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 Al DIVA recombinant vaccines for duck), we designed a H5N1 HP challenge model using 2 strains belonging to subclade 2.2.2, 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: 100 µg 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., 2003); Ct expressed in equivalent (eq) EID50/ml of swab or g of tissue using a standard curve of virus.
- 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/ 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 10^6 EID50/duck (route oculoanasal) + 5 (2 females + 3 males) contacts.

EXPERIMENT 2

- Virus: H5N1 HPAI virus A/ 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 10^6 EID50/duck (route oculoanasal) + 5 (2 females + 3 males) contacts.

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