

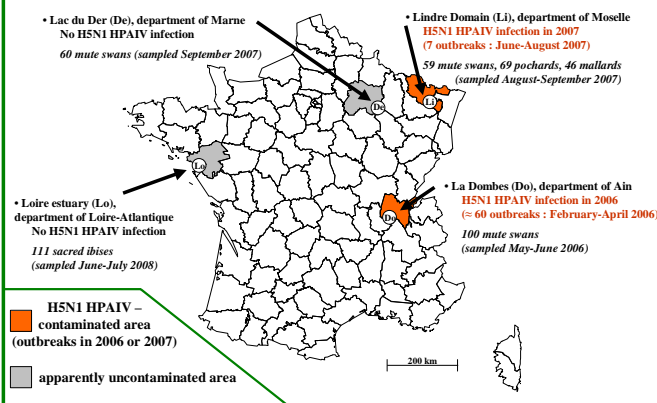
Introduction

Following the introduction of H5N1 highly pathogenic avian influenza virus (HPAIV) in Europe in 2005, this virus was detected in France :

- in February-April 2006 in the East wetlands with one exception (la Dombes essentially) in one turkey flock and wild waterfowl (mainly mute swans). Phylogenetic studies on the virus showed the existence of two distinct sub-groups, both belonging to clade 2.2.1.
- in June-August 2007 in the North-East wetlands of Moselle in wild aquatic birds (mute swans and mallards) only. The virus involved belonged to clade 2.2.3: it was close to virus circulating in Germany and Czech Republic at the same time, and different from those isolated in 2006.
- To characterize the immune status of apparently healthy wild birds present in the contaminated areas, with respect to anti-H5 and anti-N1 antibodies, and
- To compare results between species and areas (contaminated vs. apparently uncontaminated), sera were collected on four species of birds:
 - ✓ mute swans, common pochards and mallards, which came either from regions affected by the 2006 and 2007 outbreaks, or from an apparently uncontaminated region situated about 200 km westward of the Moselle wetlands. In fact, these species may act as sentinels or vectors during outbreaks.
 - ✓ sacred ibises, which were culled in 2008 in an uncontaminated area on the Loire estuary.



Figure 1 : Sampling scheme of wild birds in 2006, 2007 and 2008



Material and Methods

Wild birds were blood sampled by the technical staff of the local services of the ONCFS, resulting in the sampling scheme shown in figure 1.

Anti-H5 antibodies were detected by hemagglutination inhibition (HI) according to international standards (OIE) against five recent French antigens :

- H5N1 HP A/common pochard/France/06167/2006 (subclade 2.2.1)
 - H5N1 HP A/mute swan/France/070203tr/2007 (subclade 2.2.3)
 - H5N1 LP A/duck/France/05066b/2005
 - H5N2 LP A/duck/France/05057b/2005
 - H5N3 LP A/duck/France/02166/2002
- LP = Low Pathogenic

Categories of HI test results (Table 1 and Figure 2)

H5 -: H5-negative sera, HI titre < 4 log₂ for all five antigens tested.

H5 +: H5-positive sera, HI titre ≥ 4 log₂ against at least two antigens with different N subtype.

H5 ? : sera of uncertain status, HI titre ≥ 4 log₂ against antigens belonging to only one N subtype.

Anti-N1 antibodies were tested with a commercial competition ELISA kit (ID-Vet, Montpellier, France).

Statistical tests for (with Bonferroni correction) mean H5 HI titre pairwise comparisons : paired-t tests.
seroprevalence comparisons : χ^2 tests.

Group	% of H5 + sera ^a	Mean HI titres (log ₂) against H5 antigens ^b				
		H5N1 HP 06167	H5N1 LP 05066b	H5N2 LP 05057b	H5N3 LP 02166	H5N1 HP 070203tr
Swans - Dombes - 2006	49%***	5.6 ^{1,2}	5.6 ¹	5.4 ^{1,2}	4.8 ^{2,3}	4.2 ²
Swans - Der - 2007	69%***	6.2 ²	6.9 ¹	4.3 ⁴	5.2 ³	3.0 ²
Swans - Lindre - 2007	69%***	6.1 ¹	5.7 ^{1,2}	5.0 ^{2,3}	4.6 ³	2.9 ²
Pochards - Lindre - 2007	27%*	5.8 ¹	6.3 ¹	4.4 ^{2,3}	4.6 ²	2.5 ²
Mallards - Lindre - 2007	28%*	3.9	3.3	3.7	2.7	1.3
Ibises - Loire - 2008	64%***	3.2 ²	5.2 ²	6.1 ¹	4.8 ³	3.8 ²

Highest value homogeneous set of HI titres

Lowest value homogeneous set of HI titres

Groups sampled in areas apparently uncontaminated by H5N1 HPAIV

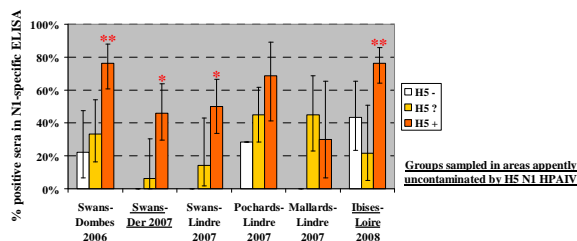
Table 1 : H5 HI tests results . Prevalence of H5-positive (H5 +) sera and variations of mean HI titres depending on the antigen, the avian species and the origin of the H5 + sera

a : Different numbers of “*” show statistically significant differences in H5+ seroprevalence between groups.

b : Only sera with complete HI test results available against all five antigens were retained for mean titre computation and comparison within each sampled group of wild birds (no mean comparisons were done for mallards, because of too small a final sample size).

Significantly different mean titres for a given group are identified with different superscript numbers across each line of the table.

Figure 2 : Comparison of anti-N1 antibody seroprevalence (determined by N1-specific ELISA) between categories of H5 HI test results, for each group of wild birds sampled



*: anti-N1 antibody seroprevalence for H5 + sera is significantly higher than for H5 ? category.

** : anti-N1 antibody seroprevalence for H5 + sera is significantly higher than for H5 ? and H5 - categories.

For birds sampled in 2007, the respective H5-negative (H5 -) categories had too small a sample size to allow meaningful statistical comparisons, so these were excluded from the analysis.

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Results – Discussion – Conclusion

➤ Moderate to high frequencies of positivity in anti-H5 antibodies by HI tests occur naturally in all groups of wild birds tested (Table 1):

Swans in 2007 > Swans in 2006 > Pochards & Mallards in 2007

Ibises in 2008 (not different from either swans' group)

- No differences in frequency between swans of different origins in 2007 (contaminated vs. apparently uncontaminated areas)

➤ Discriminating HI titre profiles / highest HI titre sets (Table 1):

▪ in contaminated areas (in 2006 and 2007): H5N1 HP 2006 = H5N1 LP

▪ in apparently uncontaminated areas: H5N1 LP > H5N1 HP 2006

and for ibises in 2008, the highest HI titre corresponds to the H5N2 LP antigen.

➤ Strong statistical association between positivity in H5-antibodies and N1-antibodies in swans and ibises irrespective of the origin of samples (contaminated and apparently uncontaminated areas) (see Figure 2)

➔ ✓ these findings point towards an acquired natural humoral response in swans vs. either H5Nx, HyN1 or H5N1 viruses.

✓ for those swans sampled in contaminated areas, this may correspond to natural immunity following infection (asymptomatic or recovery) by HPAIV H5N1.

Future Prospects

The level and length of protection mediated by the immune response detected are still to be explored. However, this response might explain the observed difference in epidemiological situations between 2006 (epizootic outbreaks of HPAIV) and 2007 (fewer sporadic cases) in France and other European countries.

Collecting more serological data on wild birds would be useful to help and model viral transmission following a new introduction of H5N1 HPAIV.