Upper and lower respiratory tract:
  - 1) Respiratory part of the nasal mucosa
  - 2) Trachea
  - 3) Bronchi
  - 4) Right apical lung lobe



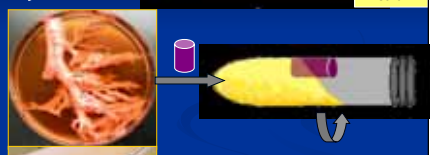
2. Optimalization of an in vitro system

➢2A) Materials and methods

1) Respiratory part of the nasal mucosa
2) Trachea
Glorieux et al. 2007



3) Bronchi



4) Right apical lung lobe filled with agarose
Porro et al. 2001

2. Optimalization of an in vitro system

- 2B) Viability parameters
  - Ciliary beating (light microscopy)
  - Ethidium monoazide bromide staining (counterstained with Hoechst) specific for necrotic and late apoptotic cells by DNA-binding.
  - Tunel staining for early and late apoptotic cells by labeling of DNA strand breaks.

2. Optimalization of an in vitro system

- Result 1:
  - Cultivation system covering the upper and lower porcine respiratory tract
  - Good viability

3. Susceptibility to influenza A viruses

- 3A) Materials and methods

The viruses:

Swine influenza viruses
Sw/Belgium/1/98 (H1N1)
Sw/Flanders/1/98 (H3N2)
Sw/Gent/7625/99 (H1N2)

Human influenza viruses
A/New Caledonia/20/99 (H1N1)
A/Panama/2007/99 (H3N2)

Avian influenza viruses
Mallard/Alberta/290/98 (H1N1-LPAI)
Mallard/Italy/3401/2005 (H5N1-LPAI)
Duck/Belgium/06936/05 (H3N6-LPAI)
Chicken/Belgium/150/99 (H5N2-LPAI)
Chicken/Italy/1067/V99 (H7N1-LPAI)

3. Susceptibility to influenza A viruses

- 3A) Materials and methods

Procedures:

NE + TOC BOC LE

tubes
24-well plates

24 hours after cultivation

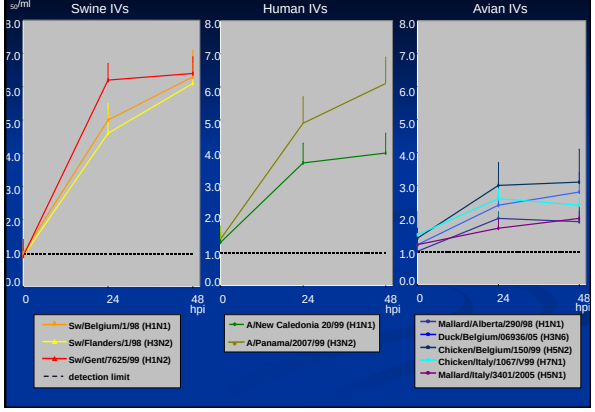
2 washes with PBS

Inoculation with 600 µl 10^6 EID_{50}

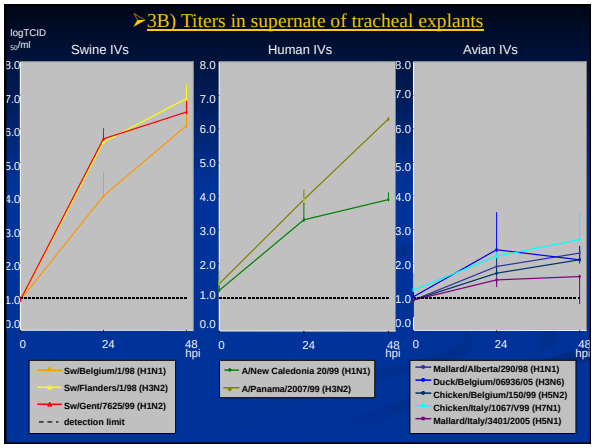
Incubation for 1 hour

Removal inoculum
3 washes with PBS
Transfer + fresh medium

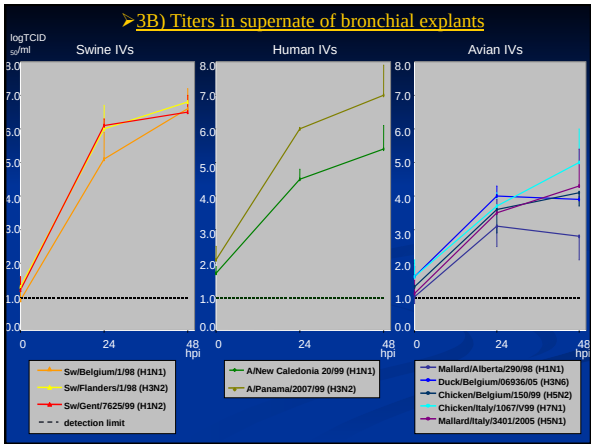
➢3B) Titers in supernate of nasal explants



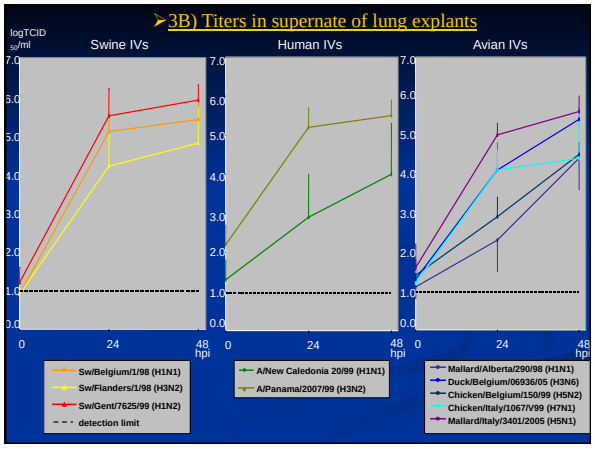
➢3B) Titers in supernate of tracheal explants



➢3B) Titers in supernate of bronchial explants



➢3B) Titers in supernate of lung explants



3. Susceptibility to influenza A viruses

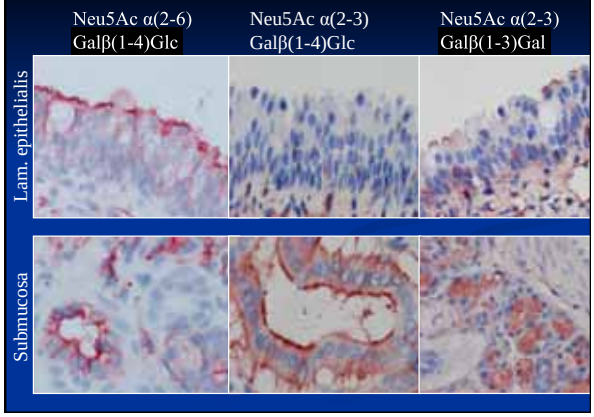
- Result 2:
  - All influenza viruses from the 3 different hosts do replicate in the 4 types of explants.
  - The in vitro systems appear to be suitable to study differences in replication capacity of genetically distinct influenza viruses.
  - Avian influenza viruses replicate best in lower respiratory tract.

4. Receptor expression

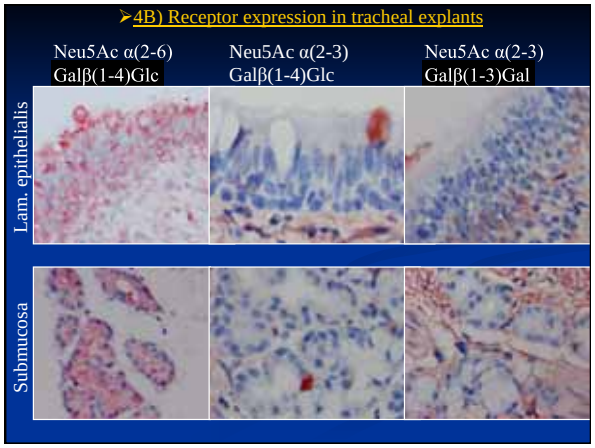
➢4A) Materials and methods

Lectine	Receptor	Substrate
Digoxigenin SNA (glycan differentiation kit: Roche)	Neu5Ac α(2-6) Galβ(1-4)Glc  **~HUMAN/SWINE IVs**	NF: red
Biotinylated MAA1 (Vectorlab)	Neu5Ac α(2-3)Galβ(1-4)Glc  **~ AVIAN IVs**	AEC: brown
Biotinylated MAA2 (Vectorlab)	Neu5Ac α(2-3)Galβ(1-3)Gal  **~ AVIAN IVs**	AEC: brown

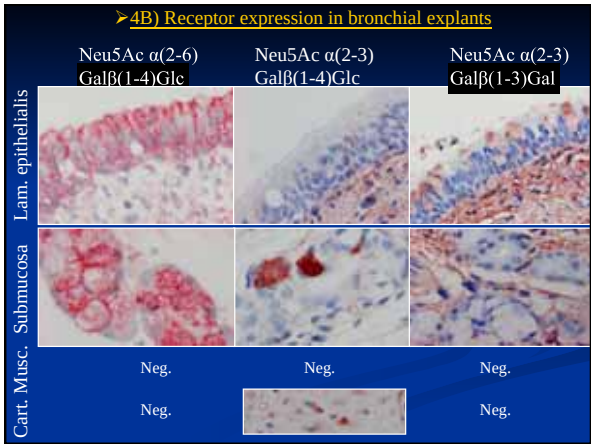
➢4B) Receptor expression in nasal explants



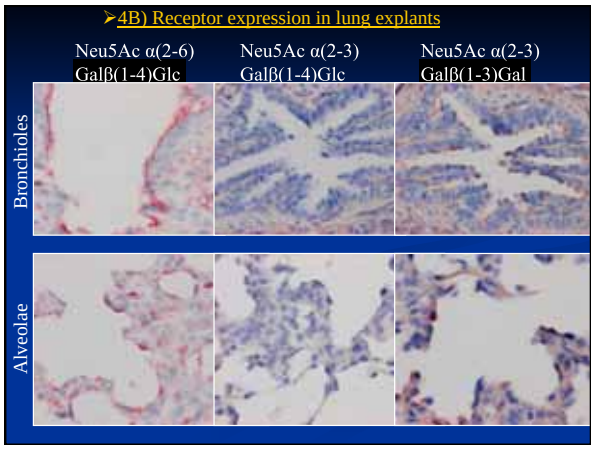
➢4B) Receptor expression in tracheal explants



➢4B) Receptor expression in bronchial explants



➢4B) Receptor expression in lung explants



4. Receptor expression

- **Summary**

	SAα2,6	SAα2,3
NE	+++	+/-
TOC	+++	+/-
BOC	+++	+
LE	++	++

+/-: Very limited expression
+: Moderate expression
++: Clear expression
+++: Abundant expression

- **Remarks**
  - MAA 1 and MAA 2 lectines should be combined to study SA-α2,3 expression.

4. Receptor expression

- Result 3:
  - Although presence of both receptor types, clear difference in expression level and pattern between SA-α(2,6) and SA-α(2,3)!!:
    - Human/swine influenza virus receptor in the entire respiratory tract.
    - Avian influenza virus receptor concentrated in the lower respiratory tract.
  - Consistency between virus yield and receptor expression in the explants.

5. Future plans

- Colocalization of virus infected cells and receptors.
  - Early phase of infection
- Differences in tissue and cell tropism
  - Cell markers
- Effect of mutations in receptor binding site on efficiency of virus replication
  - Matrosovich M. (Philipps University-Germany)

6. General conclusions

- Good CORRELATION between "in vivo" and "in vitro" results:
  - Low replication capacity of AIVs in upper respiratory tract → possible explanation for failure of pig to pig transmissions after intranasal inoculation
  - Preference of AIVs for the lower respiratory tract
  - No indications for a higher sensitivity of pigs to AIVs than humans (Nicholls et al. 2007)

6. General conclusions

- Possibilities of the "in vivo" and "in vitro" systems are COMPLEMENTARY:
  - Insight virus-cell interactions at different levels: in vitro
  - Outcome of infection in the host: in vivo
  - Impact of host factors, e.g.: immunity, genetic background,…: in vivo

With sincere thanks to: Prof. Dr. John Nicholls, Kevin Fung and Lieve Sys