Efficacy indicators of a novel DNA vaccine in Atlantic salmon infected with SAV3 using a cohabitation challenge model

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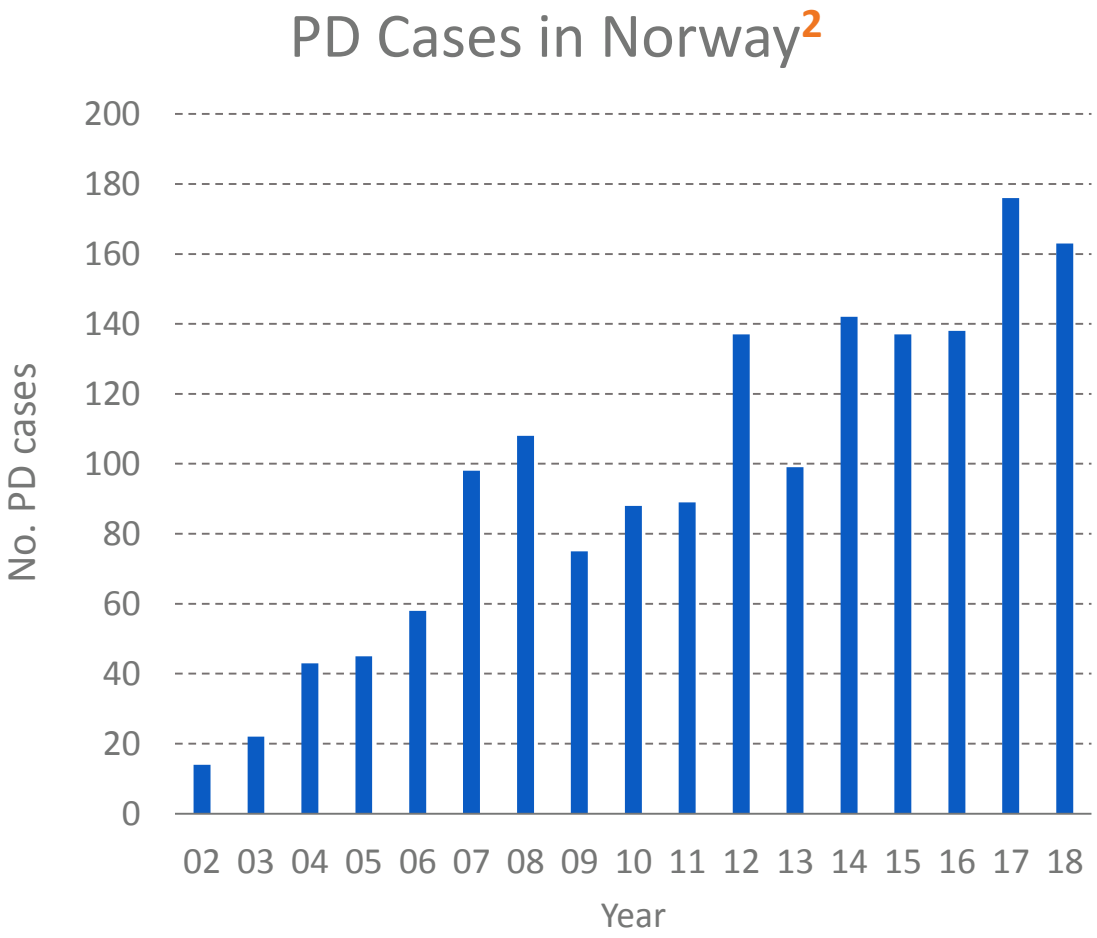
Background

PD is the most costly clinical disease in the Norwegian salmon industry[1] despite widespread use of oil-adjuvanted PD vaccines[2]



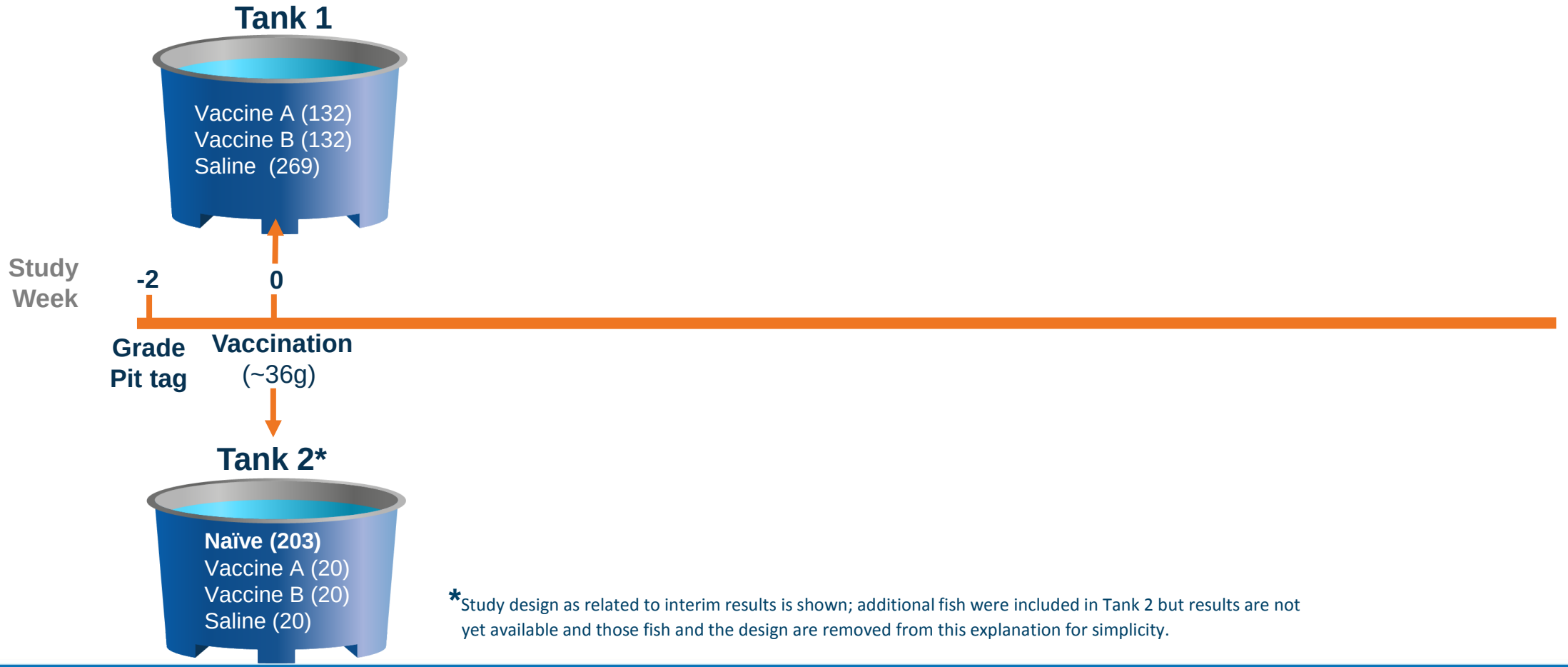
1. Pettersen, J.M. et al., 2015. The economic benefits of disease triggered early harvest: A case study of pancreas disease in farmed Atlantic salmon from Norway. Preventive veterinary medicine. 121, 314-324.
2. Annual fish health report 2018, NVI, Oslo, Norway

Study objectives

- Ascertain and compare different PD vaccines – a DNA vaccine recently licensed in EU (**Vaccine A**) to an oil-adjuvant vaccine (**Vaccine B**) with respect to different SAV3 efficacy indicators
  - Ability of immune plasma to neutralize SAV3 *in vitro*
  - Level of SAV3 transmission from vaccinated challenged fish to naïve fish
  - Efficacy against SAV3 using cohabitation challenge model in seawater
    - Viremia
    - Mortality
    - Growth
    - Histopathological analysis

Study design – Tank set up

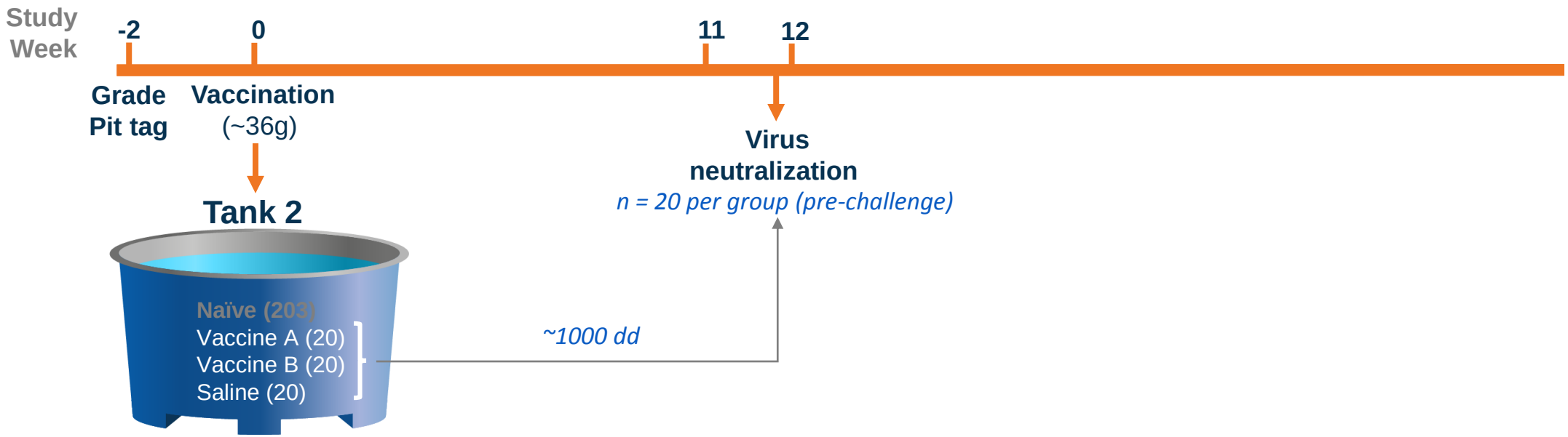
VESO Vikan, 12°C



*Study design as related to interim results is shown; additional fish were included in Tank 2 but results are not yet available and those fish and the design are removed from this explanation for simplicity.

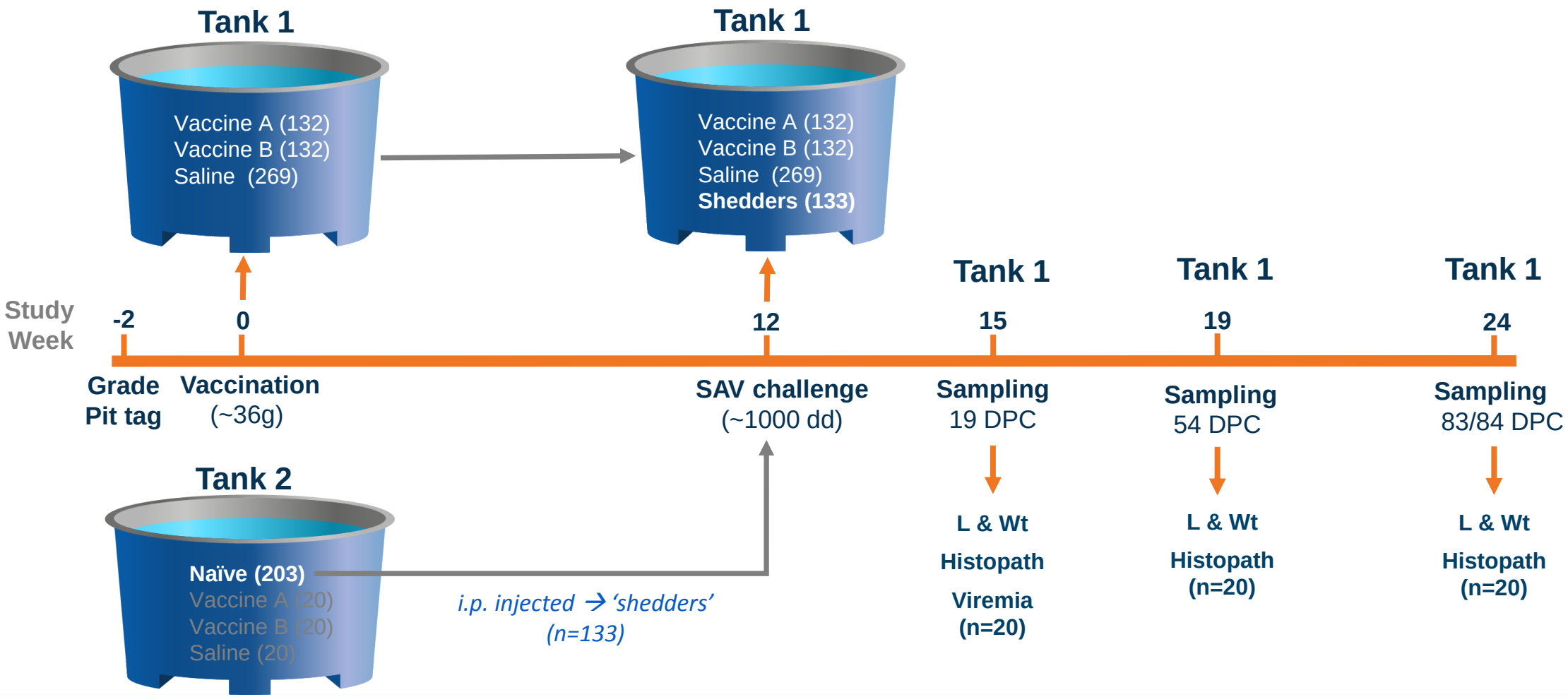
Study design – Virus neutralization

VESO Vikan, 12°C.



Study design – Efficacy

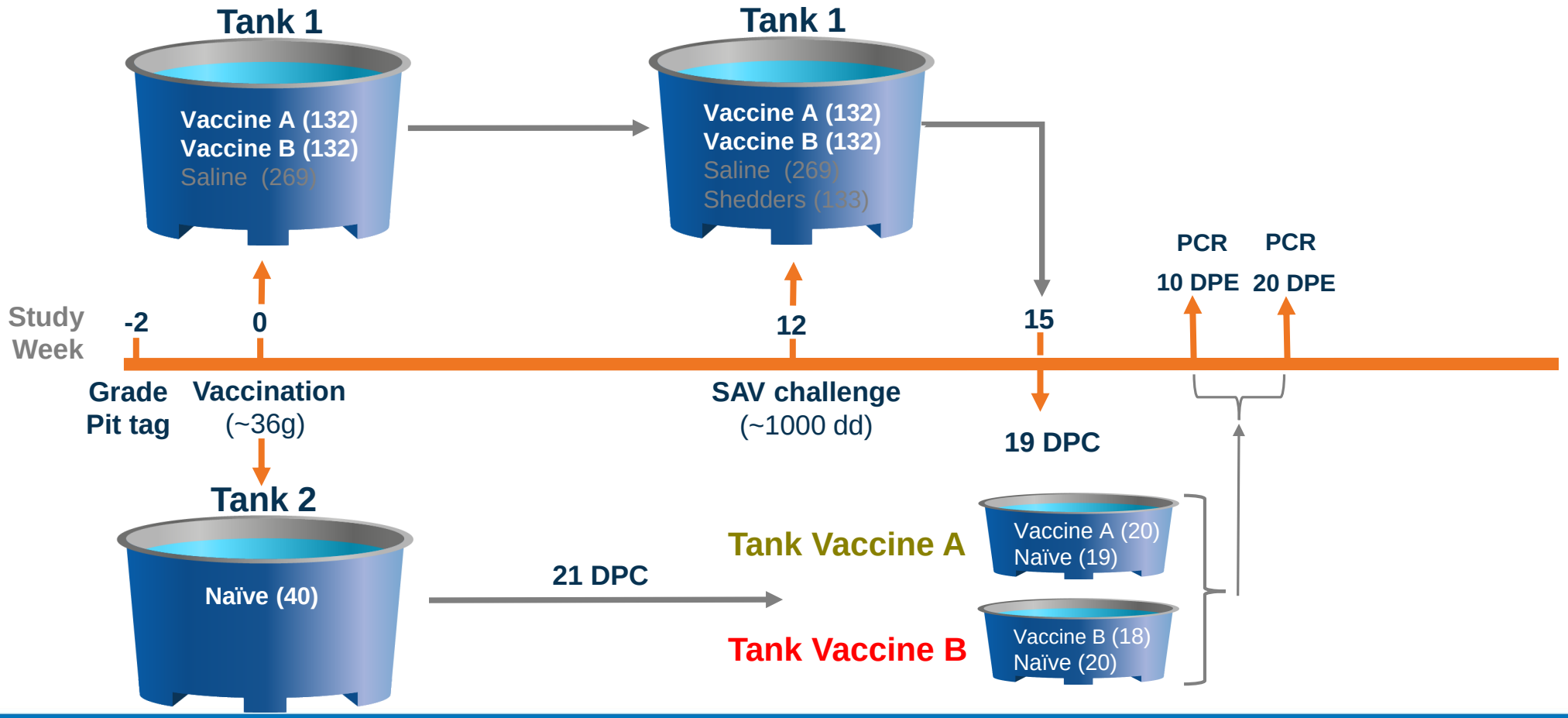
40-40-20 Elanco SAV3 cohabitation challenge model in seawater (VESO Vikan, 12°C)



DPC = Days Post Challenge

Study design – Virus transmission

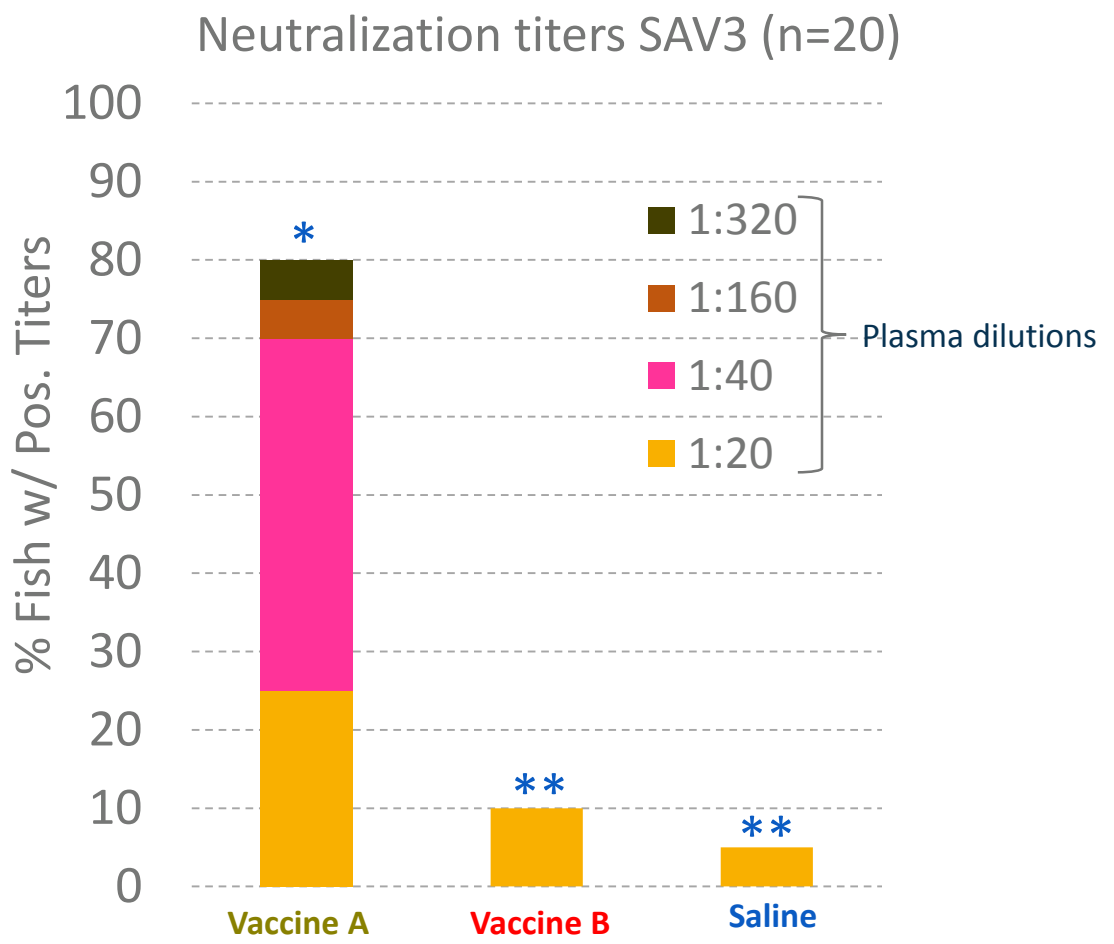
VESO Vikan, 12°C



DPC = Days Post Challenge
DPE = Days Post Exposure

Neutralization titers

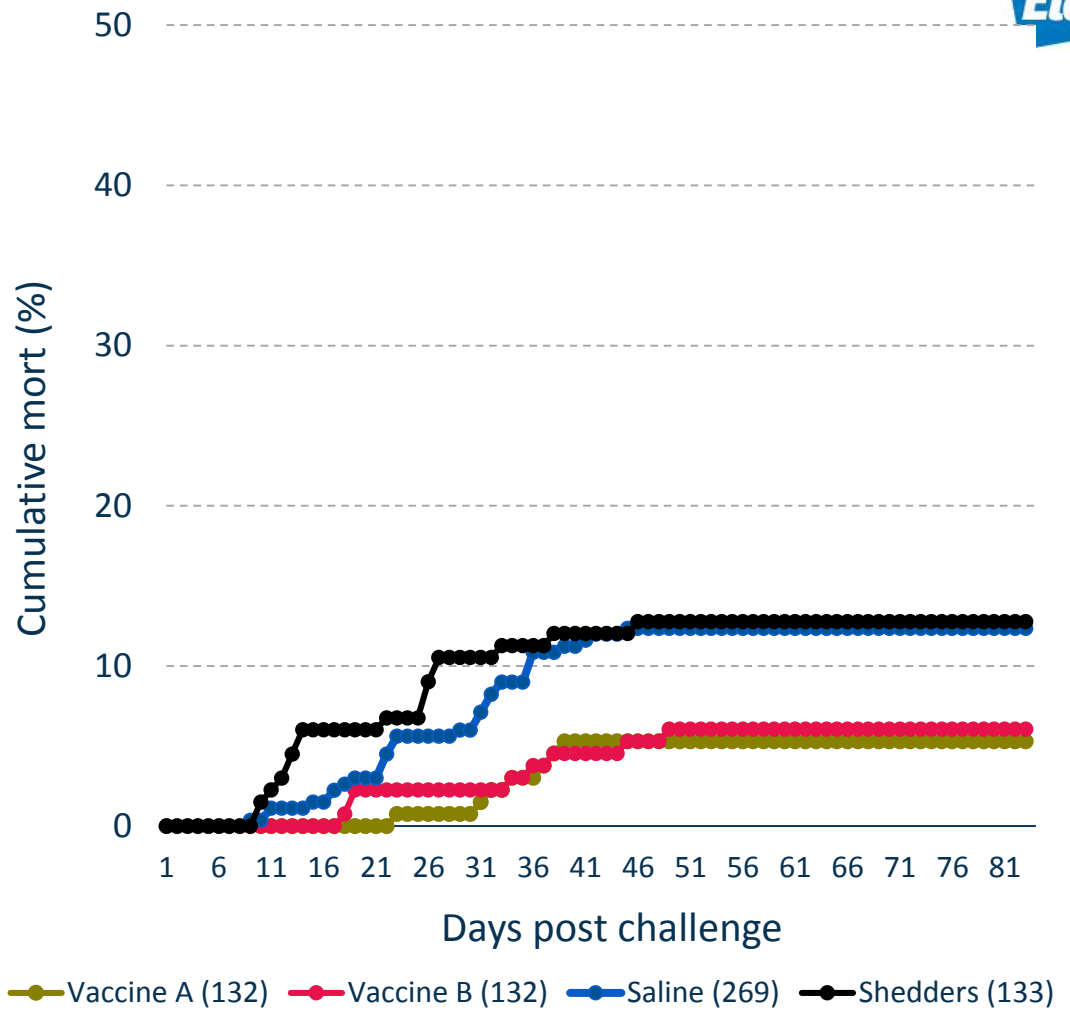
- Plasma samples taken at time of challenge (~1000 dd)
- Performed blinded at NVI
- Standardized assay based on modifications from Graham et al 2003[1]
- **Vaccine A** induced significantly higher titers than **Vaccine B** (p<0,001) and Saline (p<0,001)[2]



1. Graham, D., Jewhurst, V. M., Rowley, H., McLoughlin, M. & Todd, D., 2003. A rapid immunoperoxidase-based virus neutralization assay for salmonid alphavirus used for serological survey in Northern Ireland. Journal of fish diseases. 26. 407-13.
2. Fisher Exact Test and Ordinal Logistic Regression Model; Statistical analyses were completed with Stata/ MP 15 for Windows (StataCorp, College Station, TX)

Cumulative mortality

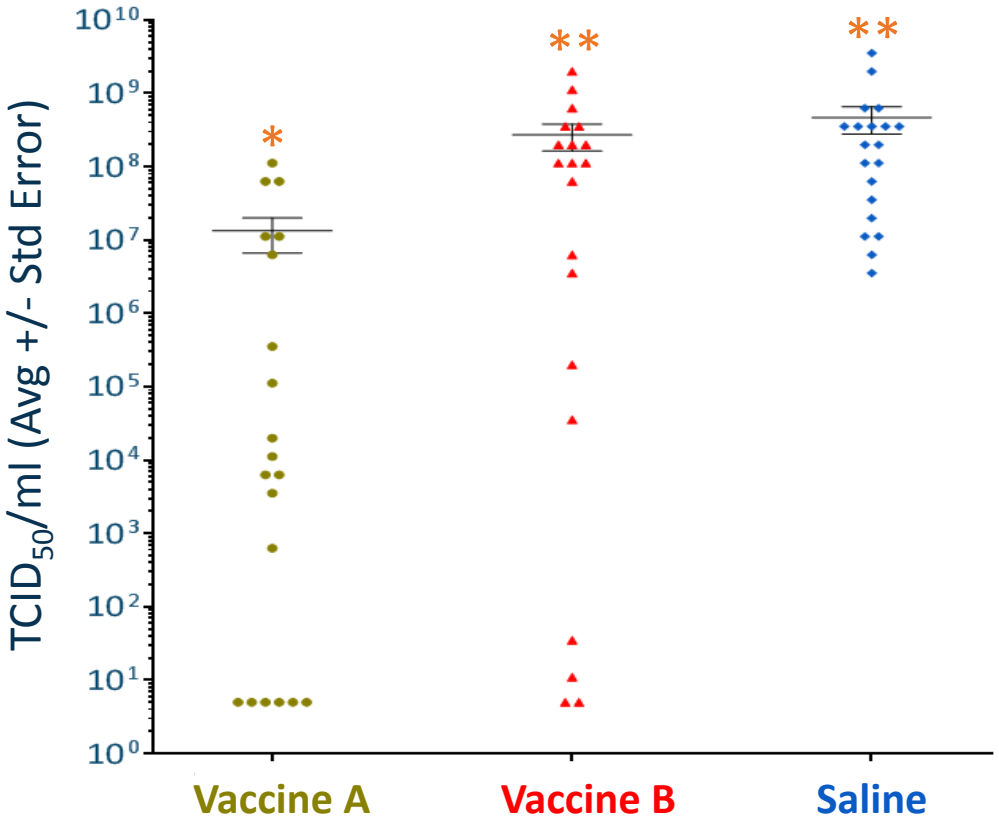
- Mortality pattern low & as expected for model
  - Vaccinates differed from Saline[1]
    - **Vaccine A vs. Saline p = 0,05**
    - **Vaccine B vs Saline p = 0,08**
  - Vaccinate mortality pattern similiar[1]
    - **Vaccine A** = 5,3%; **Vaccine B** = 6.1%; p=0.79
- Open skin wounds observed in moribund and dead fish – secondary to PD lethargy
  - Not *Moritella viscosa*
  - Not *Tenacibaculum* spp.
  - No indications of other diseases in histopathology



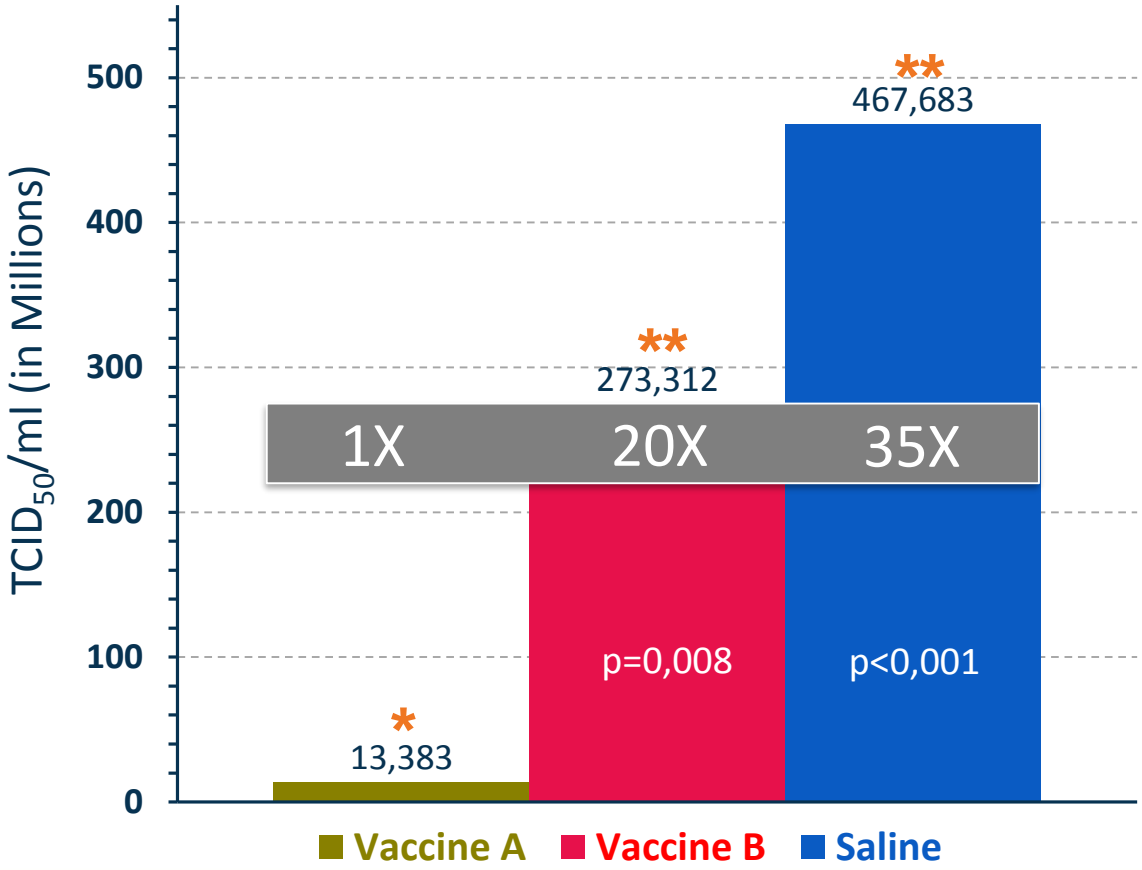
1. Cox regression analysis. Statistical analyses were completed with Stata/ MP 15 for Windows (StataCorp, College Station, TX)

PD virus in plasma (Viremia)[1] - 19 DPC

Concentration in plasma (n=20)



Avg. Concentration in plasma (in Millions)



1 Jewhurst, V A, Todd, D, Rowley M H, Walker I W, Weston J H, McLoughlin, M F & Graham, D A, 2004. Detection and antigenic characterization of salmonid alphavirus isolates from sera obtained from farmed Atlantic salmon, *Salmo salar* L., and farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of fish diseases. 27. 143-9.

* Quantile regression analysis. Statistical analyses were completed with Stata/ MP 15 for Windows (StataCorp, College Station, TX)

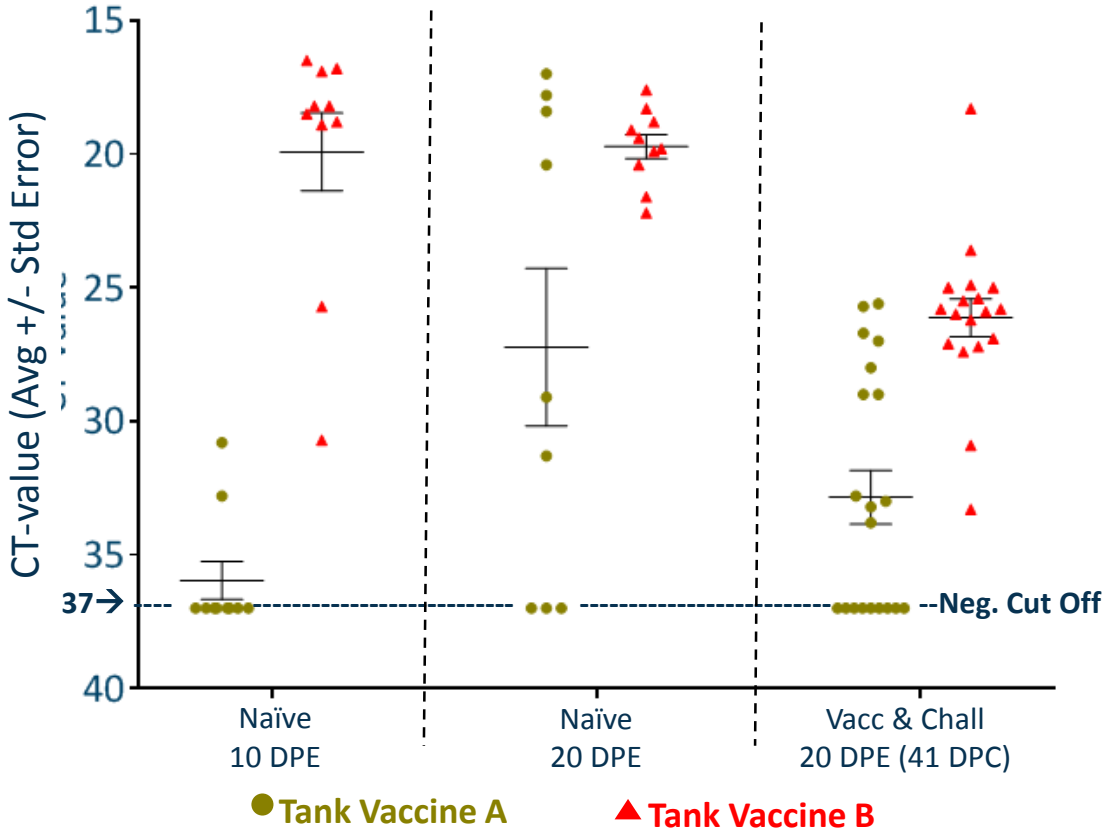
Transmission study

Tank Vaccine A — Vaccine A (20) Naïve (19)

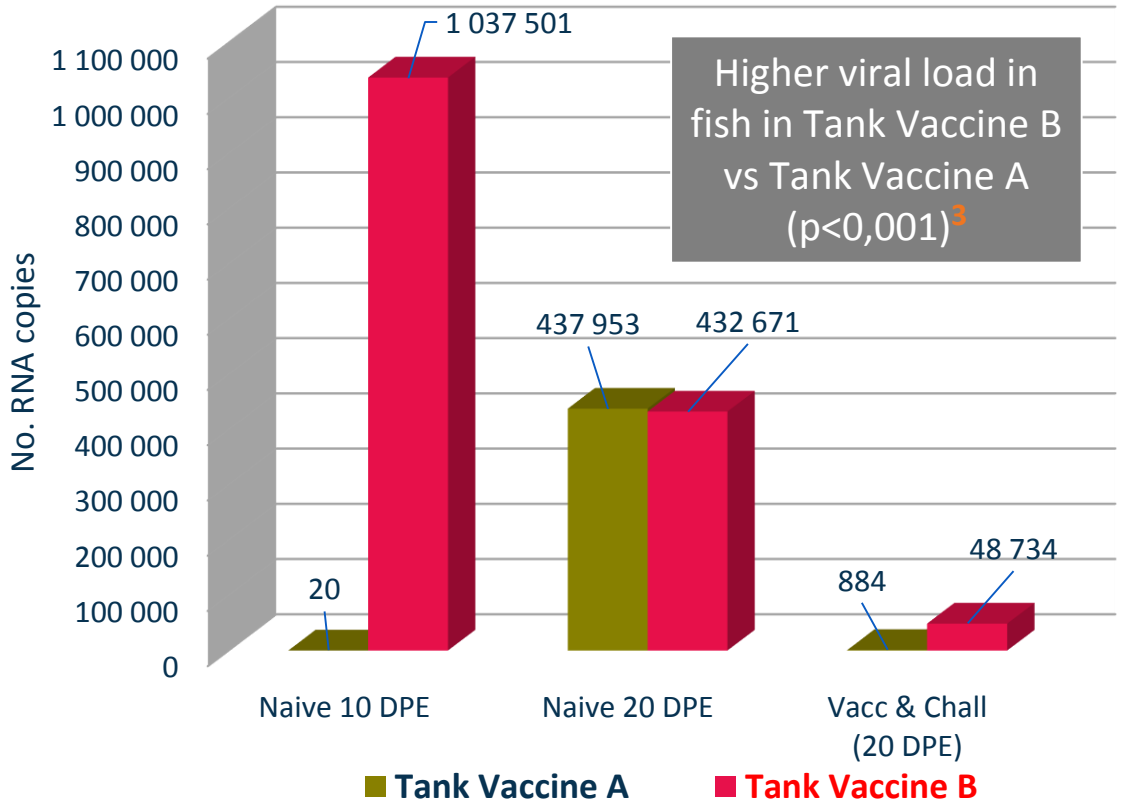
Tank Vaccine B — Vaccine B (18) Naïve (20)

Elanco

Q RT-PCR for SAV3[1]

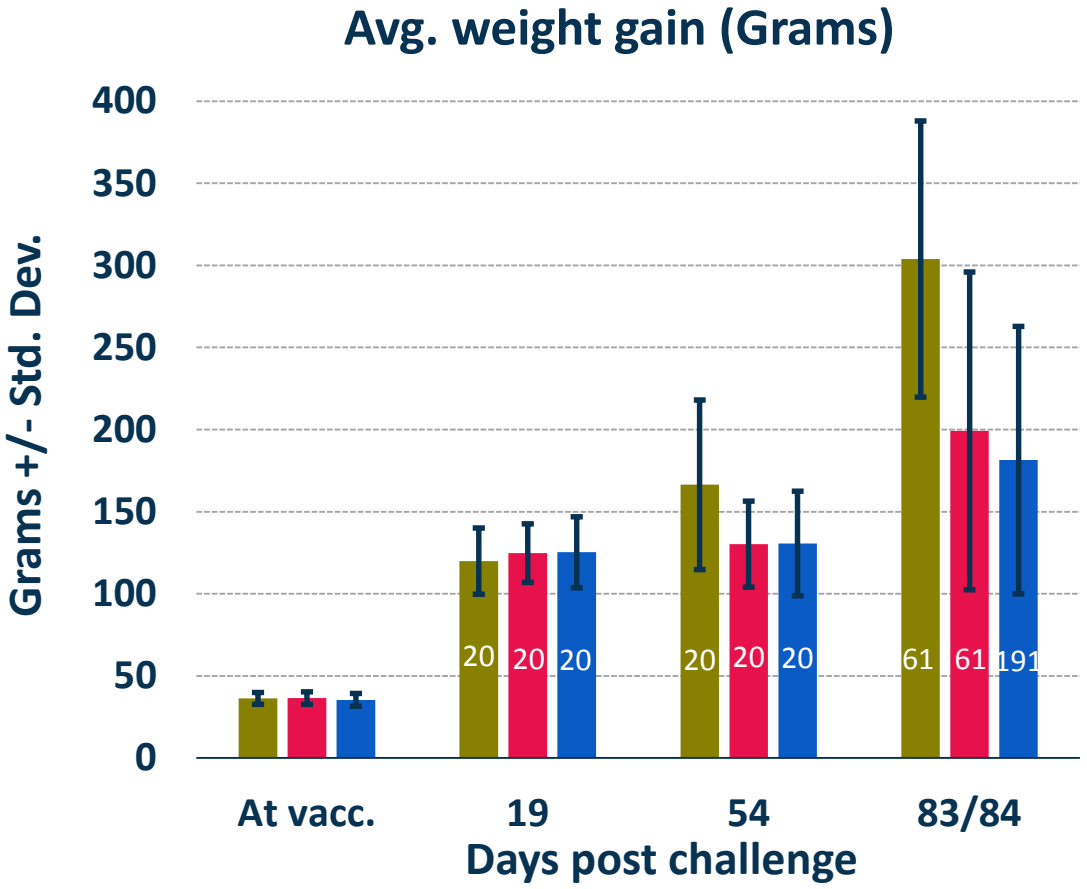


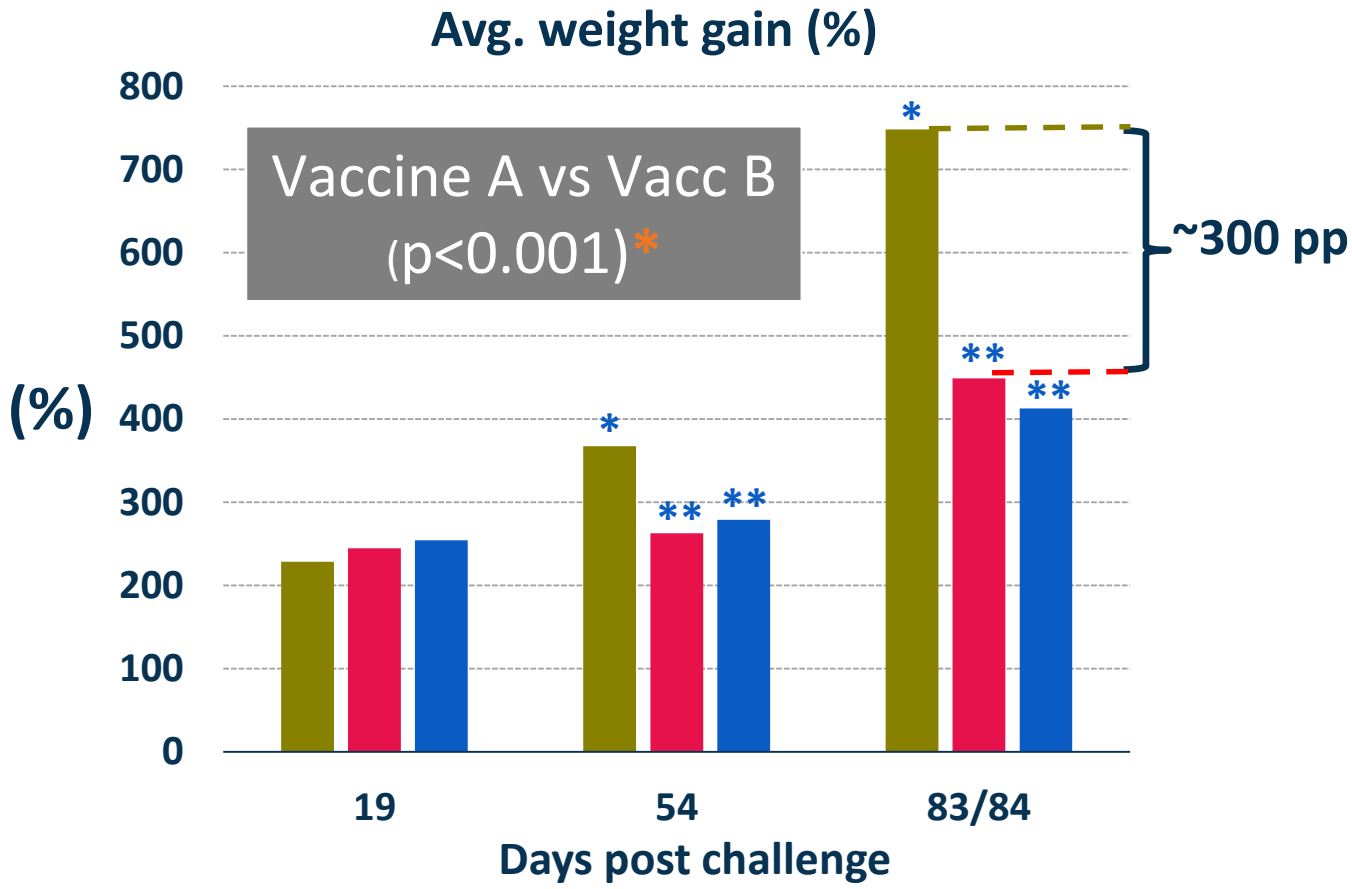
Avg. no. of SAV3 RNA copies[2]



1. Patogen AS
2. Hodneland, K & Endresen, C, 2006. Sensitive and specific detection of Salmonid alphavirus using real-time PCR (TaqMan ®). Journal of virological methods. 131. 184-92.
3. Fisher Exact & Kruskal Wallis Tests. Statistical analyses were completed with Stata/ MP 15 for Windows (StataCorp, College Station, TX)

Growth post SAV3 challenge





Vaccine A Vaccine B Saline

* Linear regression analysis. Statistical analyses were completed with Stata/ MP 15 for Windows (StataCorp, College Station, TX)

Histopathology – interim data

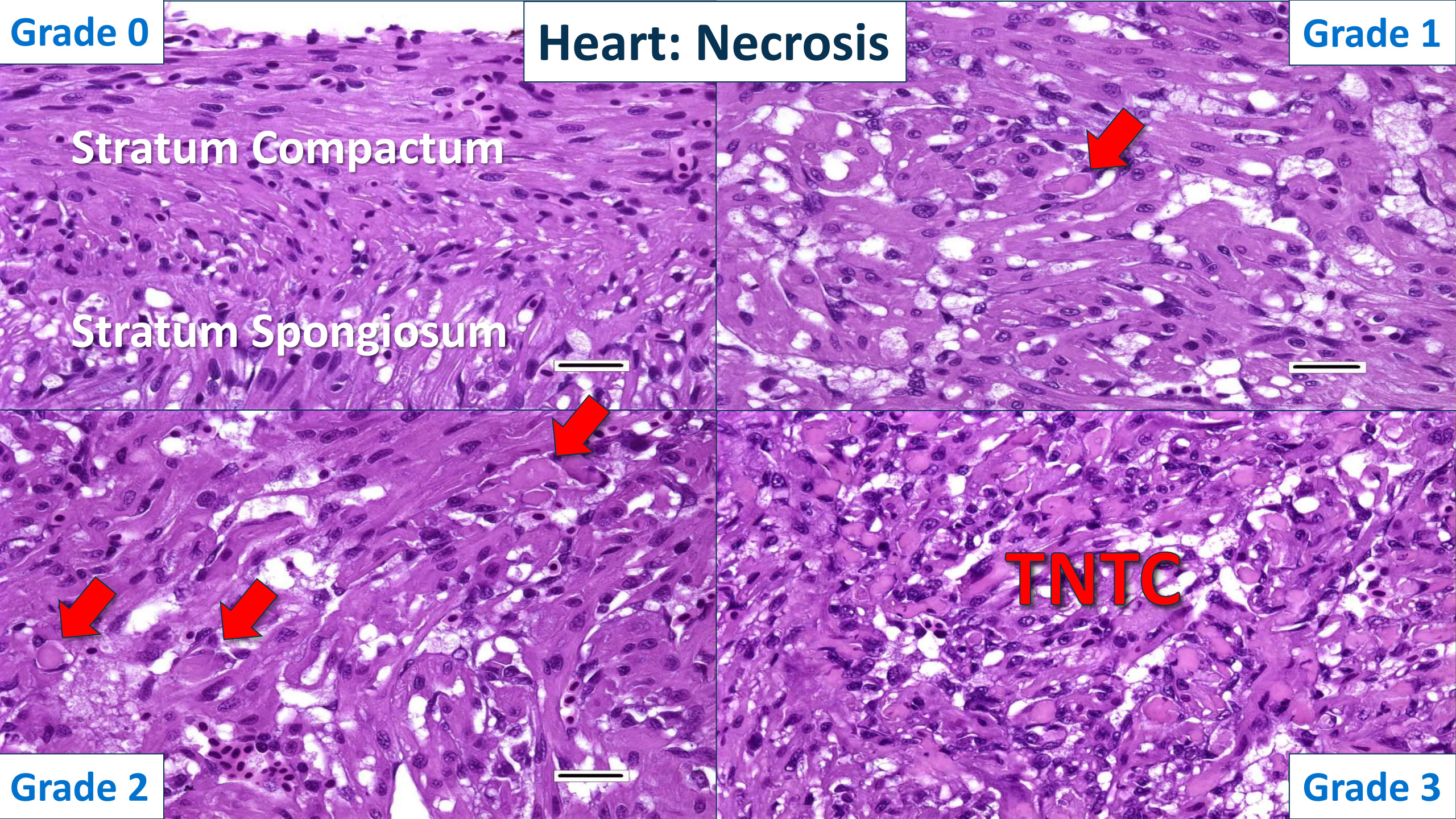
- Specimens randomized and code then evaluated without knowledge of exposure group (‘blinded’)
- Tissues: heart necrosis & loss of exocrine pancreas tissue 19, 54 and 83 DPC
- Standardized severity grading for each diagnostic criteria and tissue type

Diagnostic criteria for heart and pancreas tissues

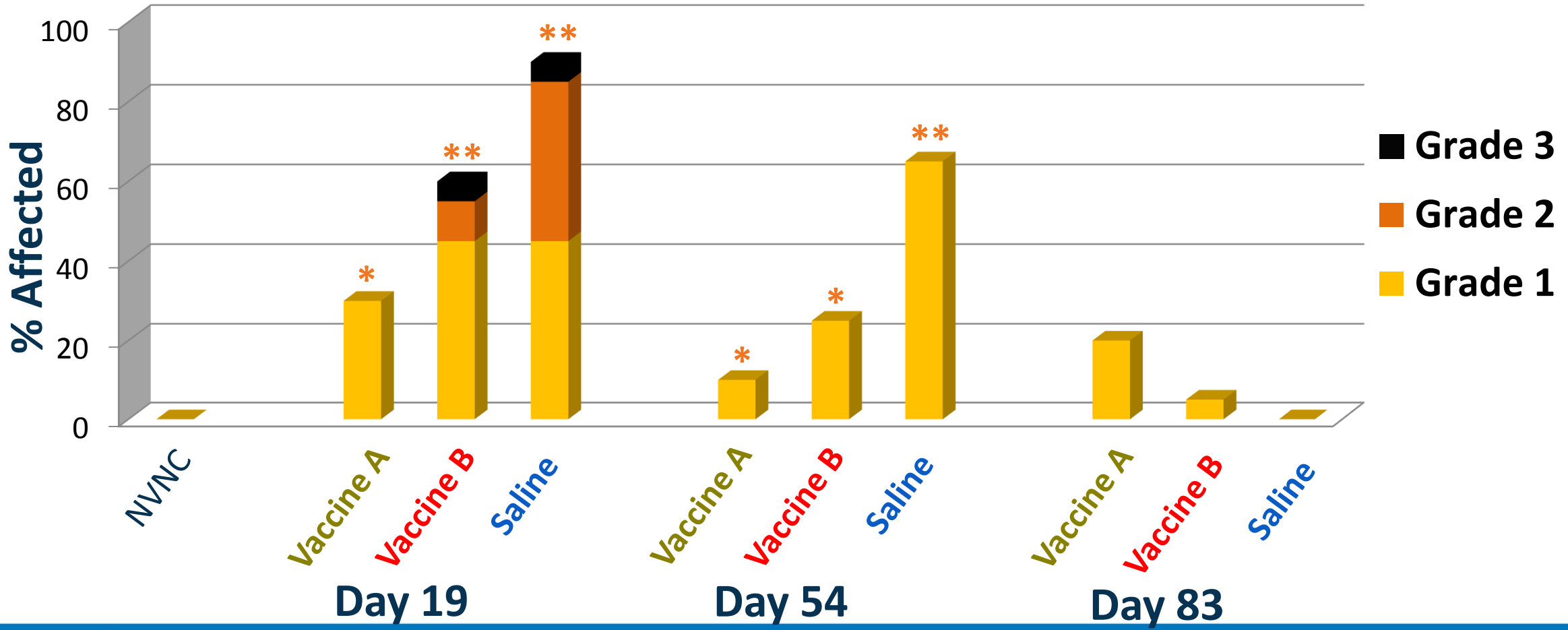
	Heart	Pancreas
Necrosis	**X**	X
Inflammation	X	X
Regeneration	X	
Fibrosis		X
Tissue loss		**X**

Standardized severity grading for diagnostic criteria

Grade 0	Not remarkable
Grade 1	Mild
Grade 2	Moderate
Grade 3	Severe


Heart: Necrosis
Grade 0
Stratum Compactum
Stratum Spongiosum
Grade 1
Grade 2
TNTC
Grade 3

Heart necrosis post SAV3 challenge

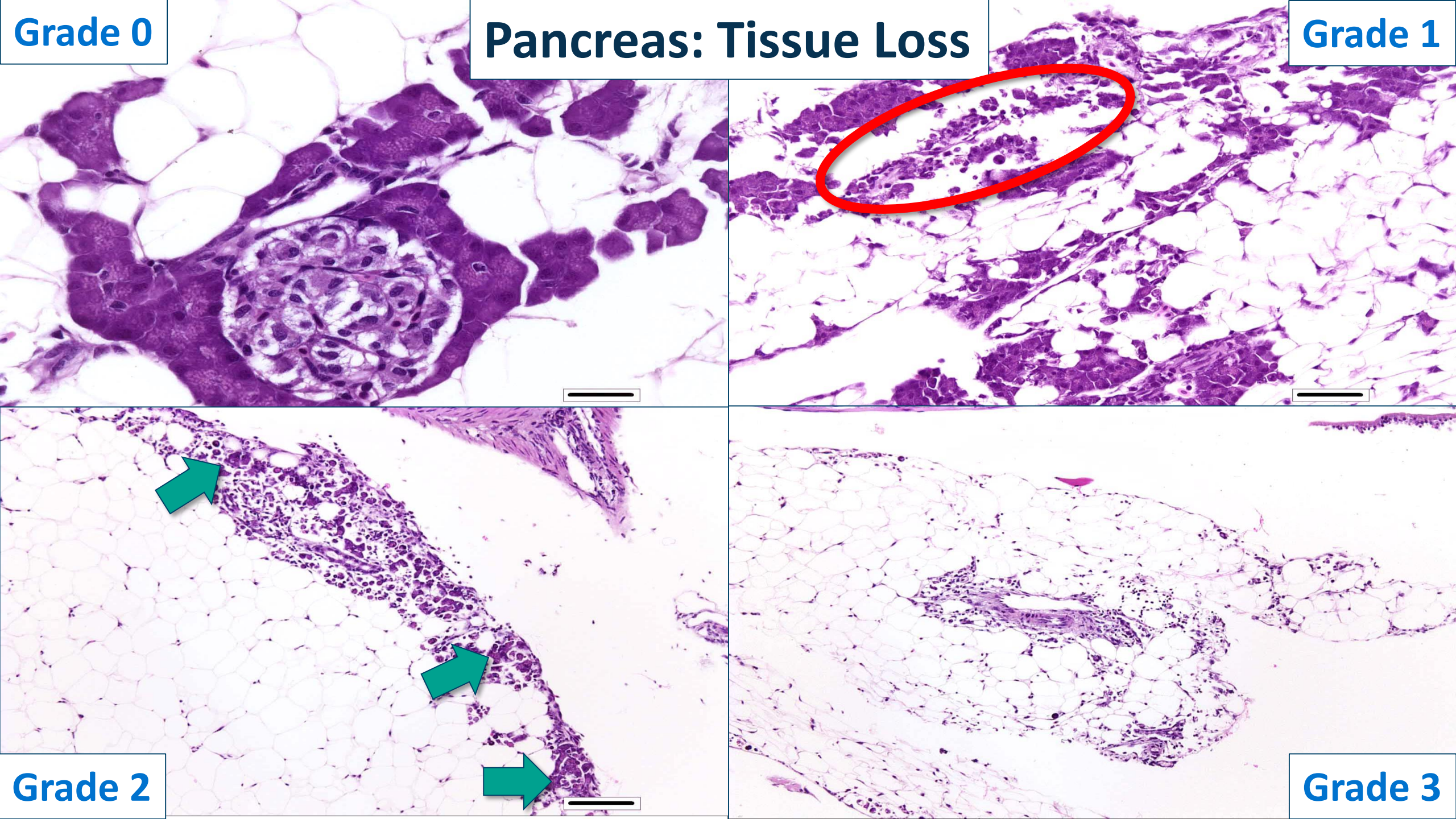


NVNC= non-vaccinated, non-challenged

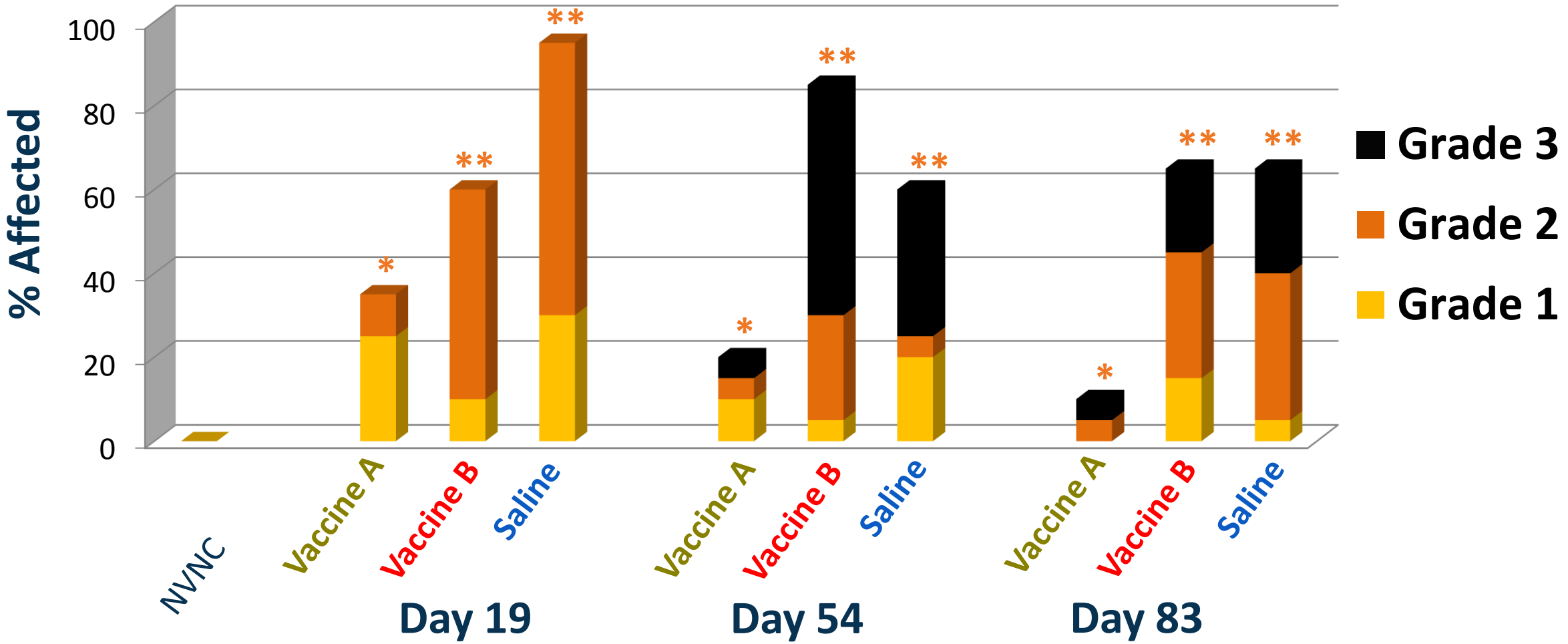
n = 20/group

*Ordinal logistic regression; * vs. ** (p < 0.05)*

Pancreas: Tissue Loss



Pancreas tissue loss post SAV3 challenge



NVNC= non-vaccinated, non-challenged
n = 20/group
*Ordinal logistic regression; * vs. ** (p < 0.05)*

Conclusions

- **Mortality**
  - ✓ Model provided low mortality in saline and vaccinate groups, as expected
  - ✓ Vaccinated groups had improved and similar survival vs. saline
- **DNA vaccine (Vaccine A) significantly outperformed the oil-based vaccine (Vaccine B) based on the following efficacy indicators;**
  - ✓ pre-challenge SAV3 neutralization capacity
  - ✓ 20X reduction SAV3 viremia levels at 19 DPC
  - ✓ reduced SAV3 transmission rates to naïve fish
  - ✓ ~300 percentage points weight gain during the 83/84 day challenge period
  - ✓ protection against SAV3-induced disease in the heart and pancreas – Interim results

Acknowledgements

Thank you for your attention!