Variable virulence in Atlantic salmon of an ISAV isolate in repeated experimental challenges and Lack of protective effects in an mRNA vaccine against ISA

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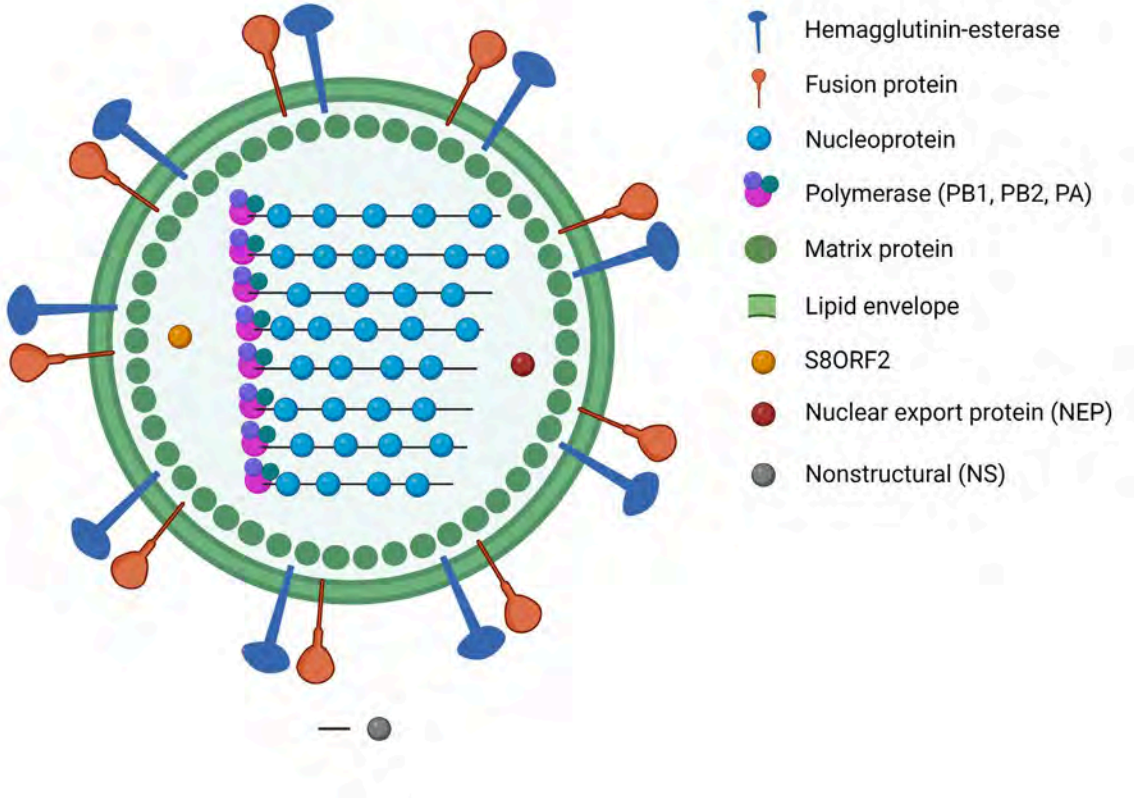
Introduction

Infectious salmon anemia virus (ISAV)

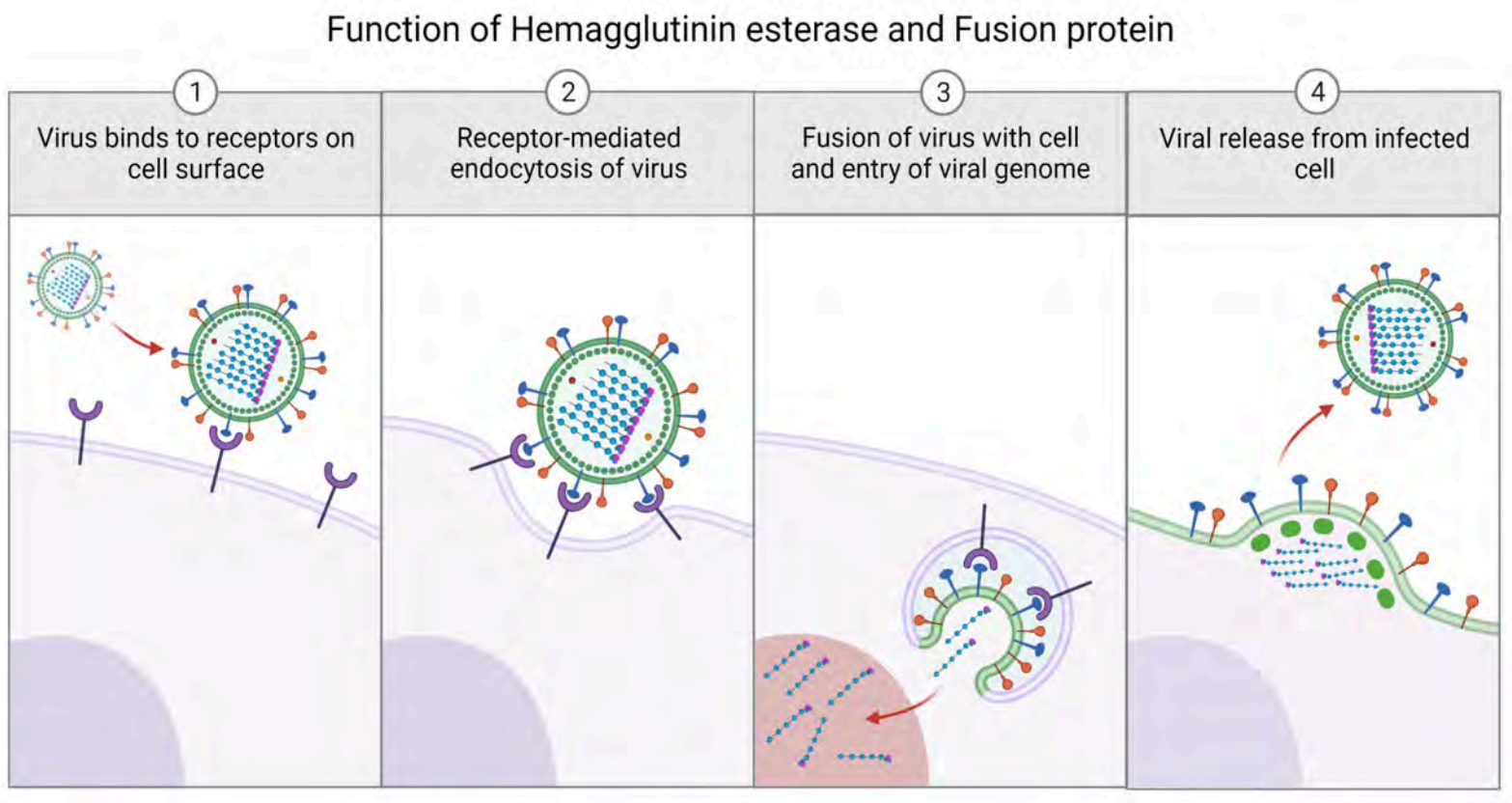
- Segmented RNA genome
- 8 segments encoding 10 proteins
- Current ISAV vaccines may be assessed as not optimal



Introduction

Hemagglutinin esterase and Fusion protein

- 2 surface glycoproteins:
- Hemagglutinin esterase (HE)
  - Receptor binding and destroying activity
- Fusion (F) protein
  - Viral fusion
- Vaccines should target these surface proteins



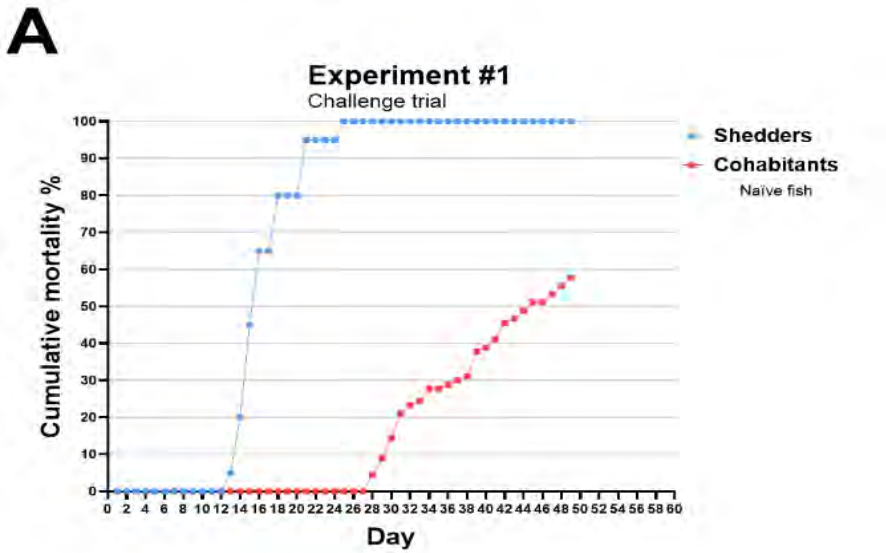
mRNA vaccination method

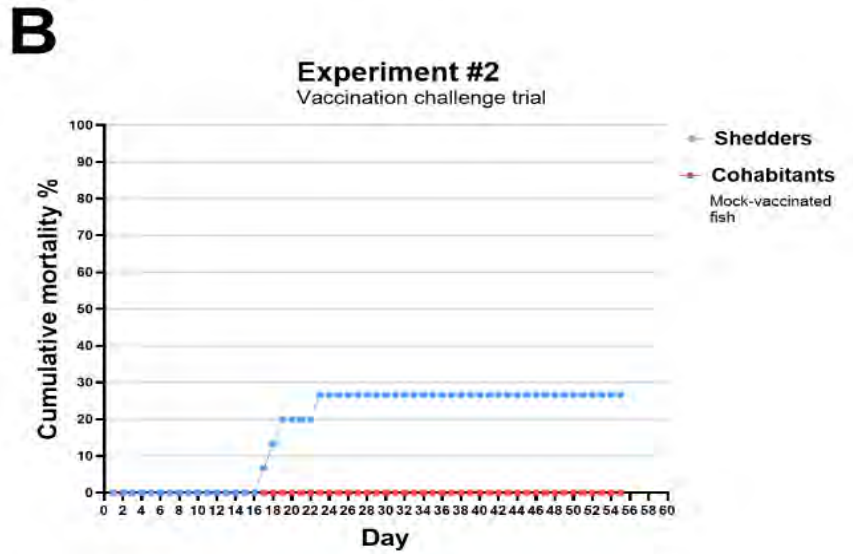
- Generate mRNA and encapsulate them in lipid nanoparticles (LNP)
- Vaccination by intramuscular injection of Atlantic salmon
- ISAV challenge
- Mortality monitoring
- Classical ISAV challenges usually uses a well characterized high virulent virus isolate
- In this experiment we wanted to use a recent isolate – assumed to be less virulent than the classical ones

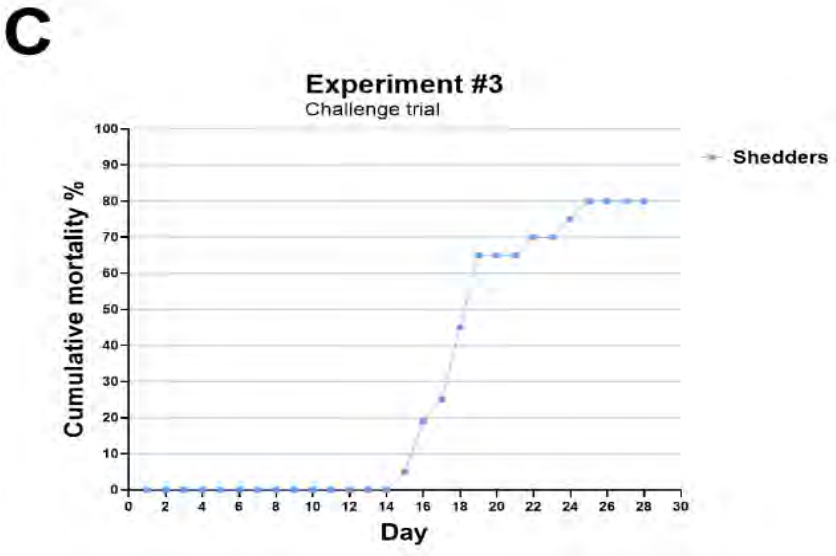
Observed variable virulence of same ISAV isolate in repeated experiments

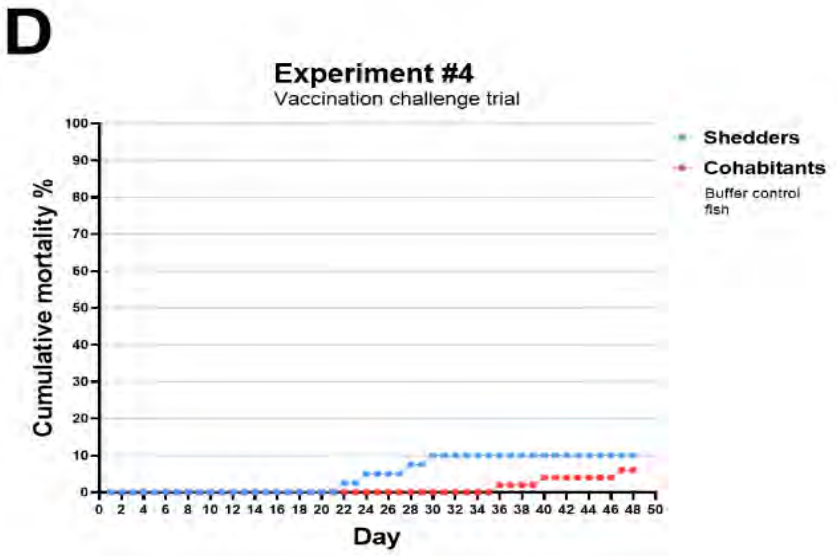
- Not all experiments go as planned…
- Originally, the experiments were conducted to assess vaccine efficacy of an mRNA vaccine against ISA using a recent virus isolate from 2020
- Four separate viral challenge trials were performed using the same virus isolate
- However, vaccine efficacy was not concludable due to lack of viral infection in the vaccination challenges
- Therefore, we sought to investigate plausible reasons for the lack of infection between challenge trials

Mortality data









Overview of parameters in all challenge experiments

- Experiment 1 and 2 were challenged with same ISAV batch
- Experiment 3 and 4 were challenged with another ISAV batch
- Viral titer was unaffected after months of storage
- Factors that may have affected shedders:
  - Fish density
  - Tank size

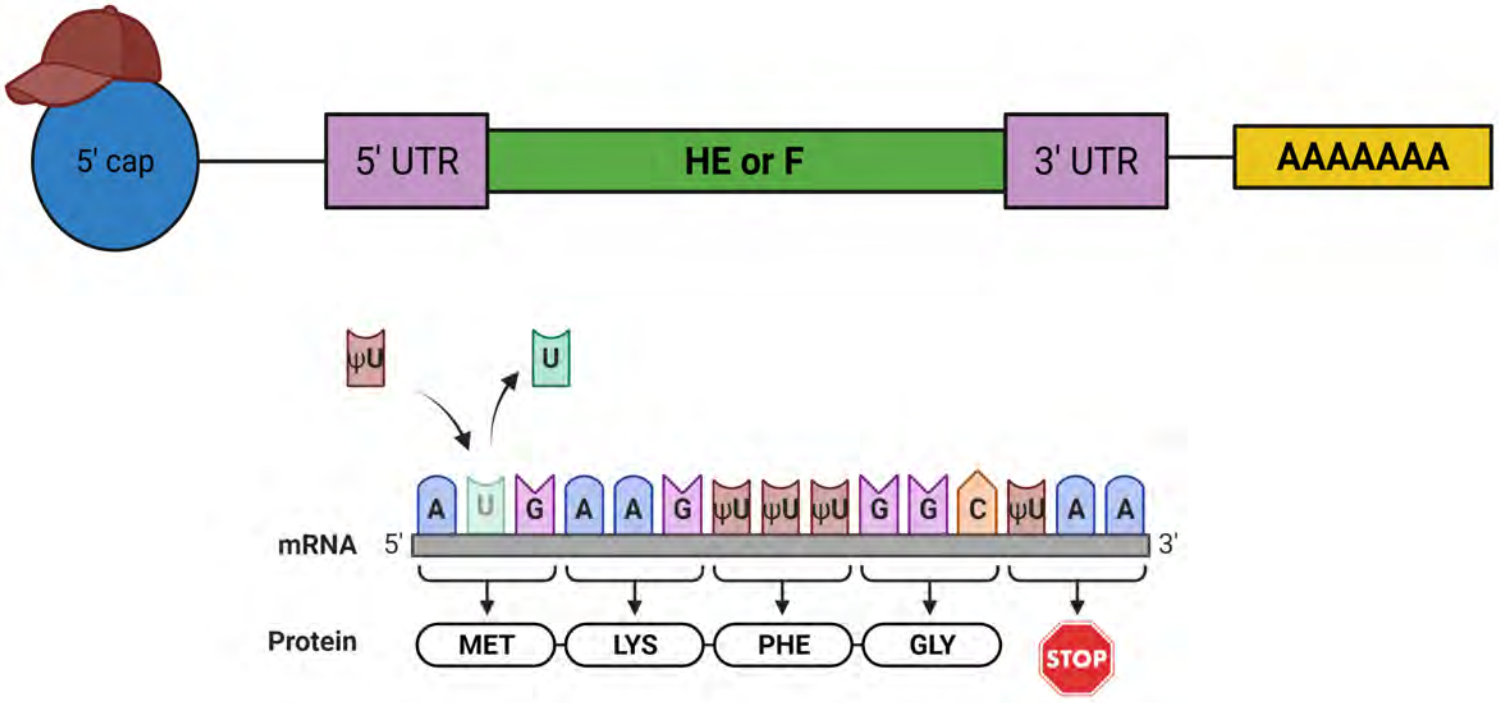
Conditions	Experiment #1 Challenge trial	Experiment #2 Vaccination challenge trial	Experiment #3 Challenge trial	Experiment #4 Vaccination challenge trial
Species	Atlantic salmon (Salmo salar)			
Strain	Stofnfiskur			
Origin	Veso Aqualab Hatchery			
Average weight	25-40 grams			
Physiological status	Parr			
Inoculum	Virus isolate: 70-72 Amount of infectious virus per fish: 10^4 $TCID_{50}$			
Number of shedder fish	**20**	**15**	**20**	**40**
Number of cohabitant fish*	**90**	**50**	**-**	**50**
Total number of fish	**110**	**165***	**20**	**240****
Cohabitant status	**Naïve**	**Mock-vaccinated**	**-**	**Tris-HCl buffer injected**
Tank size	**130L**	**450L**	**130L**	**450L**
Fish density***	**27.5 g/L**	**11.9 g/L**	**5 g/L**	**17.3 g/L**
Shedder density per volume	**0.15 fish/L**	**0.03 fish/L**	**0.15 fish/L**	**0.09 fish/L**
Salinity	Fresh water			
Stocking density	Max 50 kg/m^3			
Temperature	12ºC ± 1ºC			
Flow	0.8 l/kg fish per minute			
Water discharge	Tube overflow system			
Cleaning	Once a day			
Photoperiod regime	L:D = 24:0			
Feeding	Automatic feeders, 1% biomass per day			

Conclusion?

- Predicting virulence of uncharacterized ISAV isolates proves challenging
- Slight changes in design of challenge experiments might have considerably impact on observed virulence of assumed "low" virulent isolates
- Research of zebrafish observed elevated stress levels in small tanks compared to larger tanks (unaffected by fish densities within reasonable limits)

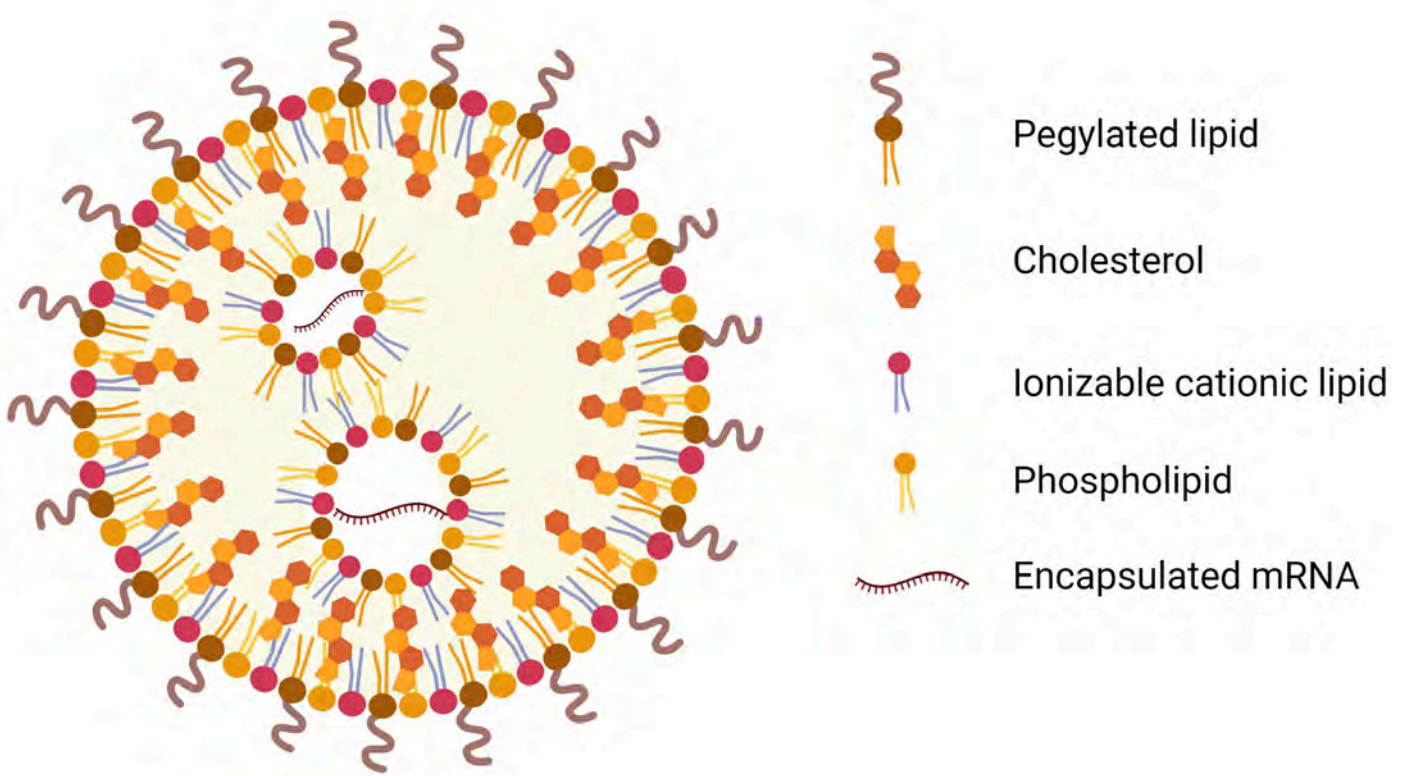
Introduction
mRNA vaccine technology

- **Construction of mRNA:**
  - Enzymatical addition of 5’ Cap
  - Poly-A tail
  - Untranslated regions (UTRs)
  - Substitution of N1-methyl pseudouridine (COVID)
    - CureVac → 40%
    - Moderna/Pfizer-BioNTech → 95%



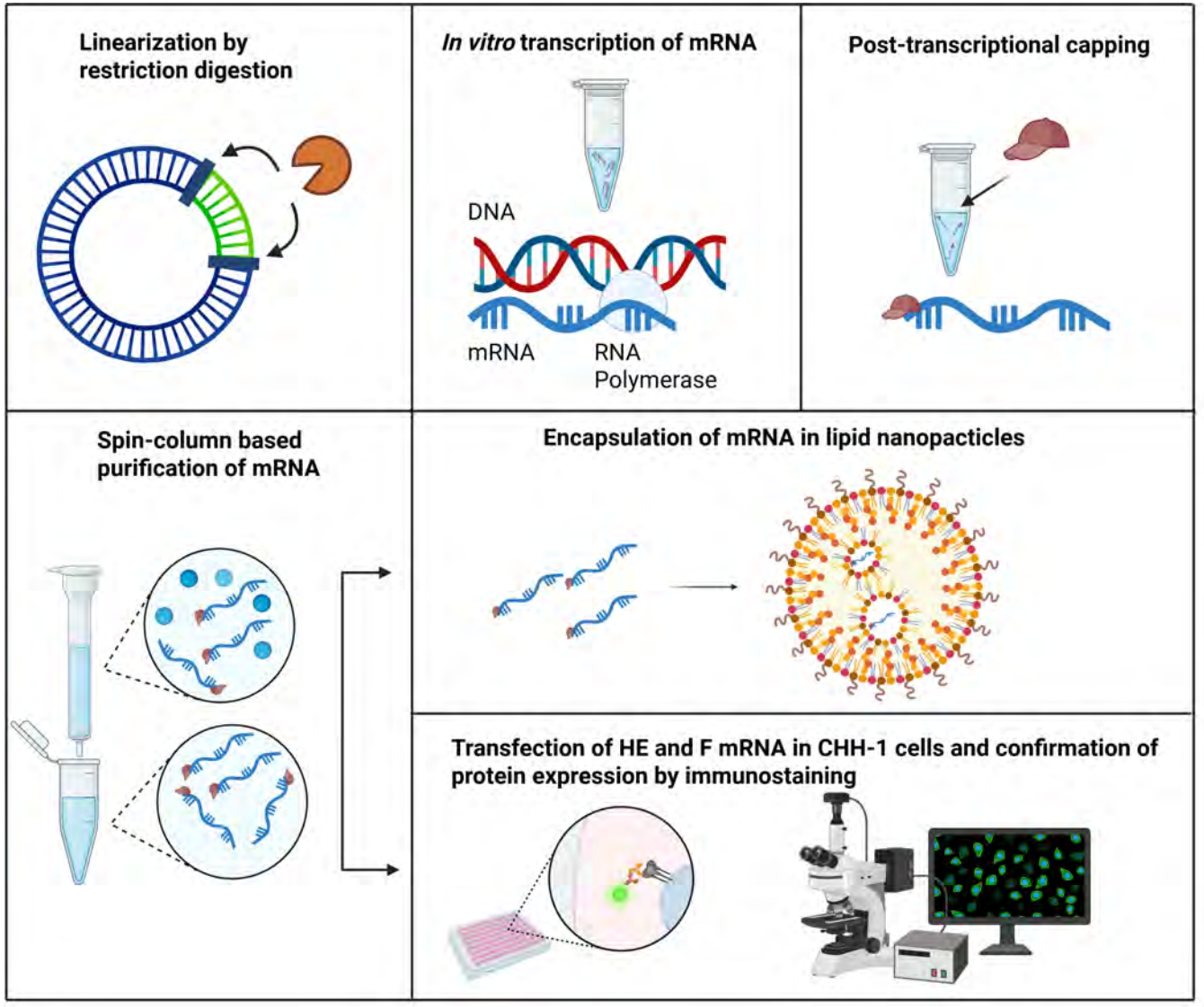
Introduction
mRNA vaccine technology

- **Lipid nanoparticles (LNPs) for mRNA delivery**
  - Consists of 4 different lipids
    - Ionizable lipids
    - "Helper lipids"
      - Cholesterol
      - Phospholipids
    - PEGylated lipids



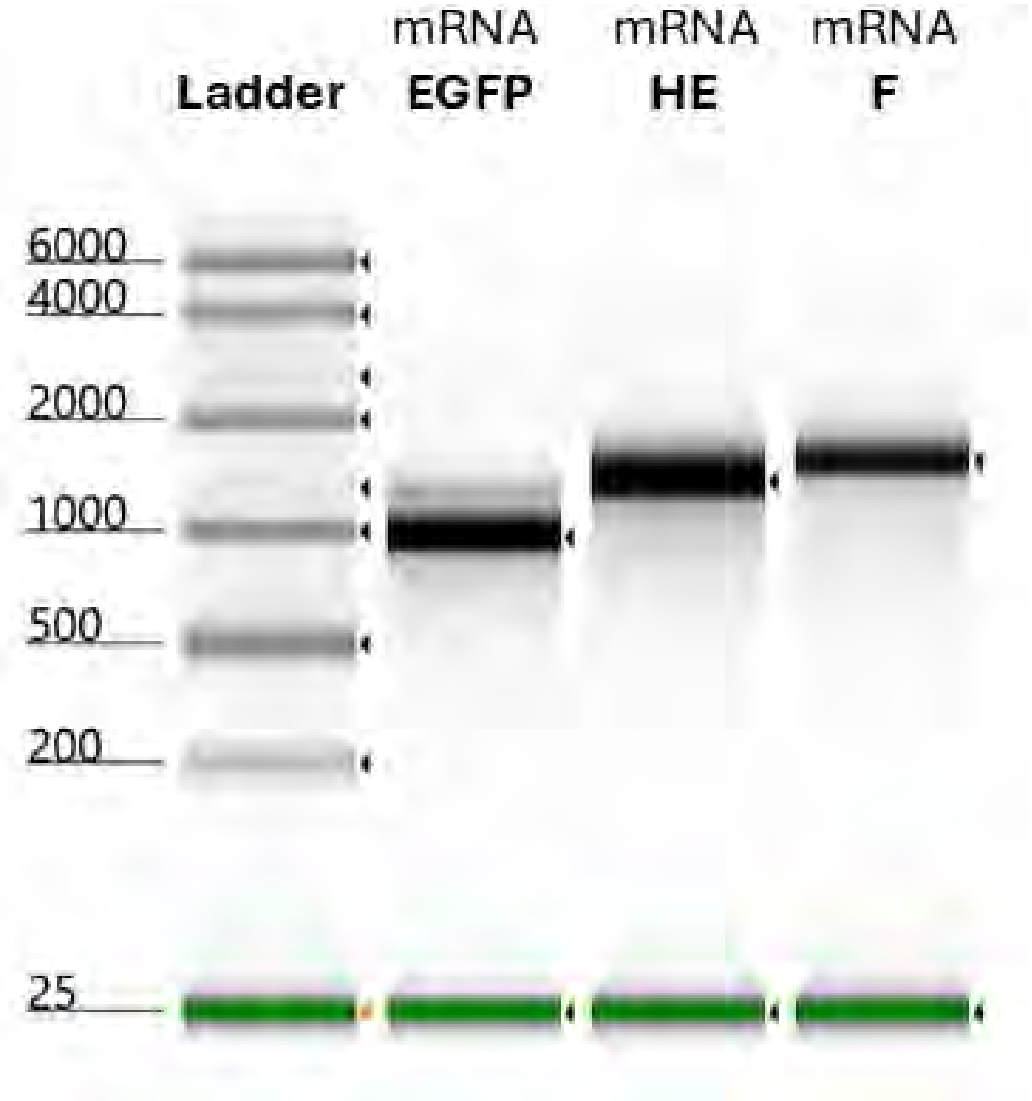
Generation of mRNA-LNPs (vaccine)

- Design of DNA plasmids encoding HE or F containing a 120 Poly(A) tail (also included EGFP mRNA as control)
- *In vitro* transcription of mRNAs with N1-methyl-pseudouridine
- Post-transcriptional capping, including Cap0 (guanosine-7-methyl) and Cap1 (methylation of the 2’-O position of the first transcribed nucleotide)
- Immunostaining of mRNA-transfected CHH-1 cells to confirm HE and F protein expression
- LNP encapsulation of mRNA by SINTEF (similar composition as Moderna's LNPs)



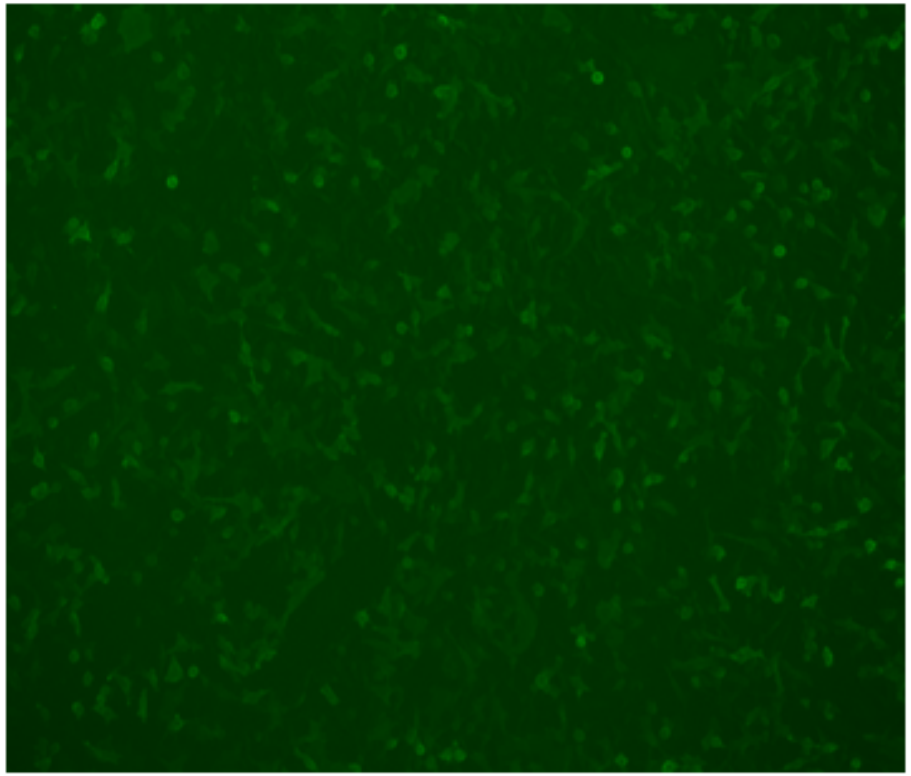
mRNA construction

- Confirmation of mRNA sizes by capillary gel electrophoresis
- Theoretical size EGFP: 988 nt
- Theoretical size HE: 1444 nt
- Theoretical size F: 1609 nt

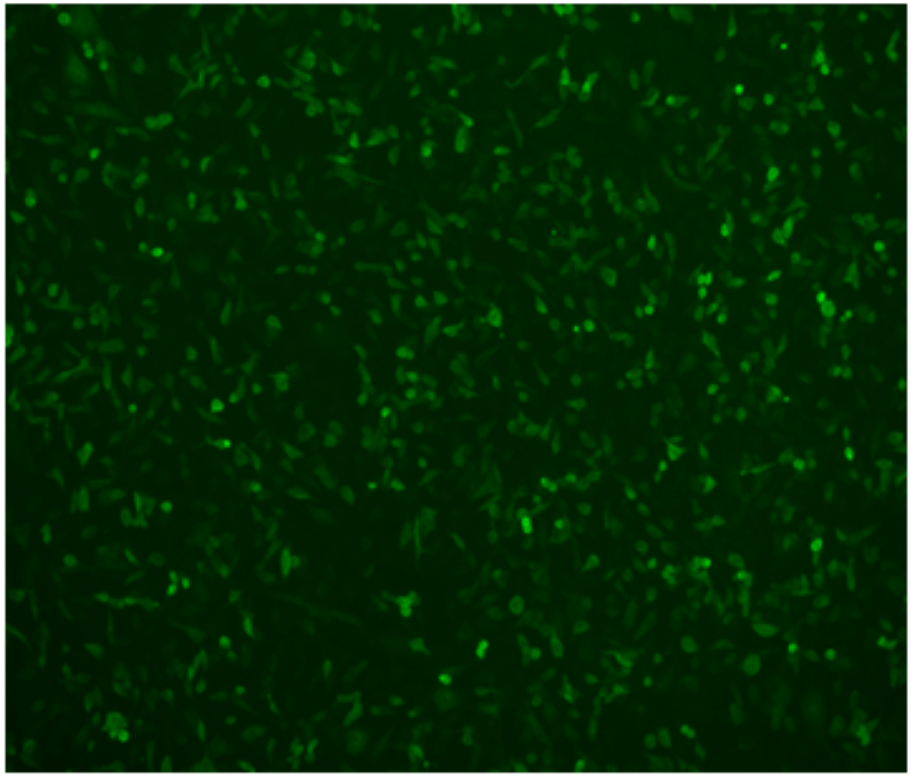


Immunostaining of CHH-1 cells transfected with HE or F mRNA (3 days post transfection)

HE mRNA



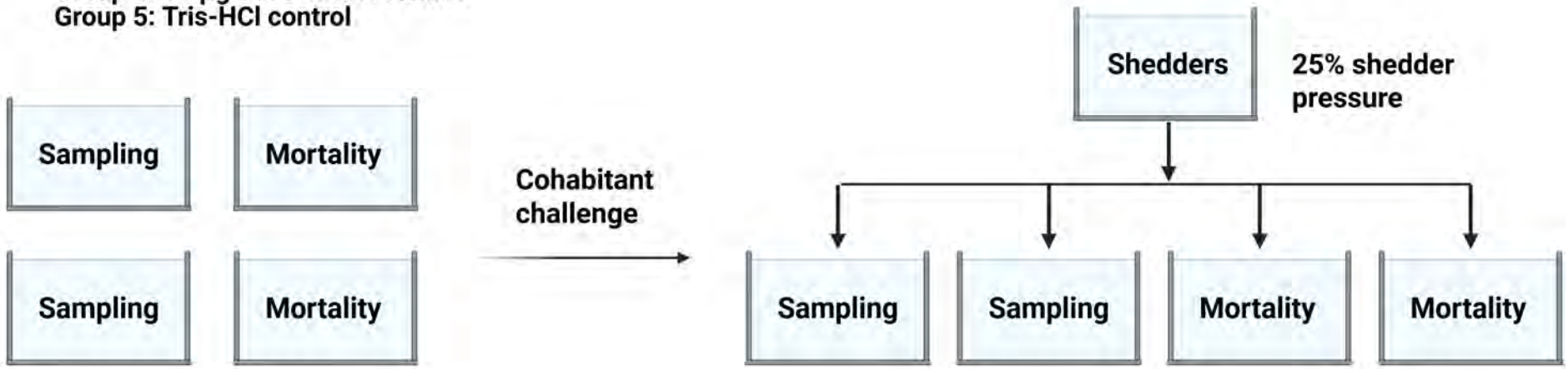
F mRNA

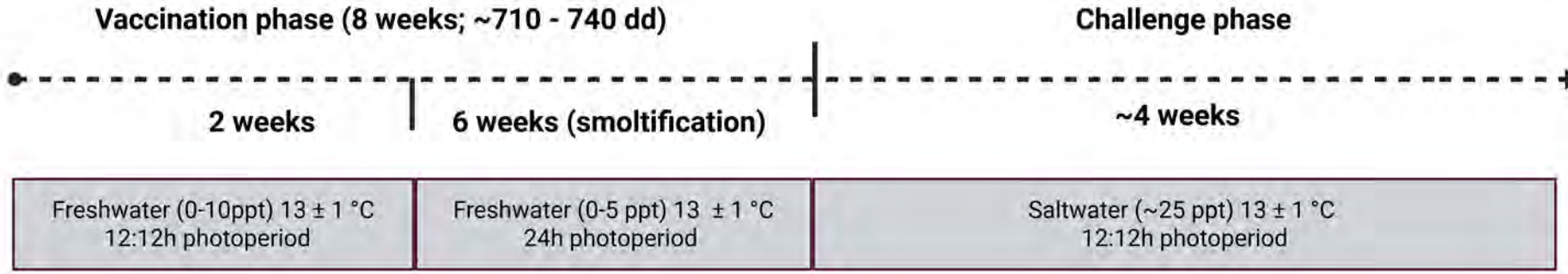


Study design

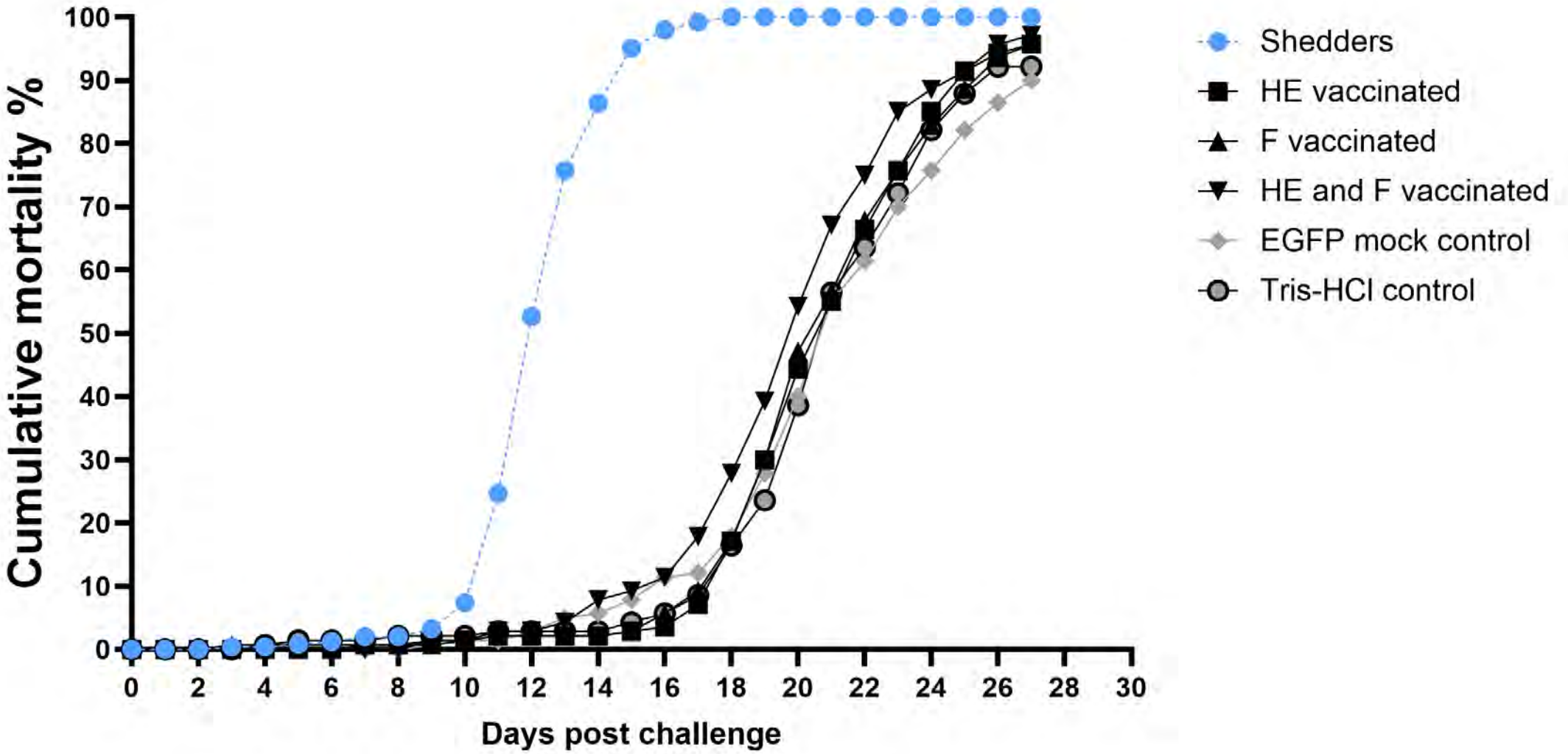
70 fish per group per tank

Group 1: 10µg HE
Group 2: 10 µg F
Group 3: 10 µg HE and 10 µg F
Group 4: 10 µg EGFP mock control
Group 5: Tris-HCl control





Mortality data from vaccine experiment



Notes

- Difference between ISAV challenge strain and mRNA sequence (NA-HPR4 and Glesvær)
- While protein expression was confirmed after IVT of mRNA, this was not checked after LNP formulation or after arrival at the fish research facility
    - LNP-encapsulated EGFP demonstrated protein expression both *in vitro* and *in vivo*
    - Vaccine shipment was still in a frozen condition at receival

Conclusion

- Our mRNA–LNP vaccines did not appear to prevent infection of ISAV
- Studies in rainbow trout have shown protection against rhabdovirus infections when administering mRNA vaccines
- Future studies should aim to optimize this vaccine technology for Atlantic salmon, including LNP formulation, mRNA construct optimization, administration route and dosage, self-amplifying mRNA(?)

FHF

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