Experimental vaccination of mule ducks under field conditions against highly pathogenic avian influenza A (H5N1) virus of clade 2.3.4.4b

Interim report 2 :

« Experimental evaluation of transmission among vaccinated ducks after challenge at 7 weeks of age »

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Executive Summary

Since 2016, five epizootics of highly pathogenic avian influenza (HPAI) caused by A(H5Nx) viruses belonging to clade 2.3.4.4b have strongly affected poultry production in France and other European countries. This was especially the case in South Western and Western France, where duck productions paid the highest tribute to the virus, as they represented up to the two-thirds of infected farms.

As a result of the magnitude of the recent epizootics, several European countries are conducting research programs to evaluate the possible contribution of vaccination against clade 2.3.4.4b highly pathogenic H5 avian influenza viruses to help control future epizootics – in addition to increased biosecurity, surveillance and stamping out of infected flocks – as laid down by new European regulations 2016/429 and 2023/361.

As part of these concerted research efforts, French authorities (Ministry of Agriculture and Food Sovereignty) coordinated a vaccination experiment in Mule ducks (the hybrid between Peking and Muscovy ducks, raised for foie-gras production), a production for which very limited experimental data are available. The experiment is being conducted by Anses (French Agency for Food Environment and Occupational Health Safety), ENVT-INRAe (National Veterinary School of Toulouse-INRAe), CIFOG (French Trade Union of Foie-gras Producers), four Regional Councils, and two manufacturers of veterinary vaccines. The study was launched in May 2022, its protocols were validated by the Ethical Committees of the participating research institutes.

The experiment was designed to check whether some vaccines, when implemented under field conditions, i) would be well tolerated in vaccinated ducks and induce an immunity allowing the differentiation between infected and vaccinated animals (DIVA principle), ii) could help reduce the excretion of the H5 HPAI virus by vaccinated birds and iii) would limit the transmission of HPAI to other vaccinated ducks. Interim report 1 presents the results of phases i) and ii) and interim report 2 presents the results on transmission (iii).

The mule ducks were vaccinated (vaccine A or vaccine B) or not in flocks kept in experimental sites under field conditions (see interim report 1) and were transferred to Anses BSL3 containment animal facilities when aged 6 weeks. The animals were distributed into three groups that were kept separately: one group of non-vaccinated control animals, one group of animals vaccinated with vaccine A and one group of animals vaccinated with vaccine B. After one week of acclimation, two animals in each group were challenged under BSL3 conditions at 7 weeks of age, by intraocular administration of a high dose ($10^6 EID_{50}$ per duck) of a A(H5N1) HPAI 2.3.4.4b clade virus isolated in 2021. Twenty-four hours post-inoculation, nine ducks with the same vaccination status as the inoculated animals were

placed in direct contact with the two inoculated animals, and nine animals of the same status were placed in indirect contact. The ducks were visited daily for clinical examination, for 32 days post inoculation. Oropharyngeal and cloacal swabbing was regularly performed for the measurement of virus excretion using qRT-PCR. Blood samples were taken before challenge (-4 days post-inoculation (dpi)) and at 14, 21 and 32 dpi to follow the post inoculation immune responses by ELISA NP, ELISA H5 and haemagglutination inhibition (HI).

The ducks transferred to Anses containment facilities did not develop any seroconversion (as detected using ELISA NP) one week post transfer, hence showing they did not get infected by influenza viruses, neither during rearing in the farm nor during transport. As already observed in the previous experiments implemented to study the reduction of excretion (see interim report 1), the transferred vaccinated birds had detectable ELISA H5 antibodies induced by vaccination prior to challenge.

All the inoculated ducks in the three groups (except one duck in the group that received vaccine A) shed virus by the oro-pharyngeal route 24 hours after inoculation. However, whereas the non-vaccinated birds excreted the challenge virus by both the oropharyngeal and the cloacal routes, the vaccinated birds receiving vaccines A or B exhibited oropharyngeal excretion only.

In non-vaccinated ducks, the onset of shedding was rapid in the inoculated animals, with viral genome detection in oro-pharyngeal and cloacal swabs as early as 1 dpi. Excretion increased up to 3 dpi and then decreased progressively over time, with oro-pharyngeal shedding still present at 32 dpi in these animals. Similar shedding profiles were obtained in non-vaccinated animals that were placed in direct and in indirect contact with the two non-vaccinated inoculated ducks, however with 24-hour and 48-hour delays respectively. These excretion profiles were very similar with those observed with unvaccinated ducks receiving an HPAI challenge during the excretion experiments reported in interim report 1.

In clear contrast with the non-vaccinated ducks, in each vaccinated group, inoculated ducks that excreted the HPAI virus, did so by the oropharyngeal route only and for seven days post inoculation. With both vaccines, only one vaccinated animal put in direct contact with vaccinated inoculated animals was detected positive, for oro-pahryngeal shedding only, and at a single time-point. None of the ducks exposed by indirect contact was found positive for viral genome, in both vaccinated groups.

The direct transmission (R_{01}=88) and airborne transmission (R_{02}=29) were very high in non-vaccinated control animals. Both transmission modes proved very well controlled by the two tested vaccines with very limited direct transmission (R_{01}<1) and no airborne transmission in both vaccinated groups.

The serological profiles observed in the different groups were consistent with the results of the virological analyses : In the non-vaccinated groups, all birds developed antibodies readily detectable by ELISA NP, ELISA H5 and haemagglutination inhibition by 14 days post inoculation or contact, consistently with the high levels of virus excretion observed in these birds. In contrast, in ducks vaccinated with vaccines A or B, only the inoculated birds developed a marked ELISA NP antibody response. Virtually no ELISA NP response developed in the contact birds and ELISA H5 and HI mean titres even decreased in the contact groups during the experiment, thus corroborating the very limited virus exposure in the vaccinated ducks exposed by contact.

Altogether, the results presented in interim report 2 are very promising as they demonstrate a very good control of the transmission of A(H5N1) HPAI clade 2.3.4.4b in vaccinated Mule ducks exposed by direct or indirect contact at 7 weeks of age.

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