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

Interim report 1 :

« Experimental evaluation of clinical protection and virus excretion »

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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).

As a first step, the two vaccines eligible for the study were selected based on the double criteria of their antigenic content being an H5 Haemagglutinin belonging to the A/goose/Guangdong/1/1996 lineage (to ensure antigenic relatedness with circulating field viruses) and of pre-existing experimental results documenting their possible efficacy in ducks under confined conditions.

As a second step (ENVT-INRAe), the vaccines were implemented according to their manufacturers' recommendations, in selected experimental farms. Three batches of Mule ducks (500 to 2000 ducks each) were vaccinated using each vaccine candidate. Unvaccinated controls were kept in each farm. The ducks were not allowed access to free range in the farms, in compliance with biosecurity regulations prevailing in France at the time when the experiment was conducted. Every two weeks,

the vaccinated and control ducks were visited for clinical examination by the farm veterinarian. At each time point, the ducks were submitted to both blood sampling (for serological examination by ELISA NP and ELISA H5) and tracheal and cloacal swabbing (for qRT-PCR analysis of possibly circulating avian influenza viruses). None of the vaccinated ducks entered the food-chain at the end of the experiment.

As a third step (Anses), vaccinated and control ducks were transferred to Anses BSL3 containment animal facilities, from two experimental farms, each of which had received one of the tested vaccines. Two duck transfers were operated at 5 and 9 weeks of age, respectively. After allowing a two-week acclimation-time, the vaccinated and control ducks were challenged under BSL3 conditions, at 7 or 11 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. For two weeks post inoculation, the inoculated ducks were visited daily for clinical examination. Oropharyngeal and cloacal swabbing was regularly performed for the measurement of virus excretion using qRT-PCR. Blood samples were taken every 4 days to follow the post inoculation immune responses by ELISA NP, ELISA H5 and haemagglutination inhibition (HI).

In the six flocks that were followed under field conditions, the results showed that the two recommended vaccination protocols could be practically implemented. Both vaccine candidates were well tolerated (no adverse reactions observed at farm level) and induced a post vaccine immunity that could be readily detected using ELISA H5 or HI (depending on the vaccine used). In all farms, the serological follow-up did not reveal any antibody responses using ELISA NP, suggesting that the followed farms remained free of infection by field avian influenza virus during the course of the experiment, an assertion that was corroborated by the lack of detection of any influenza virus by qRT-PCR at the different time points.

The ducks transferred to Anses containment facilities did not develop any seroconversion (as detected using ELISA NP) for two weeks post transfer, hence showing they did not get infected by influenza viruses during transport. Prior to challenge, the transferred vaccinated birds had detectable ELISA H5 or HI antibodies induced by vaccination, however the ELISA H5 levels and numbers of positive tended to decrease between 7 weeks and 11 weeks of age.

Following challenge, the clinical examinations and molecular analyses revealed limited signs but strong excretion (both by the oropharyngeal and cloacal routes) in the unvaccinated controls, with excretion still detectable at 10-14 days post challenge. In contrast, the two tested vaccines induced a marked reduction of oropharyngeal and cloacal excretion (the latter being nearly abrogated), as well as a shortened excretion period as compared with the unvaccinated controls, which combination resulted in statistically highly significantly different excretion patterns. These significant differences were observed at both 7 and 11 weeks of age, although the reduction of excretion after challenge at 11 weeks of age was lower than that observed at 7 weeks of age.

The post challenge serological analyses revealed stronger ELISA NP antibody responses in unvaccinated birds (consistent with a higher replication level of the challenge virus in unvaccinated ducks), whereas the ELISA H5 or HI revealed a booster effect of the challenge on the anti-H5 antibodies (consistent with the primo-immunisation against H5 by vaccination).

Altogether, the results presented in interim report 1 are very promising in terms of safety and strong shedding reduction after challenge and need to be complemented with transmission experiments (interim report 2) to check whether the reduction of excretion observed here in vaccinated ducks results in a reduced transmission between vaccinated individuals.
