

EXPERIMENTAL INFECTION OF MUSCOVY DUCKS WITH HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS (H5N1)

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INTRODUCTION

- **Highly pathogenic (HP) H5N1 avian influenza (AI)** remains enzootic in several countries of Asia and Africa, constituting a major threat, at the world level, for both animal and public health.

- **Ducks** play a major role in the epidemiology of AI, particularly HP H5N1. Although vaccination can be a useful tool to control AI, if wisely used, until now, duck vaccination is not very efficient in the field and can be improved in experimental conditions, suggesting a need to develop new vaccines and challenge model to evaluate the protection for duck species.

- **Muscovy duck** is rarely studied whereas it is the majority duck species in France, first duck producer country in Europe, and it is also produced in Asia.

- Being part of the European **NOVADUCK** project (Novel AI DIVA recombinant vaccines for duck), we designed a **H5N1 HP challenge model** using 2 strains belonging to **subclade 2.2**, the last subclade detected in Europe.

MATERIAL AND METHODS

• **Sampling:** Oropharyngeal and cloacal swabs (1 ml), organs of dead or euthanatized ducks (heart, trachea, lungs, spleen, kidney, pancreas, intestine, brain).

• **Laboratory analyses:** RNA extraction: M48 Biorobot, QIAGEN; Starting material: 100µl swab or 10 mg tissue; Elution: 100 µl RNase free water.

Quantitative RT-PCR: - Based on conserved region of the matrix gene (*Spackman et al., 2002*); Ct expressed in **equivalent (eq) EID₅₀ (µl of swab or µg of tissue)** using a standard curve of virus. - Use of β actin detection (*Van Borm et al., 2007*) to normalize Ct values obtained from organs.

• **Statistical analyses:** **Shedding:** Variance analyses (time as repeated effect, confidence interval 95%); **Titres in organs:** Variance analyses (animal as a random effect) Tukey's multiple comparisons tests; **Presence of virus in organs according to the date of death:** Nonparametric test (Kruskal-Wallis).

EXPERIMENT 1

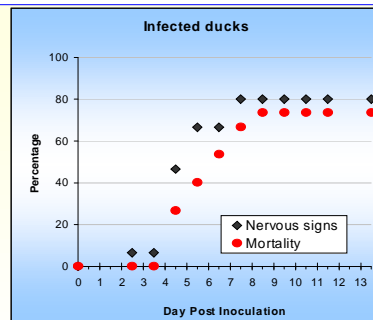
• **Virus:** H5N1 HPAI virus A/mute swan/France/070203/07 (**Sw/070203**): strain isolated in France in summer 2007.

Subclade 2.2.3

• **Experiment:** 6.5-week-old SPF Muscovy ducks:

15 (1 female + 14 males) challenged with **10⁶ EID₅₀/duck** (route oculonasal) + 5 (2 females + 3 males) contacts.

Kinetics of Mortality and Nervous Signs

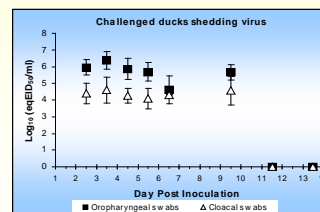


Infected ducks

Day Post Inoculation	Infectious	Contact ducks
1st clinical signs:	2.5 dpi (1/15)	2.5 dpi (1/5)
1st mortality:	4.5 dpi (4/15)	2.5 dpi (1/5)
Total mortality:	11/15 (73%) in 8.5 dpi	3/5 (60%) in 5.5 dpi
Survival at 13.5 dpi:	4/5 (27%)	2/5 (40%)

Shedding of virus in swabs evaluated by QRT-PCR

DPI	2.5	3.5	4.5	5.5	6.5	9.5	11.5	13.5
OROPHARYNGEAL								
Number	15/15	15/15	15/15	11/11	9/9	3/7	0/4	0/4
%	100%	100%	100%	100%	100%	43%	0%	0%
CLOACAL								
Number	15/15	12/15	10/15	5/11	1/9	2/7	0/4	0/4
%	100%	80%	67%	45%	11%	29%	0%	0%



Shedding: Oropharyngeal statistically higher than Cloacal (p=0.010)

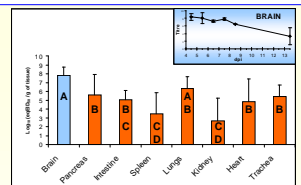
Differences between the 2 routes: 1.3 log₁₀ eqEID₅₀/ml

Peak: 2.5-3.5 dpi

Maximal values (oropharyngeal): 6.4 log₁₀ eqEID₅₀/ml

Maximal values (cloacal): 4.6 log₁₀ eqEID₅₀/ml

Amounts of RNA detected in organs (QRT-PCR)



Same letter: not significantly different (P-value<0.05)

Max Titre: Brain; 7.8 log₁₀ eqEID₅₀/g of tissue

Lowest Standard deviation: Brain

Presence of virus in organs according to the date of death (only the brain is presented in the graph above, in the upper right corner):

Not significantly \neq from 4.5 to 8.5 dpi

Presence of virus in the 4 surviving ducks at 13.5 dpi:

- Brain, Pancreas, Intestine, Spleen, Heart, Trachea: Lower titres (compared to other dates)
- Lungs & Kidney: Not significantly \neq at 13.5 dpi (but high Standard Deviation)

The weakest virus loads in brain compared to the other ducks
A few or no virus detected in the other organs

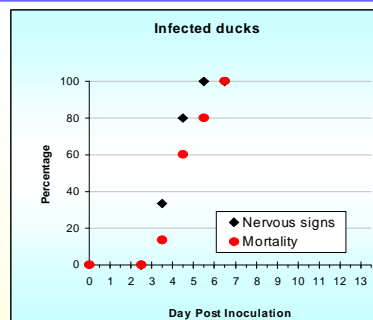
EXPERIMENT 2

• **Virus:** H5N1 HPAI virus A/mute swan/France/06299/06 (**Sw/06299**): strain isolated in France during winter 2006 French outbreaks (*Le Gall-Recule et al., 2008*).

Subclade 2.2.1

• **Experiments:** 5.5-week-old SPF Muscovy ducks:

15 (7 females + 8 males) challenged with **10⁶ EID₅₀/duck** (route oculonasal) + 5 (2 females + 3 males) contacts.

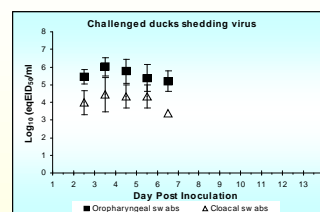


Infected ducks

Day Post Inoculation	Infectious	Contact ducks
1st clinical signs:	3.5 dpi (5/15)	3.5 dpi (3/5)
1st mortality:	3.5 dpi (2/15)	3.5 dpi (2/5)
Total mortality:	15/15* (100%) in 6.5 dpi	5/5 (100%) in 6.5 dpi
Survival at 13.5 dpi:	0/5 (0%)	0/5 (0%)

* 3 ducks euthanatized at 6.5 dpi because of serious nervous signs.

DPI	2.5	3.5	4.5	5.5	6.5
OROPHARYNGEAL					
Number	15/15	15/15	13/13	7/7	3/3
%	100%	100%	100%	100%	100%
CLOACAL					
Number	13/15	12/15	12/13	6/7	1/3
%	87%	80%	92%	86%	33%



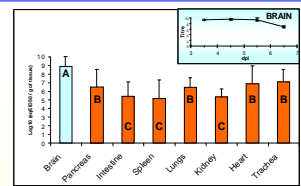
Shedding: Oropharyngeal statistically higher than Cloacal (p=0.0033)

Differences between the 2 routes: 1.5 log₁₀ eqEID₅₀/ml

Peak: 3.5-4.5 dpi

Maximal values (oropharyngeal): 6.0 log₁₀ eqEID₅₀/ml

Maximal values (cloacal): 4.4 log₁₀ eqEID₅₀/ml



Same letter: not significantly different (P-value<0.05)

Max Titre: Brain; 8.9 log₁₀ eqEID₅₀/g of tissue

Lowest Standard deviation: Brain

Presence of virus in organs according to the date of death (only the brain is presented in the graph above, in the upper right corner):

- Pancreas & Spleen: Not significantly \neq from 3.5 to 6.5 dpi
- Brain, Intestine, Kidney, Heart, Trachea: Decrease at 6.5 dpi
- Lungs: Decrease from 5.5 dpi to 6.5 dpi

Presence of virus in the 3 ducks humanely killed at 6.5 dpi (serious nervous signs):

The lowest virus loads in brain compared to the other dead ducks
No trace of virus in Pancreas, Intestine, Spleen or Heart

CONCLUSION-DISCUSSION

- Both challenges worked well. The strain from subclade 2.2.1 was more virulent than the strain belonging to subclade 2.2.3 (No survival, higher virus loads in organs).

- Cloacal viral shedding was lower than oropharyngeal in the 2 trials.

- With both viruses the brain was the organ presenting the highest virus load. All ducks that died with clinical symptoms presented virus in their brain, being probably the primary cause of death.

- In both experiments, challenged ducks and contacts shed the same amount of virus, showing the fast and efficient virus transmission using a H5N1 HPAI virus.

- All these data suggested the use of strain from subclade 2.2.1 as a putative challenge model to test several vaccines.

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References:

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