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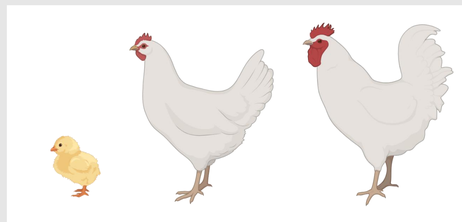


EVALUATION OF SELF-AMPLIFYING MRNA VACCINATION IN CHICKENS USING A LUCIFERASE REPORTER AND AN H5N1 VACCINE

Introduction

Industrial poultry are routinely vaccinated against infectious diseases to decrease their impact on both poultry health and poultry farm profitability. In addition, some infectious avian diseases are of global concern due to their zoonotic potential and their ability to evolve into more virulent variants. Recently, avian influenza (AI), especially highly pathogenic variants (HPAI), came into the spotlight, as the virus showed an increasing ability to expand and persist in Western Europe. Thanks to the new European Regulation (2023/361), mRNA vaccines could aid in limiting the ramifications of this disease. Hence, we aimed to assess feasibility of self-amplifying RNA (saRNA) vaccination in broilers and optimize the route of administration.

In vitro research

Materials and methods

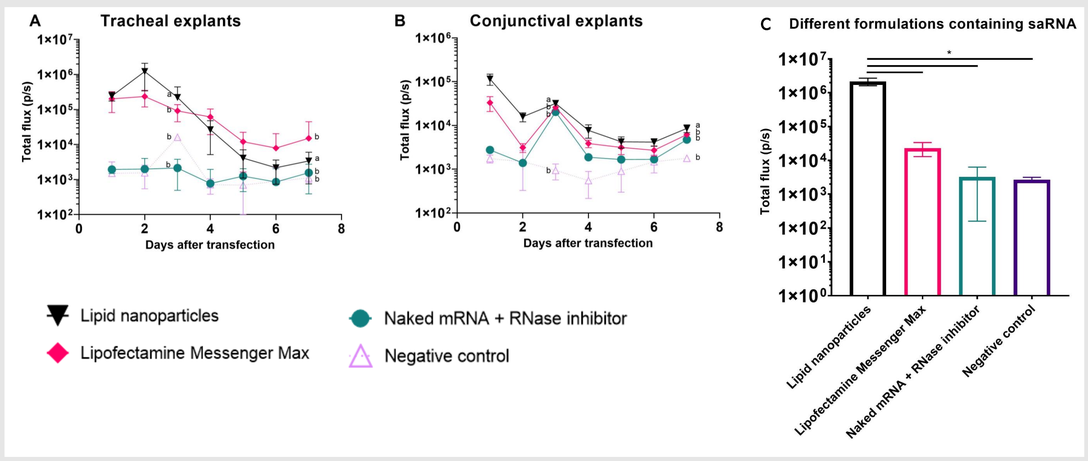
Luciferase-encoding saRNA (100 ng) was administered to three different avian cell types, each mimicking a specific administration route:

Tissue type	Mimicked administration route
Tracheal explants	Spray
Conjunctival explants	Eye drops
Primary cecal chicken cells	Drinking water

Different delivery vehicles were used: LNPs, Lipofectamine MessengerMAX, and naked saRNA in the presence of an RNase inhibitor. After the administration of luciferin, luciferase expression was determined using the IVIS Lumina III.

Results

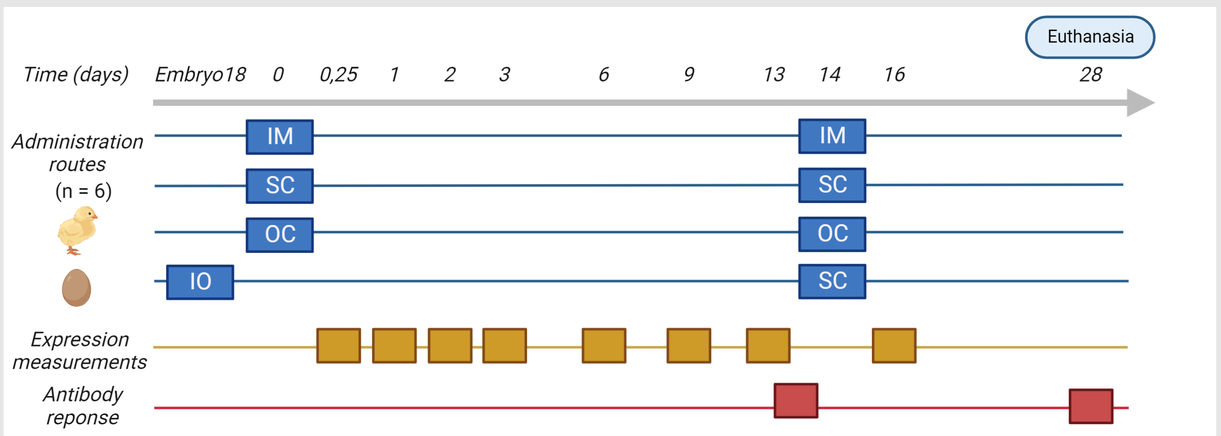
translation of the luciferase sequence in all selected avian cell types can be confirmed. .As all selected cell types showed luciferase expression, no unsuitable administration route was determined. The naked saRNA proved not to be suitable in any of the tested cell types, highlighting the need for a delivery vehicle. Additionally, the LNPs outperformed the lipofectamine Messenger Max



In vivo trial 1: Optimizing the administration route

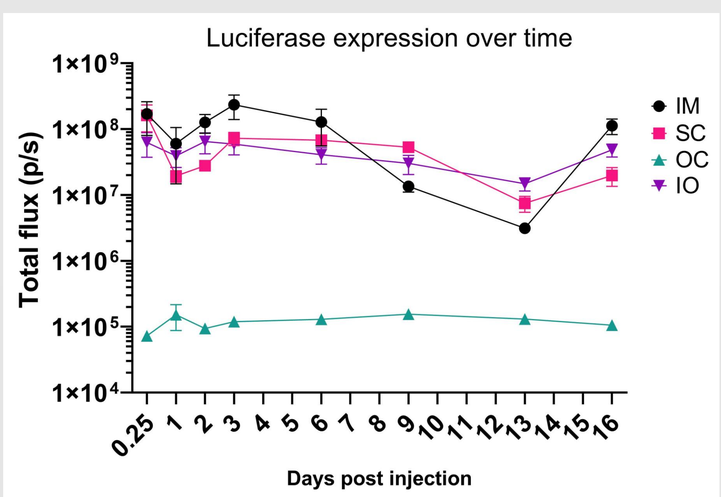
Material and methods

ROSS 308 broiler chicks or eighteen-day embryonated eggs were randomly allocated to 6 groups of 6 animals and injected with 1 µg luciferase-encoding saRNA-LNPs. Different administration routes were used: intramuscular injection (IM), subcutaneous injection (SC), ocular eye drops (OC) or *in ovo* injection (IO). Booster administration occurred on day 14 using their respective administration routes except the IO group, which was boosted subcutaneously. Control groups were mock treated with sterile PBS. *In vivo* bioluminescence imaging was performed as described below at 6h, 1, 2, 3, 6, 9, 13 and 16 days post hatch. Serum samples were collected two weeks after both prime and boost injection.



Results *in vivo* trial 1

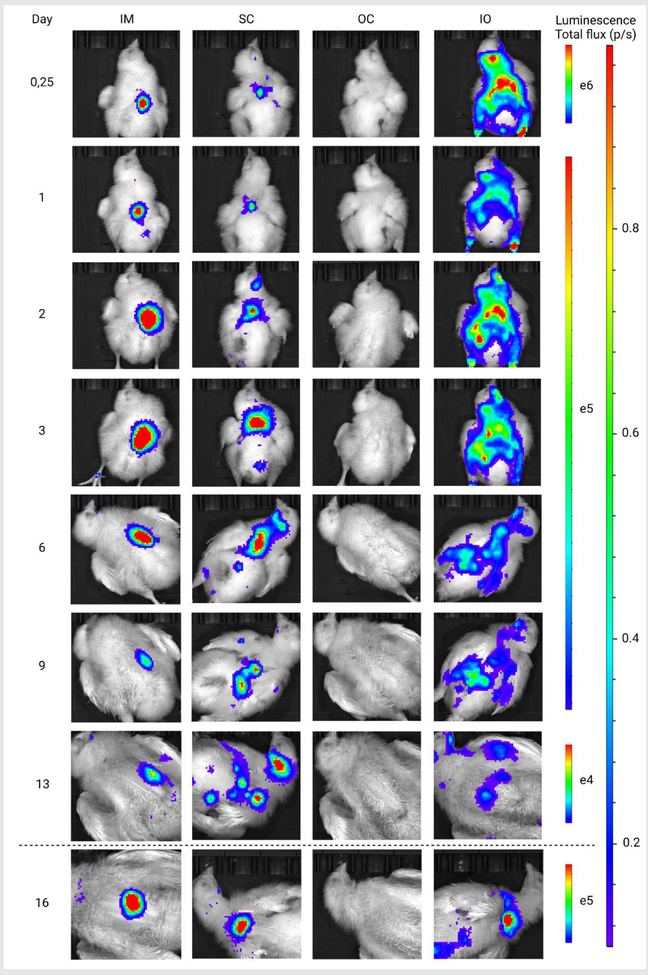
Luciferase expression: IM, SC and IO administration resulted in clear, high luciferase expression. The amount of protein produced was highest in the IM group, followed closely by the SC and IO group. OC administration was unsuccessful. The IM, SC and IO groups displayed similar expression kinetics

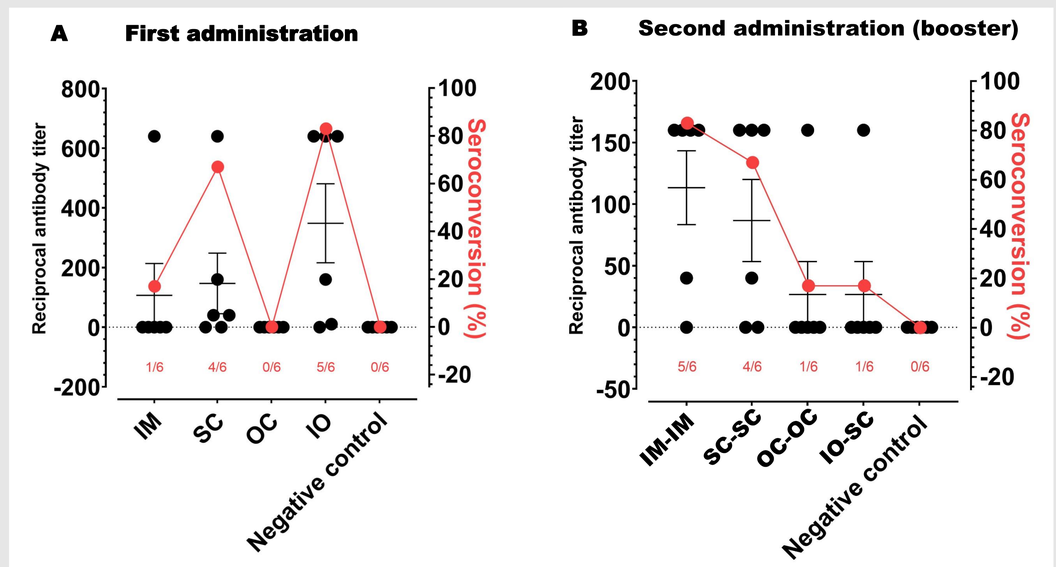


Results *in vivo* trial 1 (continued)

Biodistribution:: The biodistribution of the saRNA differs between the IM, SC and IO group. IM and SC injection of saRNA-LNPs generated a local expression pattern near the injection site, whereas IO injection resulted in a more diffuse luciferase expression spread over the entire body.

Antibody production: Despite using a very weak immunogen, antibodies were raised against this protein, albeit at limited levels. Antibodies were produced using the IM, SC and IO route, but not the ocular route. Seroconversion rates were higher after a second shot, hence showcasing a trend towards the need for a booster shot





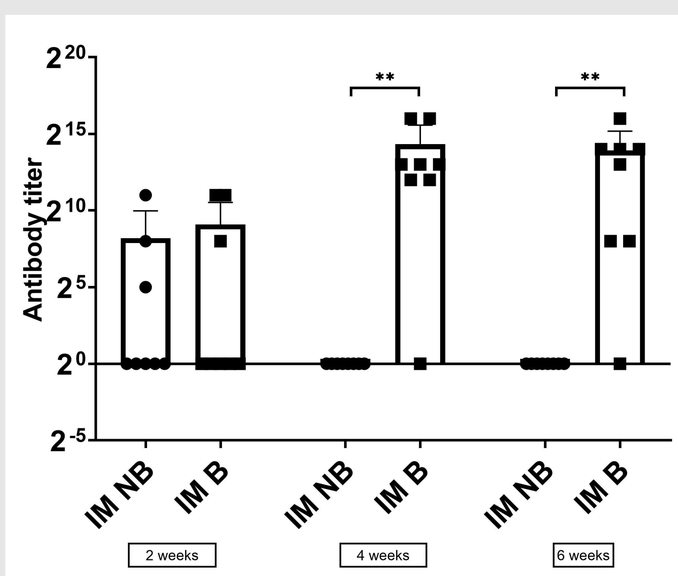
In vivo trial 2: Administration of H5-encoding saRNA-LNPs in broilers

Material and methods

ROSS 308 broilers were randomly allocated into 3 groups of 8 animals. Two of three groups received 1 µg H5-encoding saRNA-LNPs IM. One of these groups (B) received a booster after 14 days, whereas the second group (NB) received a mock injection. The control group received a mock prime and boost injection. Serum samples were collected two weeks after both prime and boost injection and an additional third time four weeks after boost injection.

Results *in vivo* trial 2

Our data shows high anti-HA antibody production, but only when a booster shot was administered. Four weeks after prime injection, the titers of the NB group decrease to 1, whereas the B group results in titers up to 65 536. As the mean antibody titer of the B group remained higher than 17 000, this suggest that broiler chickens could be protected until the end of their lifecycle.



Conclusion

In conclusion, this study was able to confirm a very efficient *in vivo* translation of the saRNA platform after IM, SC and IO administration in poultry using lipid nanoparticles. Subsequent experiments employing H5-encoding saRNA confirmed clear antibody production when employing a strong immunogen.