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# Pathogenesis and transmission of LPAI viruses in pigs

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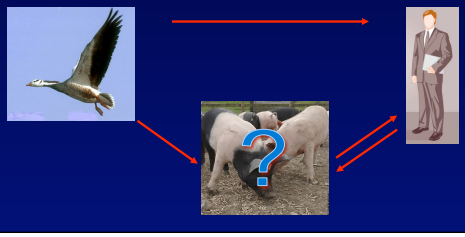
## Classical hypothesis

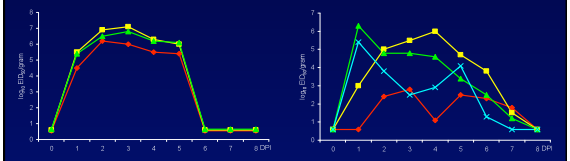
The pig as intermediate host for avian influenza viruses and "mixing vessel" for potentially pandemic reassortants?



## Evidence for species barrier

- AI viruses seem to replicate less efficient in pigs than SIVs
  1. No or moderate virus excretion in pigs experimentally infected with HPAI H5N1 viruses (Shortridge *et al*., 1998; Choi *et al*., 2005; Isoda *et al*., 2006 )
  2. Moderate virus excretion titres in pigs experimentally infected with LPAI viruses of different subtypes; no excretion with 9/38 AI isolates (Kida *et al*., 1994)
  3. Lower virus excretion levels with LPAI viruses than with SIVs in pigs (own results)

## Nasal virus excretion of pigs intranasally inoculated with LPAI viruses



SIV H1N1
H3N2
H1N2

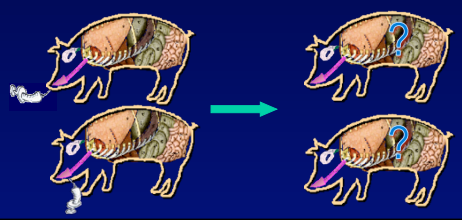
LPAI H1N1
H3N6
H5N2
H7N1

## Aim of our research

Study the pathogenesis of a LP H5N2 virus isolate in pigs

## Pathogenesis: aims

1. Examine tissue tropism of a LP H5N2 AI virus in pigs
2. Influence of inoculation method on extent of virus replication



## Materials and methods

chicken/Belgium/150/99, LP H5N2 virus

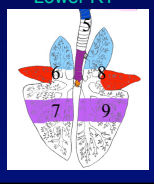
12 pigs inoculated intranasally ($10^7$ $EID_{50}$)
12 pigs inoculated intratracheally ($10^{7.5}$ $EID_{50}$)
euthanasia of 2 pigs per day, 1-6 dpi

clinical monitoring
collection of respiratory tract and other tissues for virus titration and immunofluorescence (IF)

## Materials and methods

Upper RT


Lower RT




1. Nasal mucosa respiratory
2. Nasal mucosa olfactory
3. Nasopharynx
4. Tonsil
5. Trachea
6. Lung apical/cardiac right
7. Lung diaphragmatic right
8. Lung apical/cardiac left
9. Lung diaphragmatic left

+ brain stem, spleen, intestine and serum

## Intranasal inoculation LPAI H5N2

| Tissue | Virus titres at … DPI | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Upper RT | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
| 1. Nasal muc. Resp. | + | + | + | + | - | + | - | - | - | - | - | + |
| 2. Nasal muc. Olf. | + | + | + | + | + | + | + | + | - | + | + | + |
| 3. Nasopharynx | + | + | + | + | - | + | + | + | - | + | + | + |
| 4. Tonsil | + | + | + | + | + | + | - | + | - | + | - | - |
| Lower RT | | | | | | | | | | | | |
| 5. Trachea | + | + | + | + | - | + | + | + | - | + | - | + |
| 6. Lung a+c R | + | NA | + | NA | + | NA | + | + | - | + | - | + |
| 7. Lung D R | + | NA | + | NA | - | NA | - | + | - | - | - | + |
| 8. Lung a+c L | - | - | - | + | - | + | - | + | - | + | - | + |
| 9. Lung DL | + | + | - | + | - | + | + | < | - | + | - | - |

-: no virus isolation +: virus isolation; NA: not available

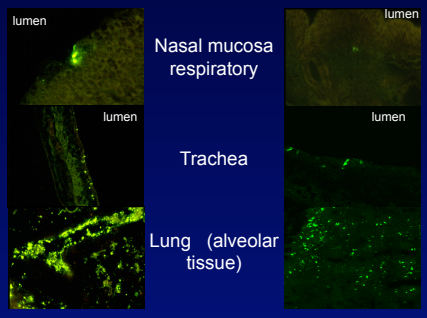
## Intranasal inoculation SIV H1N1

| Tissue | Virus titres at … DPI | | | | | |
|---|---|---|---|---|---|---|
| Upper RT | 1 | 2 | 3 | 4 | 5 | 6 |
| 1. Nasal muc. Resp. | ++ | ++ | ++ | ++ | ++ | - |
| 2. Nasal muc. Olf. | ++ | ++ | ++ | ++ | - | - |
| 3. Nasopharynx | ++ | ++ | ++ | ++ | + | + |
| 4. Tonsil | ++ | ++ | ++ | ++ | + | - |
| Lower RT | | | | | | |
| 5. Trachea | ++ | ++ | ++ | ++ | ++ | - |
| 6. Lung a+c R | ++ | ++ | ++ | ++ | ++ | ++ |
| 7. Lung D R | ++ | ++ | ++ | ++ | ++ | - |
| 8. Lung a+c L | ++ | ++ | ++ | ++ | ++ | - |
| 9. Lung DL | ++ | ++ | ++ | ++ | ++ | ++ |

-: no virus isolation ++: virus isolation; <: no virusisolation; NA: not available

## Intranasal inoculation

SIV H1N1 LPAI H5N2



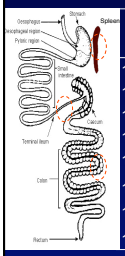

Nasal mucosa respiratory

Trachea

Lung (alveolar tissue)

## Intranasal inoculation LPAI H5N2

Extra respiratory tissues



| Tissue | Virus titres … DPI | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
| 10. Duodenum | - | - | - | - | - | - | - | - | - | - | - | - |
| 11. Jejunum | - | - | - | - | - | - | - | - | - | - | - | - |
| 12. Ileum | - | - | - | - | - | - | - | - | - | - | - | - |
| 13. Colon | - | - | - | - | - | - | - | - | - | - | - | - |
| 14. Rectum | - | - | - | - | - | - | - | - | - | - | - | - |
| 15. Spleen | - | - | - | - | - | - | - | - | - | - | - | - |
| 16.Brain stem | + | - | - | - | + | - | - | + | - | - | - | - |
| 17 Serum | - | - | - | - | - | - | - | - | - | - | - | - |

-: no virus isolation; +: virus isolation

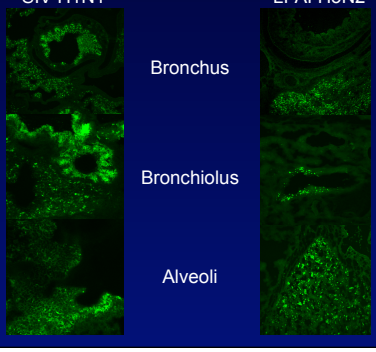
# Intratracheal inoculation LPAI H5N2

| Tissue | Virus titres at … DPI | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Upper RT | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
| 1. Nasal muc. Resp. | - | - | - | - | - | - | - | - | - | - | - | - |
| 2. Nasal muc. Olf. | + | - | - | - | - | - | - | - | - | - | - | - |
| 3. Nasopharynx | - | - | - | - | + | - | + | + | - | - | - | - |
| 4. Tonsil | - | - | - | - | + | - | + | - | + | + | - | - |
| Lower RT | | | | | | | | | | | | |
| 5. Trachea | + | + | + | + | + | - | + | + | + | - | - | - |
| 6. Lung a+c R | + | + | + | NA | + | NA | + | + | + | - | + | - |
| 7. Lung D R | + | + | + | NA | + | NA | + | + | + | - | - | - |
| 8. Lung a+c L | + | NA | + | - | + | + | + | + | + | - | - | - |
| 9. Lung DL | + | NA | + | - | + | + | + | + | - | - | - | - |

-: no virus isolation +: virus isolation; NA: not available

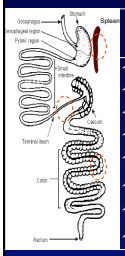
# Intratracheal inoculation

SIV H1N1 LPAI H5N2




# Intratracheal inoculation LPAI H5N2

Extra respiratory tissues



| Tissue | Virus titres … DPI | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
| 10. Duodenum | - | - | - | - | - | - | - | - | - | - | - | - |
| 11. Jejunum | - | - | - | - | - | - | - | - | - | - | - | - |
| 12. Ileum | - | - | - | - | - | - | - | - | - | - | - | - |
| 13. Colon | - | - | - | - | - | - | - | - | - | - | - | - |
| 14. Rectum | - | - | - | - | - | - | - | - | - | - | - | - |
| 15. Spleen | - | - | - | - | - | - | - | - | - | - | - | - |
| 16.Brain stem | - | - | - | - | - | - | - | - | - | - | - | - |
| 17 Serum | - | - | - | - | - | - | - | - | - | - | - | - |

-: no virus isolation

## Pathogenesis: conclusions

- Replication of LPAI H5N2 virus is limited to the respiratory tract, with indication for neurotropism
- LPAI H5N2 virus replicates less eficiently than SIVs, resulting in
  - lower virus titres
  - fewer virus positive cells
  - restricted virus spread from the upper to the lower respiratory tract and vice versa

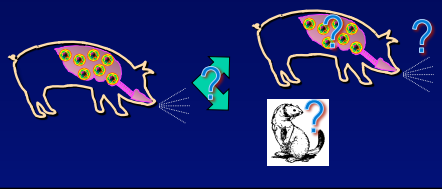
Are levels of virus excretion sufficient to allow transmission of LPAI viruses?

Transmission of AI viruses between pigs has not been shown under experimental conditions

1. No virus transmission of HP H5N1 or H7N7 AI viruses between pigs under experimental conditions (Shortridge *et al*., 1998; Loeffen *et al*., 2004; Choi *et al*., 2005)
2. Wholly AI viruses rarely become established in pigs; exception avian-like H1N1 SIVs in Europe

## Transmission: Aim

Can LPAI viruses spread between pigs and from pigs to other mammals

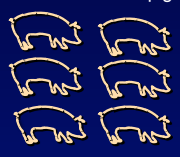


## Transmission: Materials

- Seronegative piglets; 6-8 weeks
- Seronegative adult ferrets
- 2 SIVs: - A/swine/Belgium/1/98 (H1N1)
  - A/swine/Flanders/1/98 (H3N2)
- 6 LPAIs - A/duck/Italy/1447/05 (H1N1)
  - A/duck/Belgium/06936/05 (H3N6)
  - A/mallard/Alberta/47/98 (H4N1)
  - A/mallard/Italy/3401/05 (H5N1)
  - A/chicken/Belgium/150/99 (H5N2)
  - A/chicken/Italy/1067/V99 (H7N1)

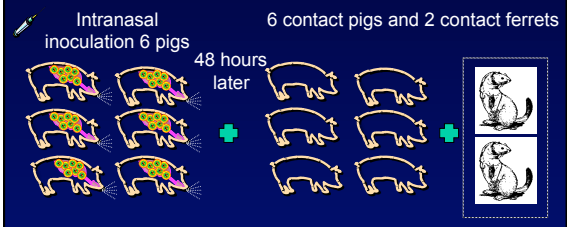
## Transmission : experimental design

Intranasal inoculation 6 pigs



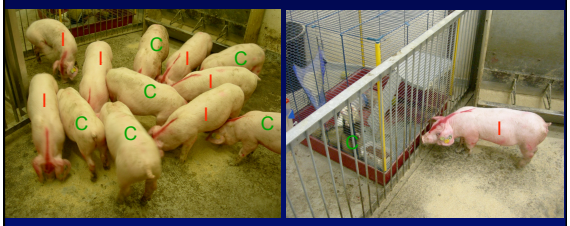
Stable climate: 20-22°C and R.H. 50-70%

## Transmission : experimental design




Parameters:
- nasal swabs 1-10 DPI or DPC for virus isolation
- blood for serology 0, 14 and 28 DPI or DPC

## Transmission: Experimental design



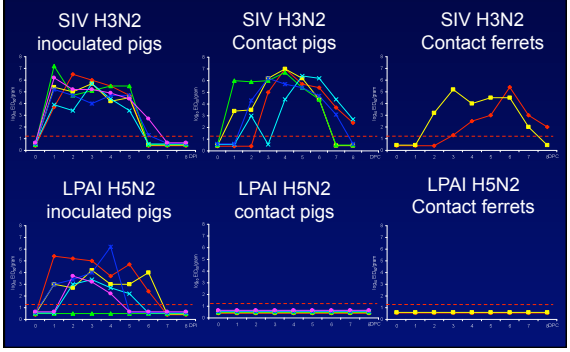

## Results transmission SIVs

| Virus | # animals / total with | | | | | |
|---|---|---|---|---|---|---|
| | Inoculated piglets | | Contact piglets | | Contact ferrets | |
| | Virus excr. | Anti-bodies | Virus excr. | Anti-bodies | Virus excr. | Anti-bodies |
| H1N1 | 6/6 | 6/6 | 6/6 | 6/6 | 2/2 | 2/2 |
| H3N2 | 6/6 | 6/6 | 5/5 | 5/5 | 2/2 | 2/2 |

## Results transmission LPAIs

| Virus | # animals / total with | | | | | |
|---|---|---|---|---|---|---|
| | Inoculated piglets | | Contact piglets | | Contact ferrets | |
| | Virus excr. | Anti-bodies | Virus excr. | Anti-bodies | Virus excr. | Anti-bodies |
| H1N1 | 6/6 | 6/6 | 0/6 | 0/6 | 0/2 | 0/2 |
| H3N6 | 6/6 | 6/6 | 0/6 | 0/6 | 0/2 | 0/2 |
| H4N1 | 6/6 | 5/5 | 0/6 | 0/5 | 0/2 | 0/2 |
| H5N1 | 1/6 | 6/6 | 0/6 | 0/6 | 0/2 | 0/2 |
| H5N2 | 5/6 | 6/6 | 0/6 | 0/6 | 0/2 | 1/2 |
| H7N1 | 5/6 | 6/6 | 0/6 | 2/5 | 0/2 | 0/2 |

## Nasal virus excretion




## Transmission: conclusion

- No or only very limited spread of AI viruses between pigs or from pigs to ferrets
- Lower virus excretion with LPAI viruses than with SIVs might give a partial explanation
- Transmission model can be used to study the effect of well-defined genetic alterations on transmission capacity of LPAI viruses

## Conclusions

- Pathogenesis of LP H5N2 virus is similar to that of SIV, but lower replication efficiency
- LPAI viruses fail to transmit between pigs and from pigs to ferrets
  - There is a clear species barrier for LPAI viruses to infect pigs
  - Risk of pigs to spread LPAI viruses to man is probably overestimated in the past, but may not be ruled out completely