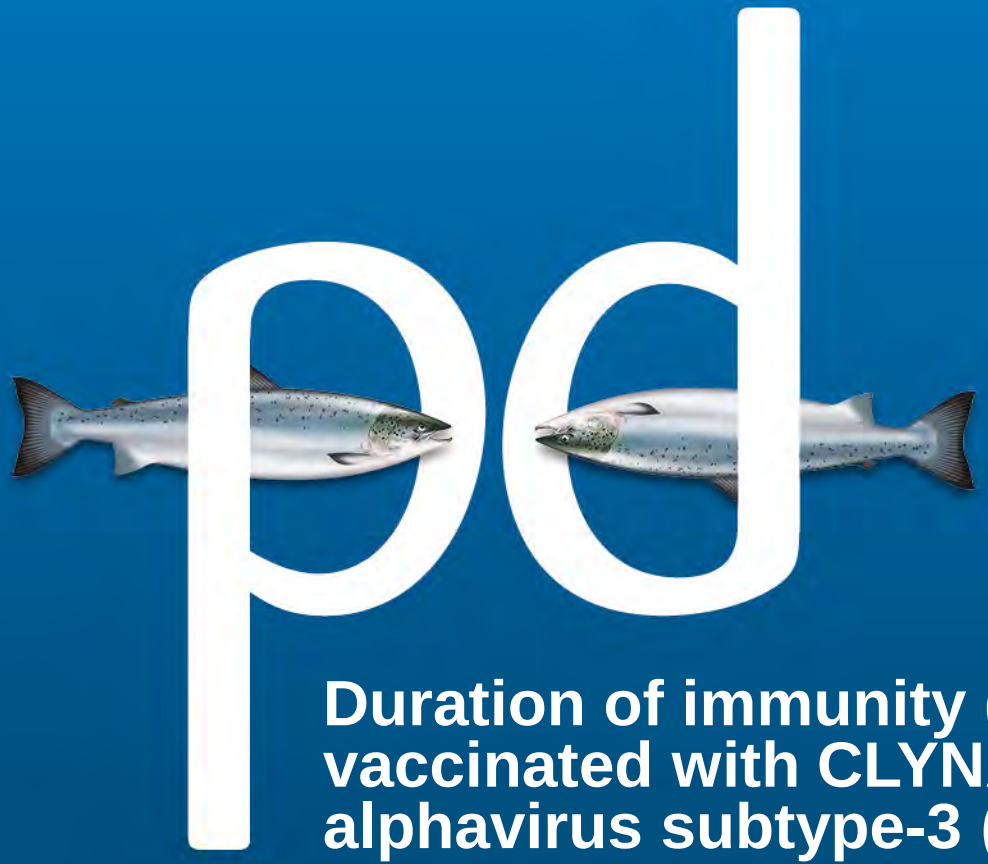


Duration of immunity (DOI) study in Atlantic salmon parr vaccinated with CLYNAV using a saltwater (SW) Salmonid alphavirus subtype-3 (SAV3) cohabitation challenge model

[1]Lisa Phillips , [1]Ginny Jones , [1]Jennifer Harkness, [1] Paul Tonita , [2] Jeffrey C. Wolf, [3]Ana Silva

[1] Elanco Canada Ltd.,
[2] Experimental Pathology Laboratories Inc., USA
[3] Elanco Denmark ApS Norway Branch

PD TriNation, 21-22 April, 2021

CLYNAV… a great step forward

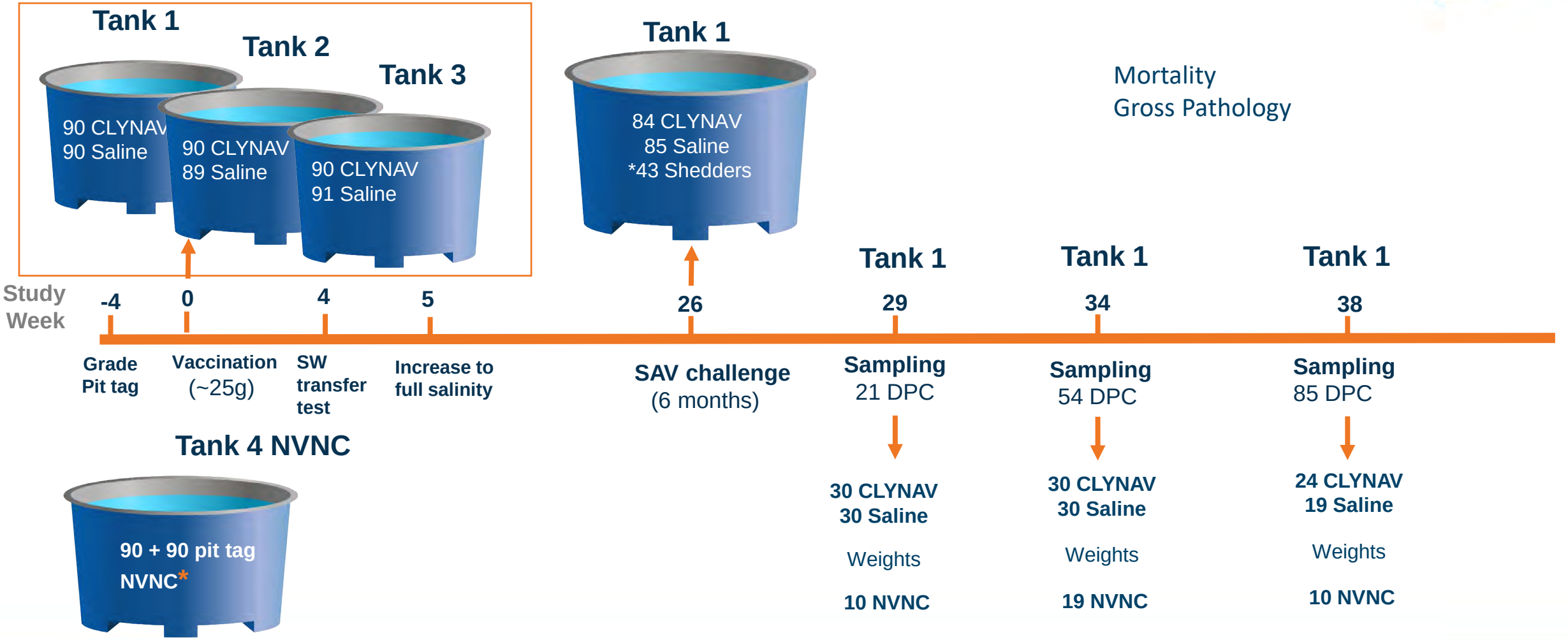
To Re-Cap

- Used the SW cohabitation model and demonstrated efficacy using relevant clinical endpoints for 1 and 3 months ….
  - Impact on average weight gain and mortality
  - Target organ histopathologic findings (heart, pancreas, red and white skeletal muscle)
  - Appearance and timing of gross lesions
- Used challenge parameters of ~35 kg/m^3 at challenge, temperature 14 ºC +/- 2°C with ratios of 40% vaccinates + 40% controls + 20% shedders (IP injected with SAV3)
- Optimized use of tank space and maximized viral pressure by using freshly smolted Trojan shedders

Reference NAH-14-138 & NAH-16-055

Study design

SAV3 cohabitation challenge in SW 6 months post vaccination



*NVNC= Not Vaccinated Not Challenged

DPC = Days Post Challenge

* Newly smolted fish ~ 50g i.p. injected Trojan shedders

Study design

SAV3 cohabitation challenge in SW 9.5 months post vaccination

Mortality
Gross Pathology

Tank 2.G1 | Tank 2.G3 | Tank 2.G2

Study Week: -4 | 0 | 4 | 5 | 33 | 41 | 44 | 49 | 53

Grade Pit tag | **Vaccination** (~25g) | **SW transfer test** | **Increase to full salinity** | **Tank 2 split into 3 tanks** | **SAV challenge** (9.5 months) | **Sampling** 21 DPC | **Sampling** 55 DPC | **Sampling** 84 DPC

Tank 4 NVNC

29 CLYNAV
26 Saline
Weights
10 NVNC

28 CLYNAV
22 Saline
Weights
10 NVNC

26 CLYNAV
18 Saline
Weights
10 NVNC

*NVNC= Not Vaccinated Not Challenged

DPC = Days Post Challenge

* Newly smolted fish ~ 39g i.p. injected Trojan shedders

Study design

SAV3 cohabitation challenge in SW 12 months post vaccination

Mortality
Gross Pathology

Tank 3.I2 Tank 3.I1 Tank 3.I3

Study Week: -4 | 0 | 4 | 5 | 41 | 52 | 55 | 60 | 65

Grade Pit tag (-4)

Vaccination (~25g) (0)

SW transfer test (4)

Increase to full salinity (5)

Tank 3 split into 3 tanks (41)

SAV challenge (12 months) (52)

Sampling 19 DPC (55)
- **28 CLYNAV**
- **29 Saline**
- Weights
- **Histopathology**
- **10 NVNC**

Sampling 54 DPC (60)
- **28 CLYNAV**
- **29 Saline**
- Weights
- **Histopathology**
- **10 NVNC**

Sampling 89 DPC (65)
- **28 CLYNAV**
- **27 Saline**
- Weights
- **Histopathology**
- **9 NVNC**

*NVNC= Not Vaccinated Not Challenged

DPC = Days Post Challenge

* Newly smolted fish ~ 57g i.p. injected Trojan shedders

Gross internal observations 6-month challenge

Prevalence (percentage) of occurrence by group and sampling time (Days Post-challenge (DPC))*

DPC	Group	# sampled	Emaciated / thin / No fat	Fluid / mucus filled intestines	Petechial hemorrhage in cecal fat	Ascites	Pale / abnormal heart	Raspberry-like or swollen spleen	Yellow / pale liver
21	Saline	30		83%	93%	3%		17%	
	CLYNAV	30		20%	27%	3%			
	NVNC	10							
54	Saline	30	57%	93%			3%		10%
	CLYNAV	30							
	NVNC	19							
85	Saline	19	89%	79%				32%	
	CLYNAV	24							
	NVNC	10							

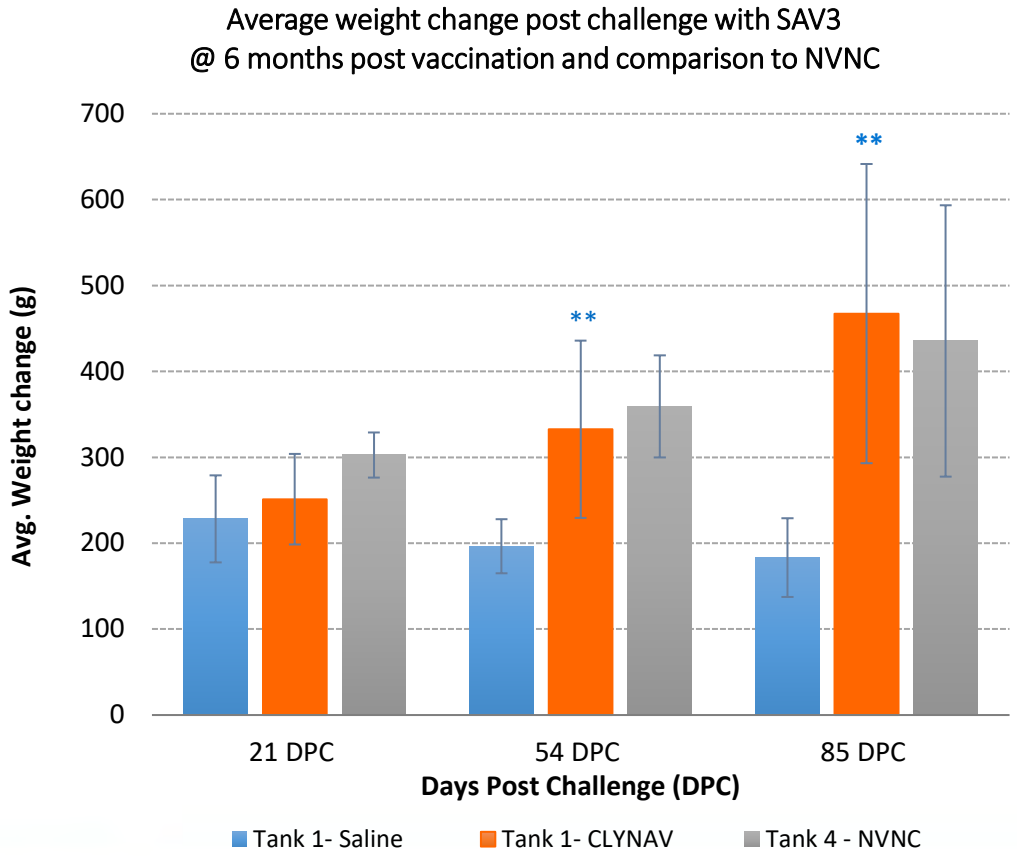
* Empty cell represents 0% prevalence

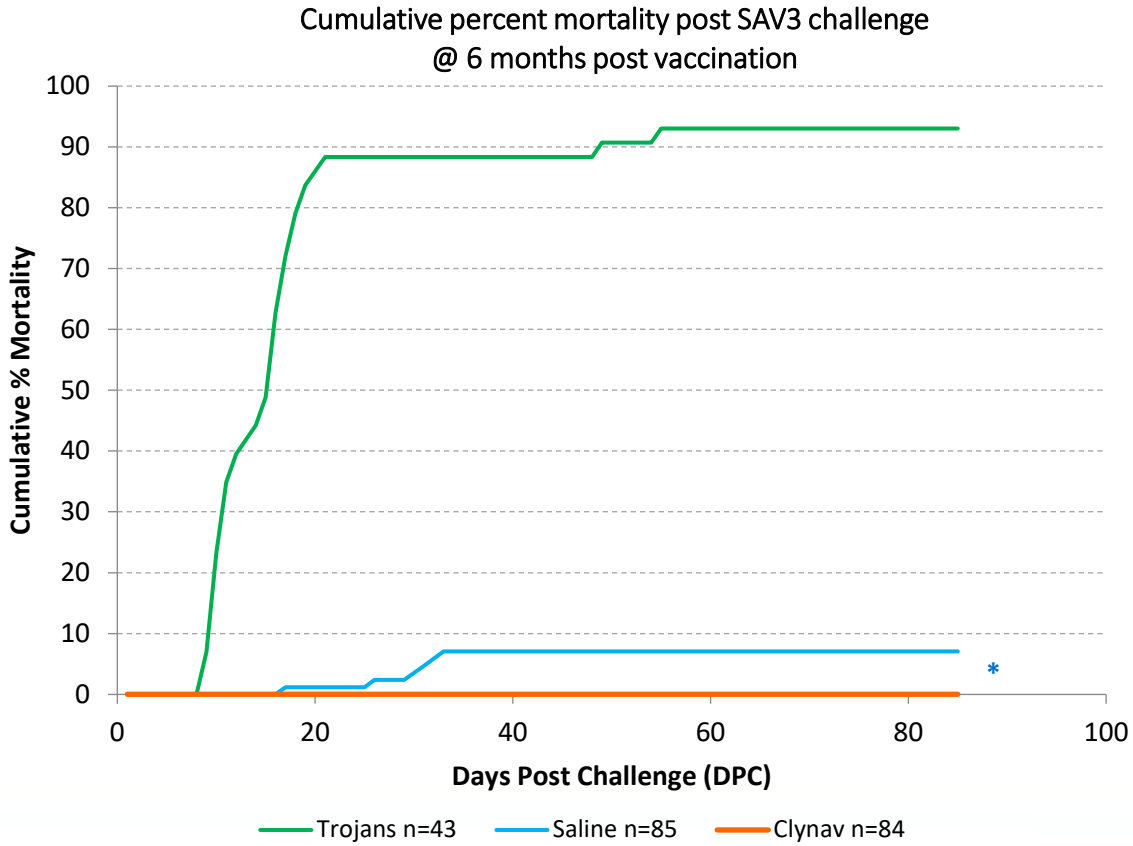
Gross internal observations 6-month challenge

Prevalence (percentage) of occurrence by group and sampling time (Days Post-challenge (DPC))

6-month duration of immunity (DOI) data

Bodyweight change and cumulative mortality post challenge

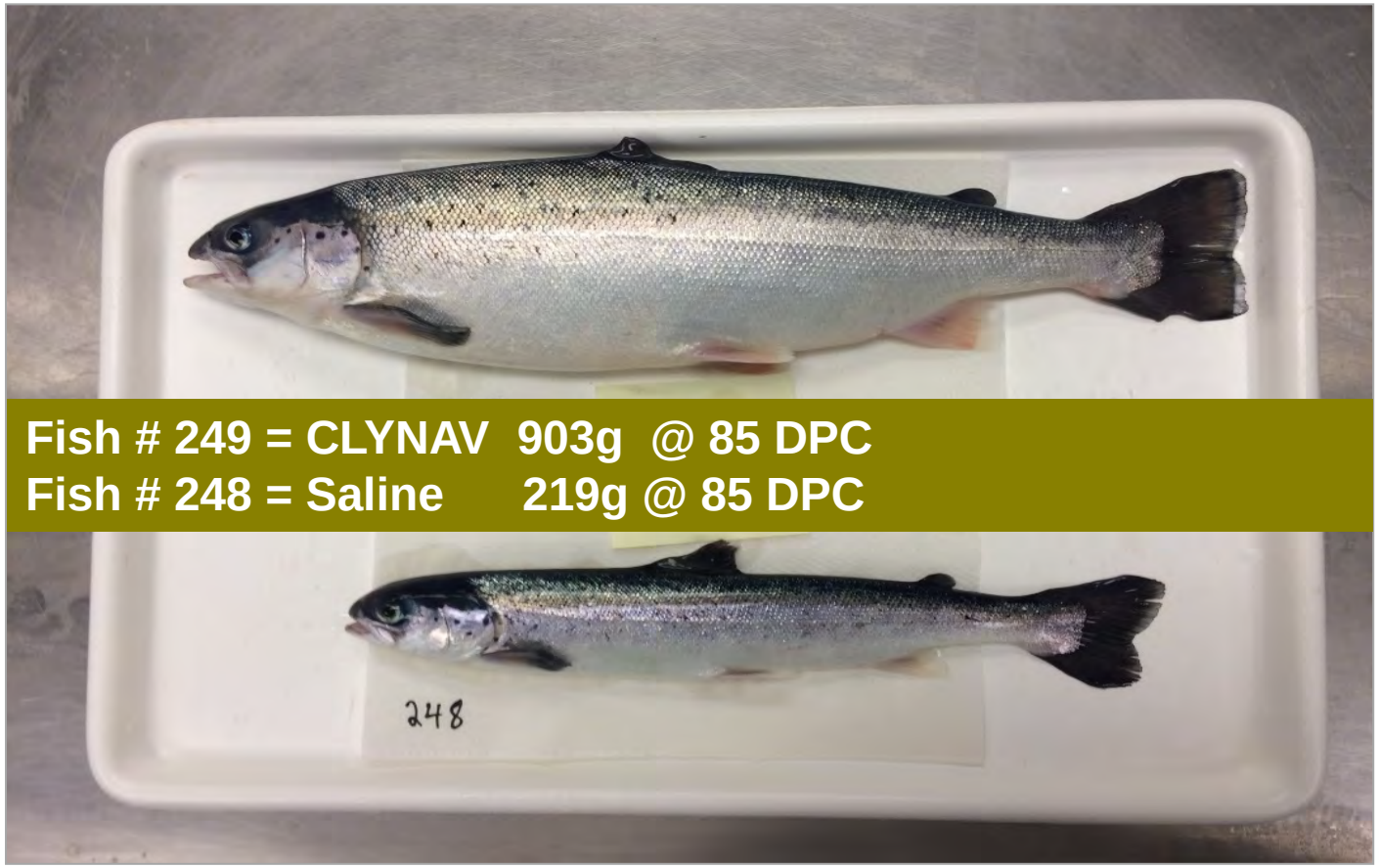




*Statistically significant at p≤ 0.05 (Cox-Tarone test)
**Statistically significant at p≤ 0.01 (ANOVA test)

6-month duration of immunity (DOI) data

Bodyweight change and cumulative mortality post challenge



Gross internal observations 9.5-month challenge

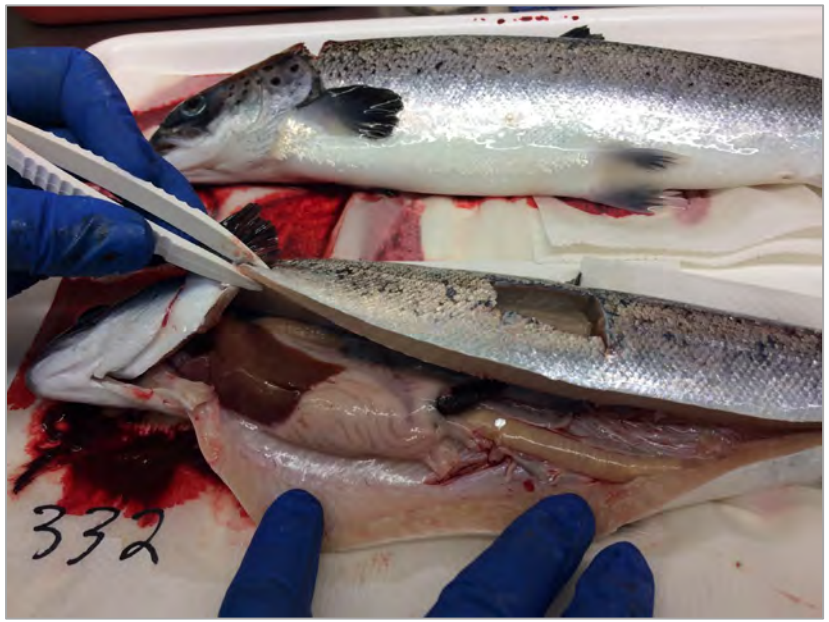
Prevalence (percentage) of occurrence by group and sampling time (Days Post-challenge (DPC))*

DPC	Group	# sampled	Emaciated / thin / No fat	Fluid / mucus filled intestines	Petechial hemorrhage in cecal fat	Ascites	Pale / abnormal heart	Raspberry-like or swollen spleen	Yellow / pale liver
21	Saline	26	4%	69%	69%		8%	50%	35%
	CLYNAV	29						3%	
	NVNC	10							
55	Saline	22	73%	95%				27%	50%
	CLYNAV	28	4%		4%			4%	
	NVNC	10							20%
84	Saline	18	83%	83%				44%	
	CLYNAV	26					4%		
	NVNC	10							

* Empty cell represents 0% prevalence

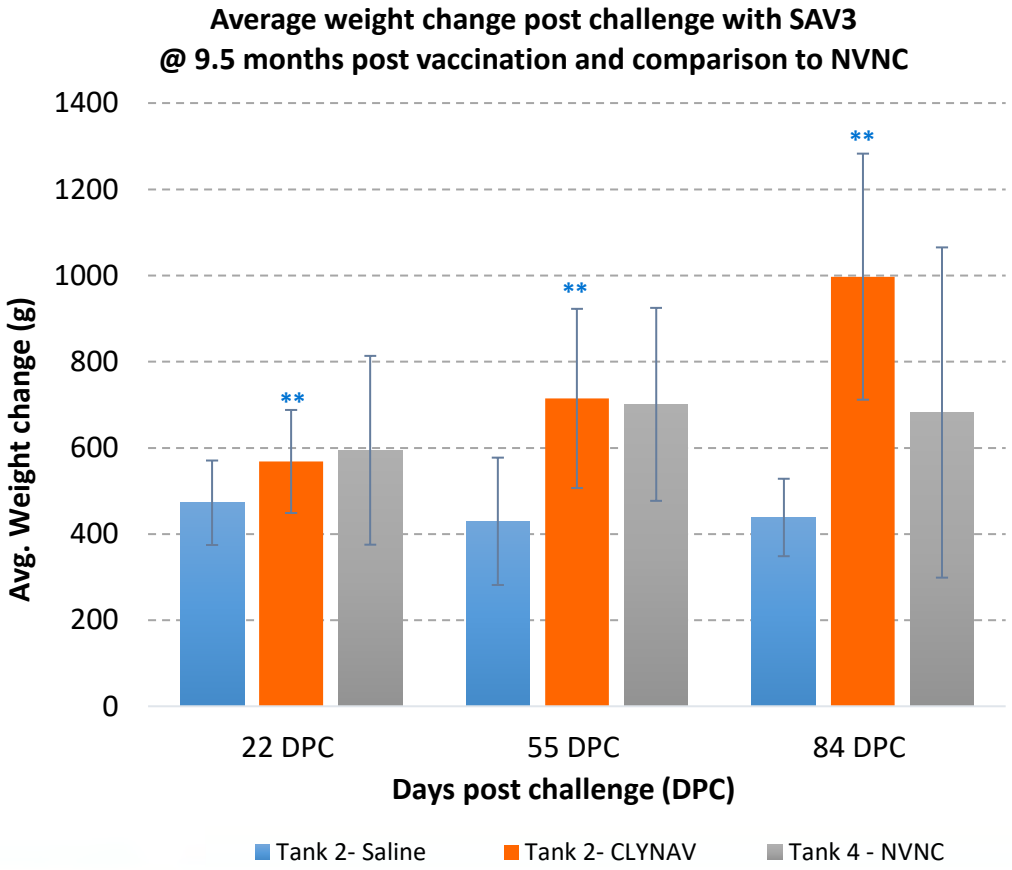
Gross internal observations 9.5-month challenge

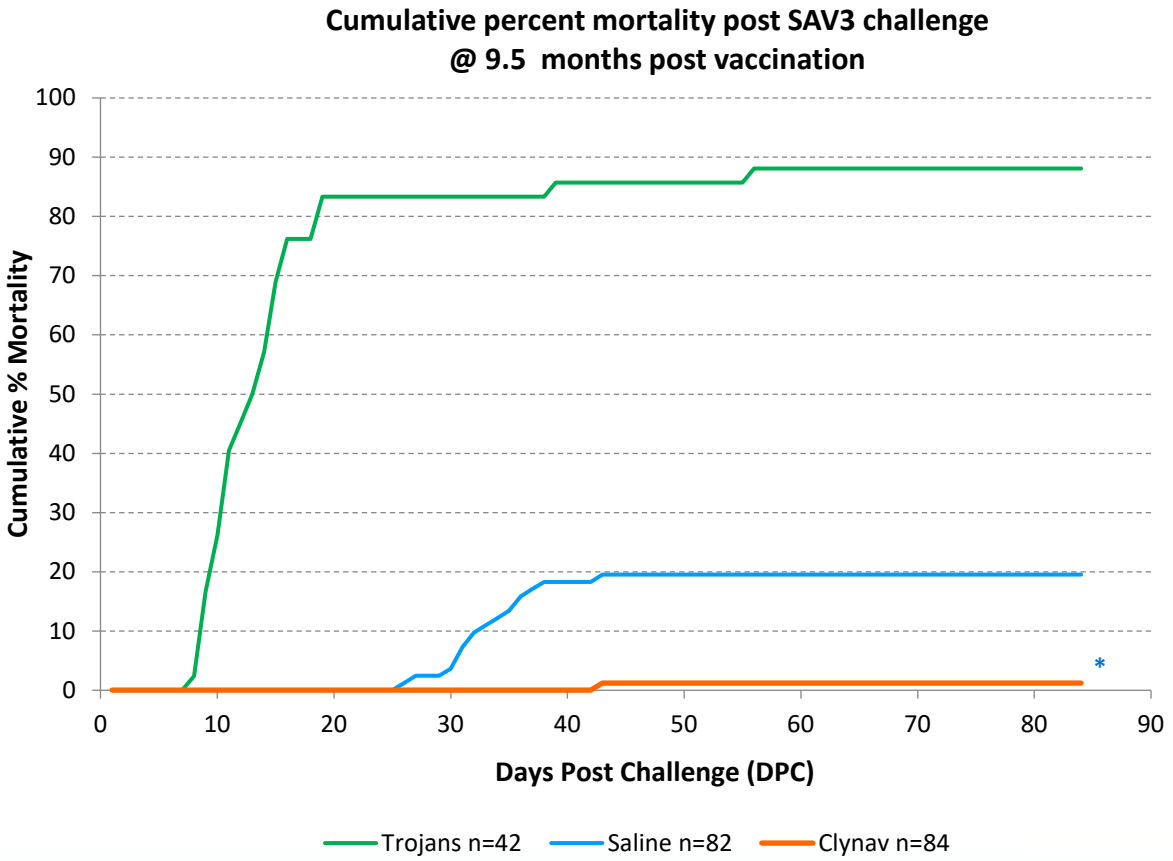
Prevalence (percentage) of occurrence by group and sampling time (Days Post-challenge (DPC))



9.5-month duration of immunity (DOI) data

Bodyweight change and cumulative mortality post challenge





*Statistically significant at p≤ 0.01 (Cox-Tarone test)
**Statistically significant at p≤ 0.01 (ANOVA test)

9.5-month duration of immunity (DOI) data

Bodyweight change and cumulative mortality post challenge

Fish # 487 = CLYNAV 1155 g @ 84 DPC
Fish # 488 = Saline 569g @ 84 DPC

Gross internal observations 12-month challenge

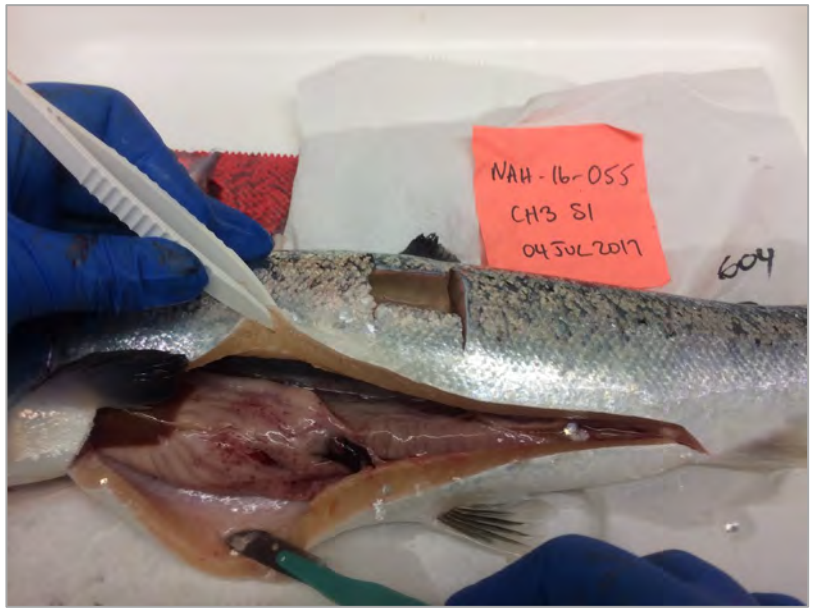
Prevalence (percentage) of occurrence by group and sampling time (Days Post-challenge (DPC))*

DPC	Group	# sampled	Emaciated / thin / No fat	Fluid / mucus filled intestines	Petechial hemorrhage in cecal fat	Ascites	Pale / abnormal heart	Raspberry-like or swollen spleen	Yellow / pale liver
19	Saline	29		79%	69%	7%		21%	
	CLYNAV	28							
	NVNC	10							
54	Saline	29	41%	86%	21%			21%	3%
	CLYNAV	28		4%					
	NVNC	10							
89	Saline	27	52%	89%		4%	19%	44%	
	CLYNAV	28		4%				11%	
	NVNC	9		11%					11%

* Empty cell represents 0% prevalence

Gross internal observations 12-month challenge

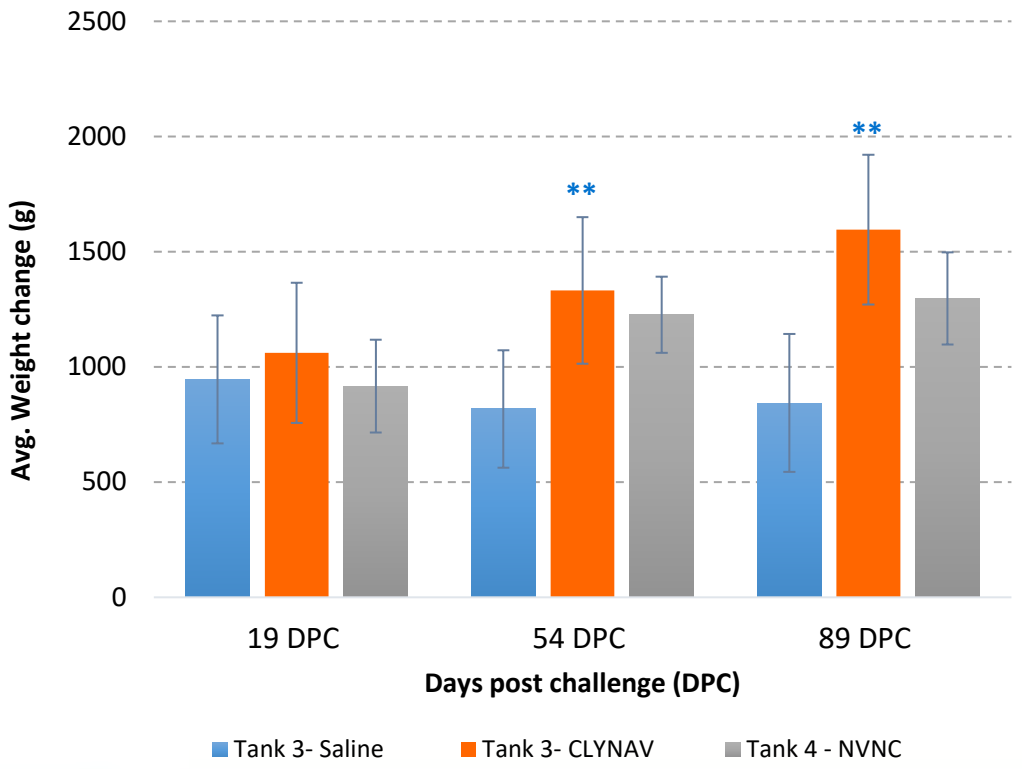
Prevalence (percentage) of occurrence by group and sampling time (Days Post-challenge (DPC))



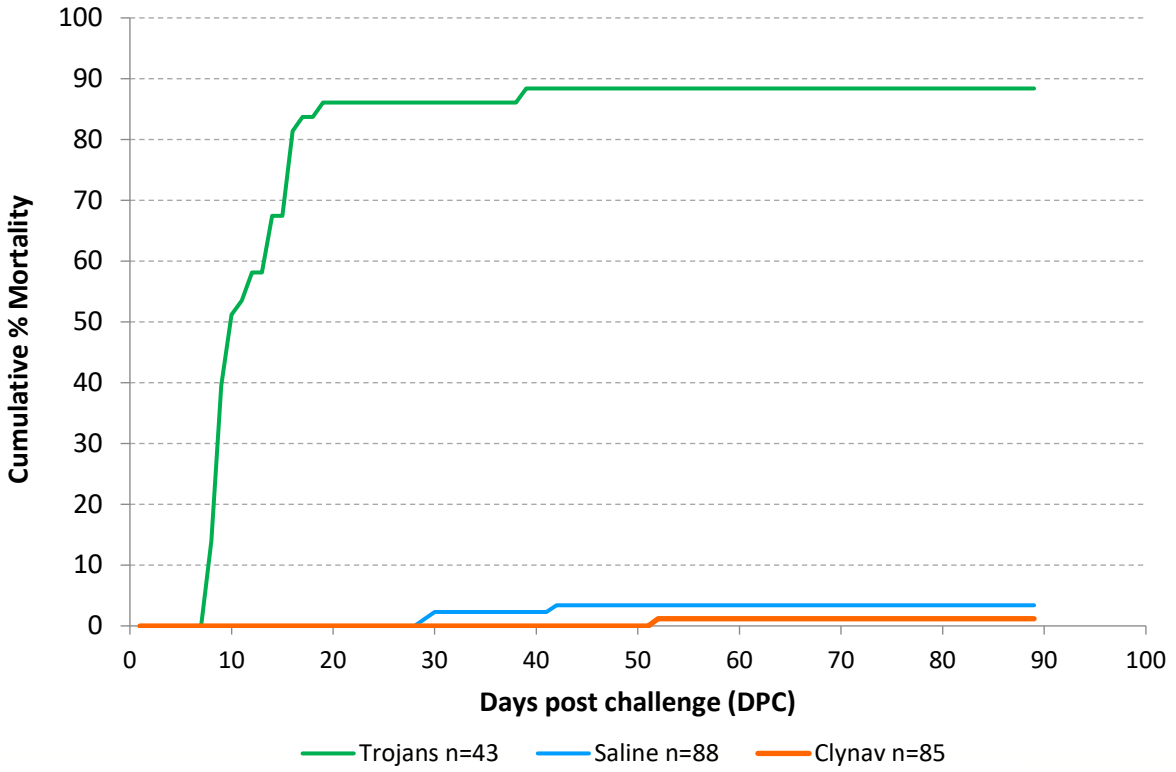
12-month duration of immunity (DOI) data

Bodyweight change and cumulative mortality post challenge

Average weight change post challenge with SAV3 @ 12 months post vaccination and comparison to NVNC



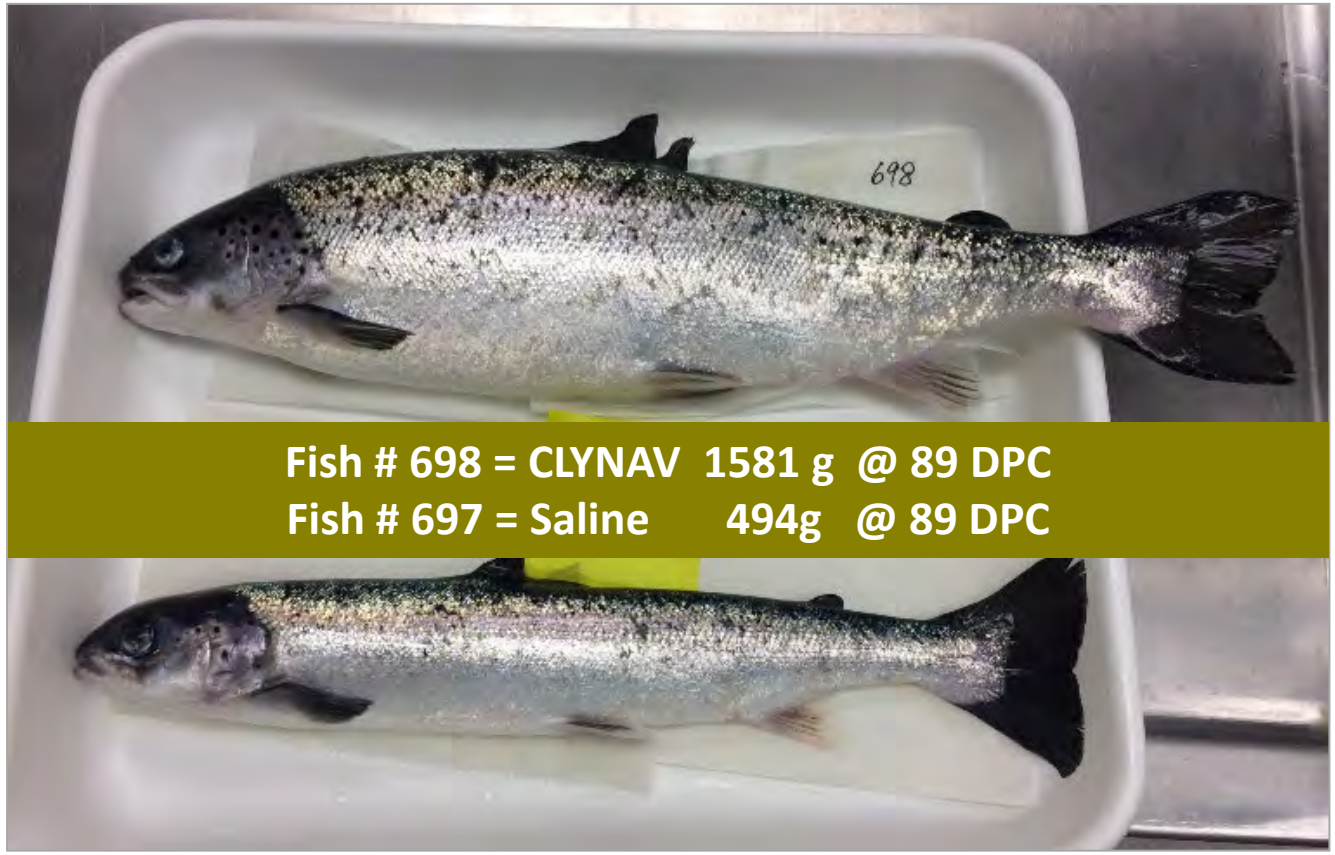
Cumulative percent mortality post SAV3 challenge @ 12 months post vaccination



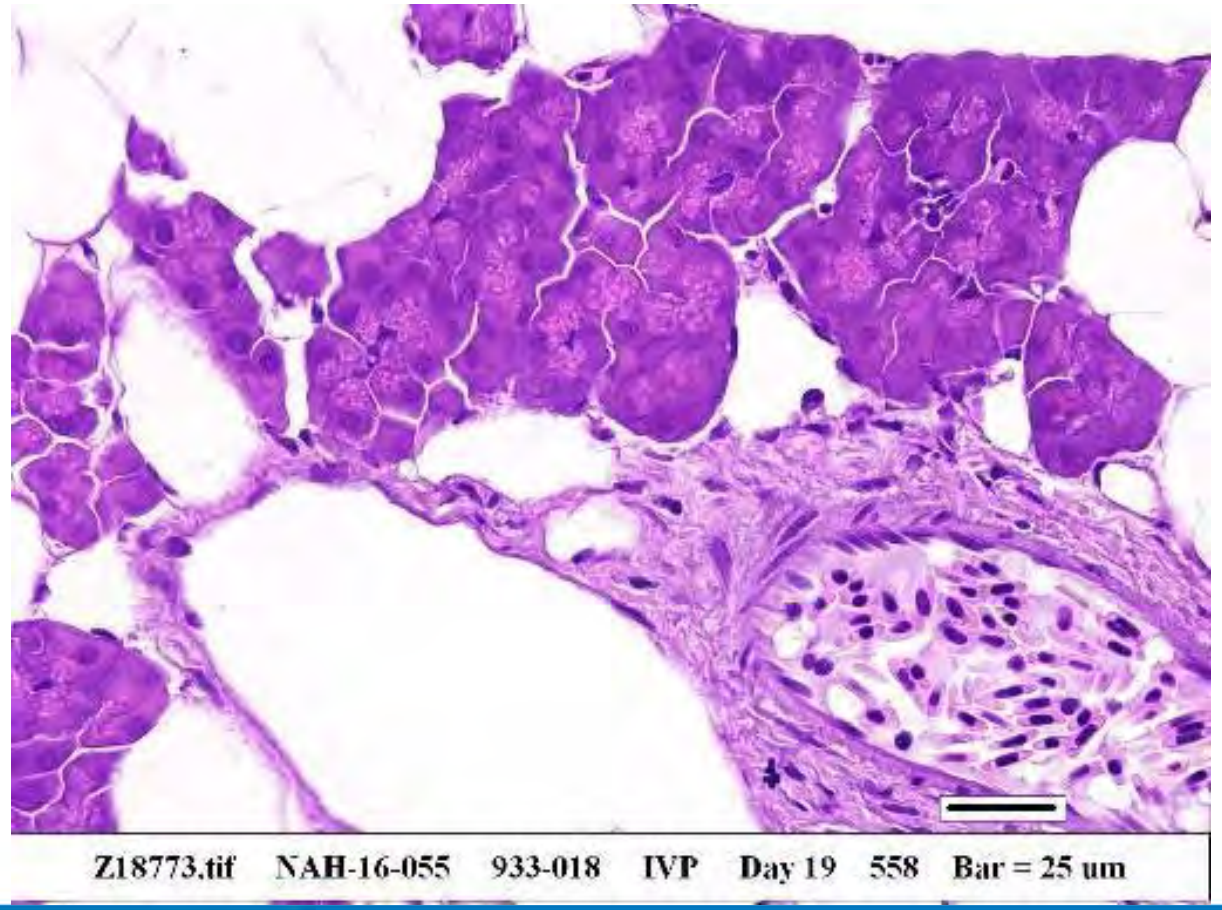
**Statistically significant at p≤ 0.01 (ANOVA test)

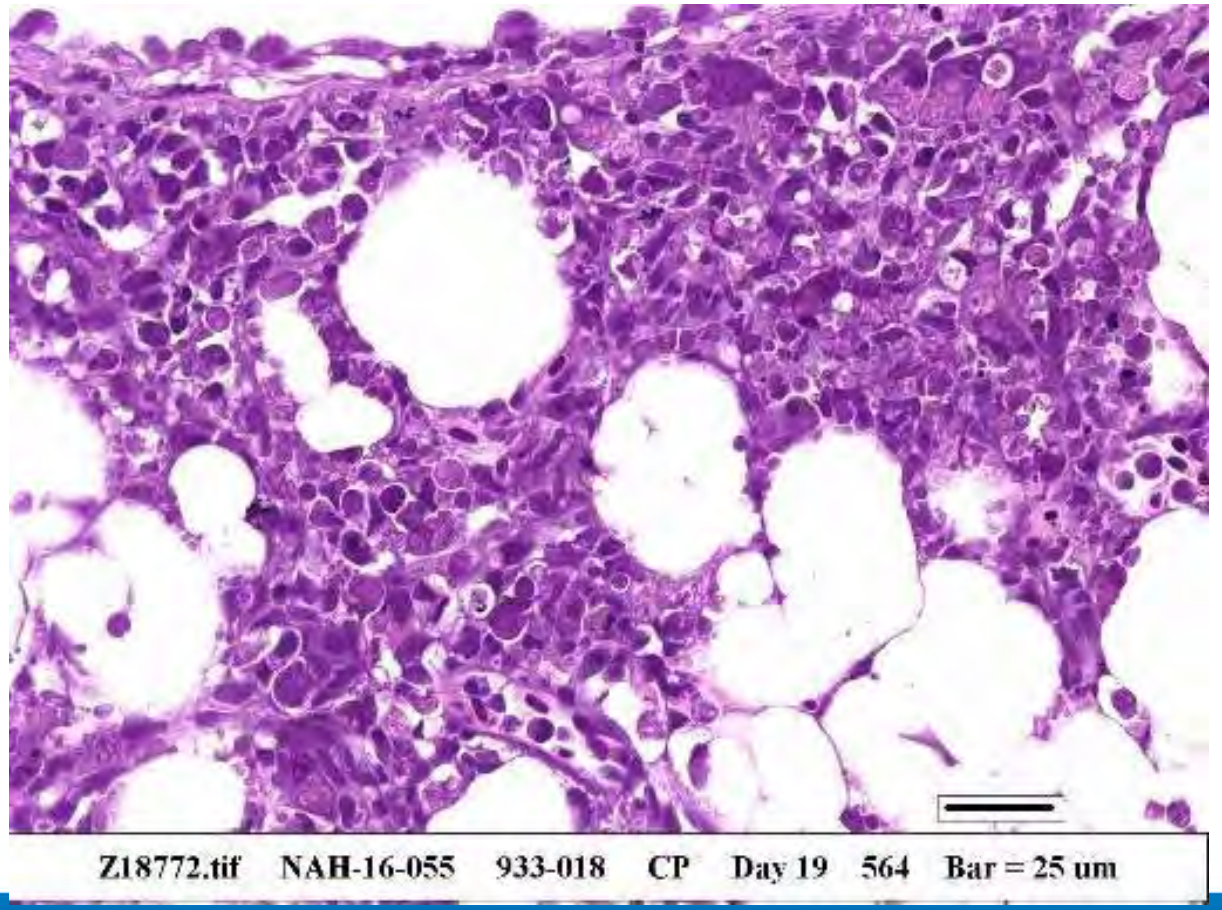
12-month duration of immunity (DOI) data

Bodyweight change and cumulative mortality post challenge

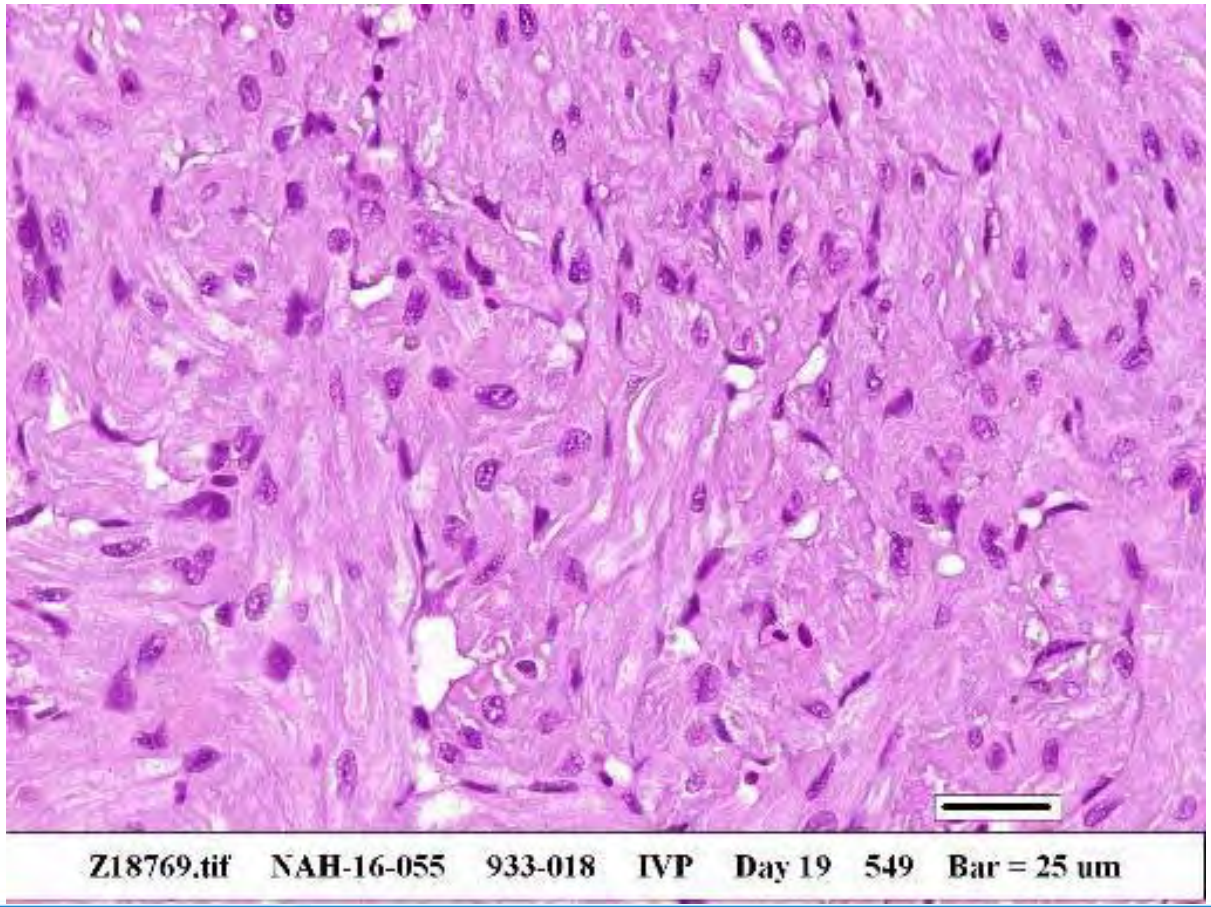


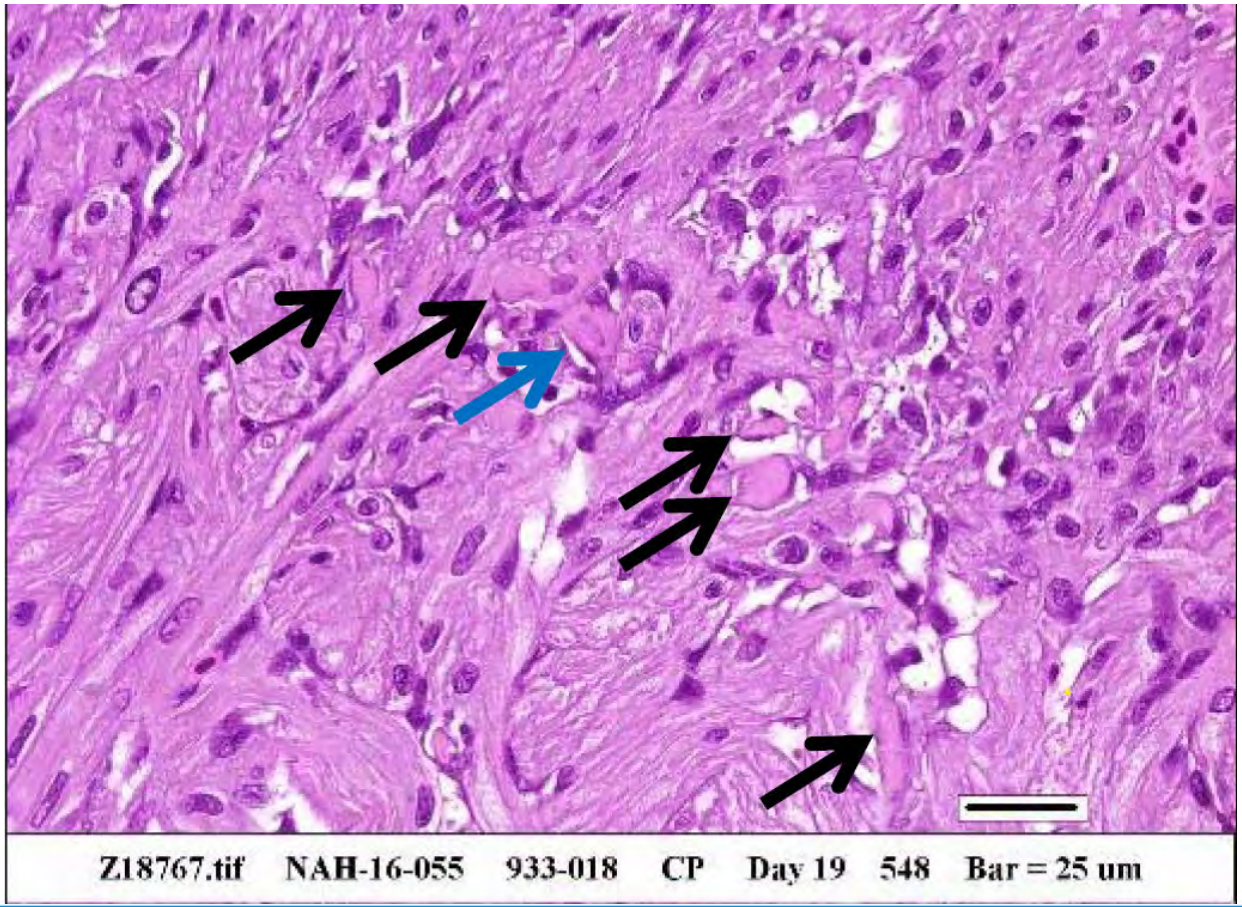
12 month DOI data – Pancreas histopatholgy scores



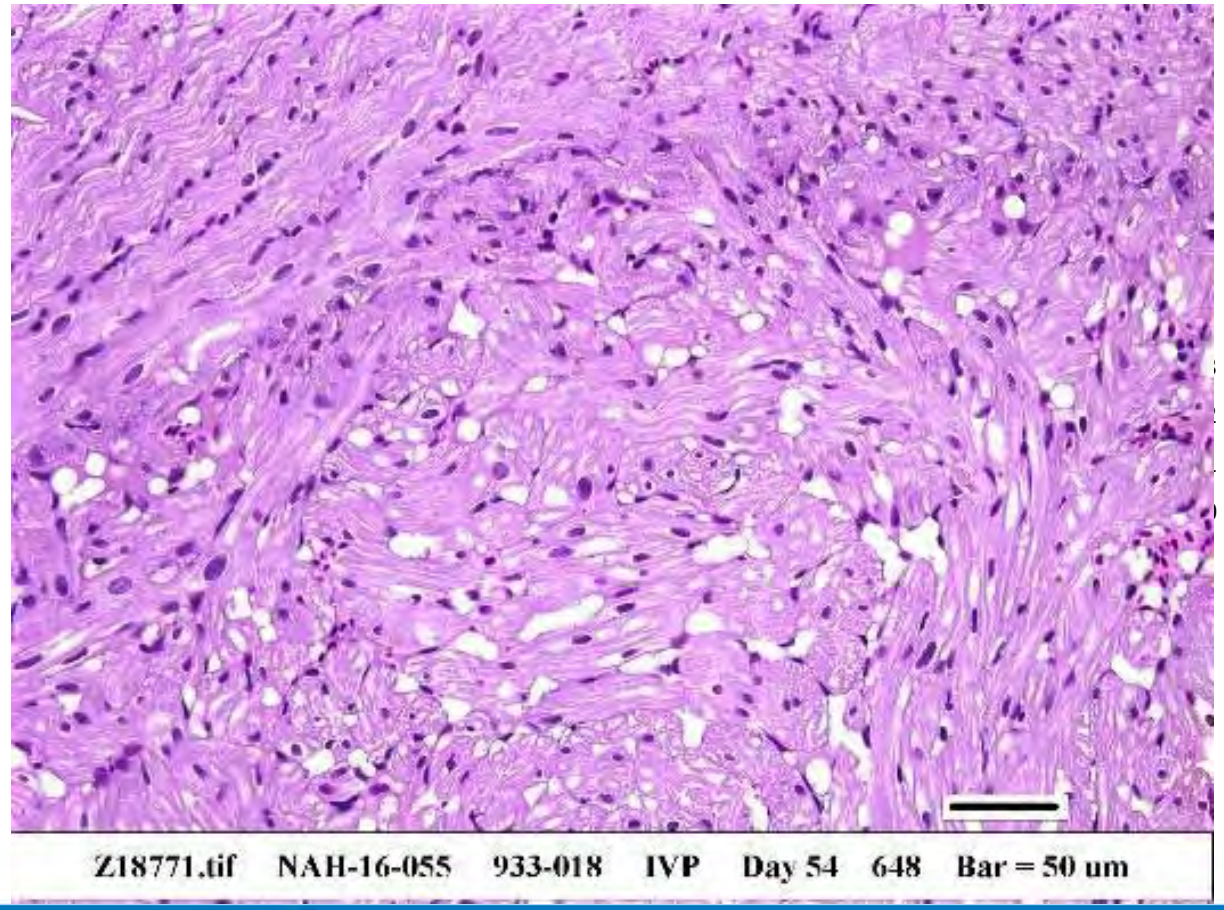


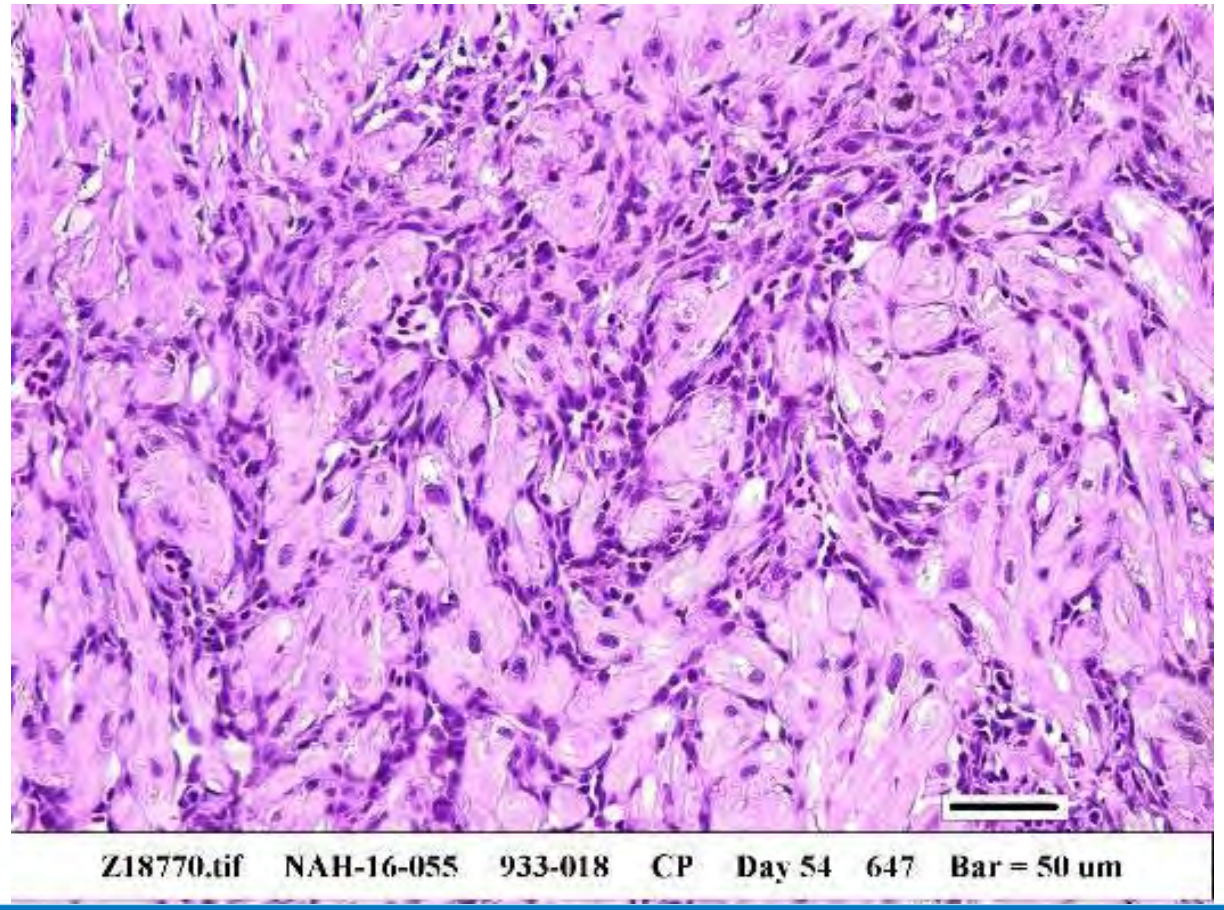
12 month DOI data – Heart histopatholgy scores



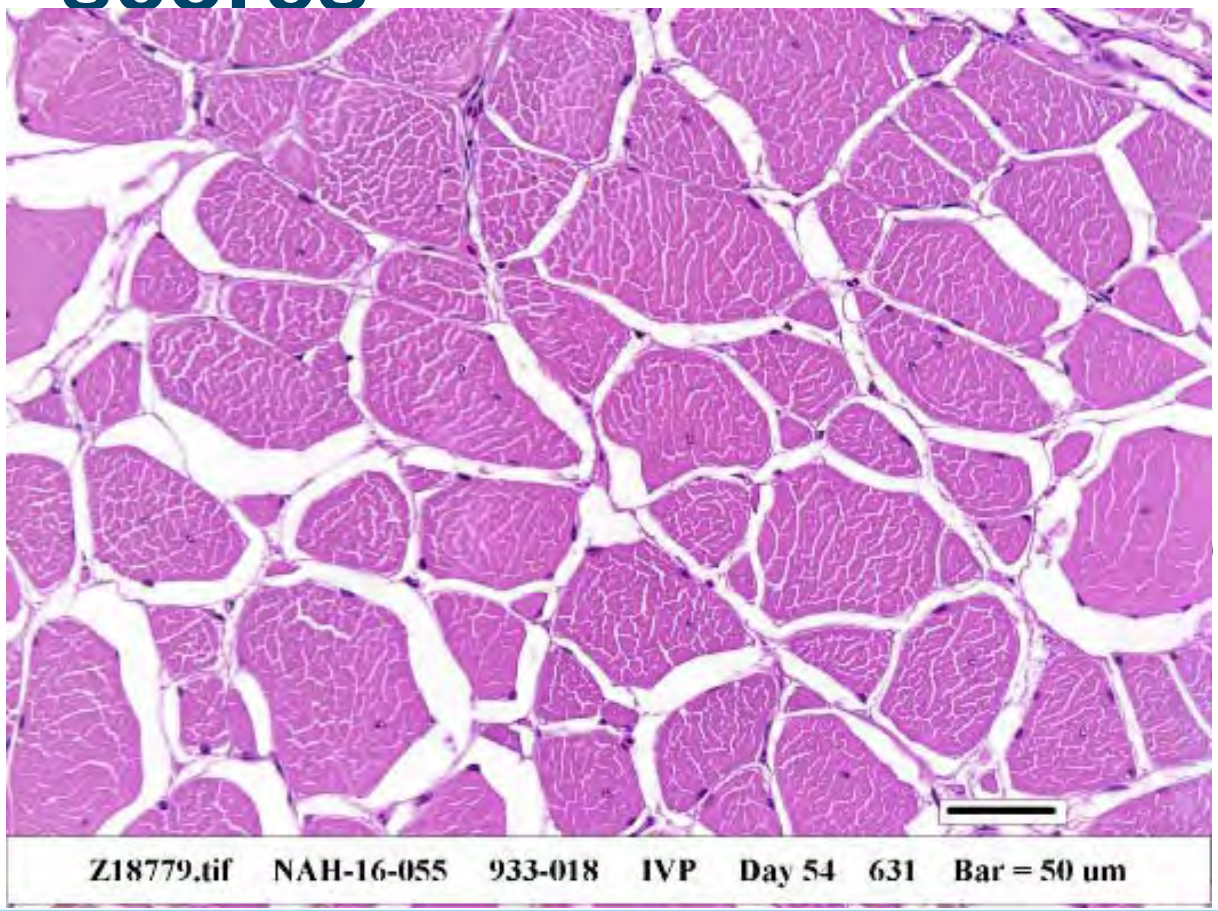


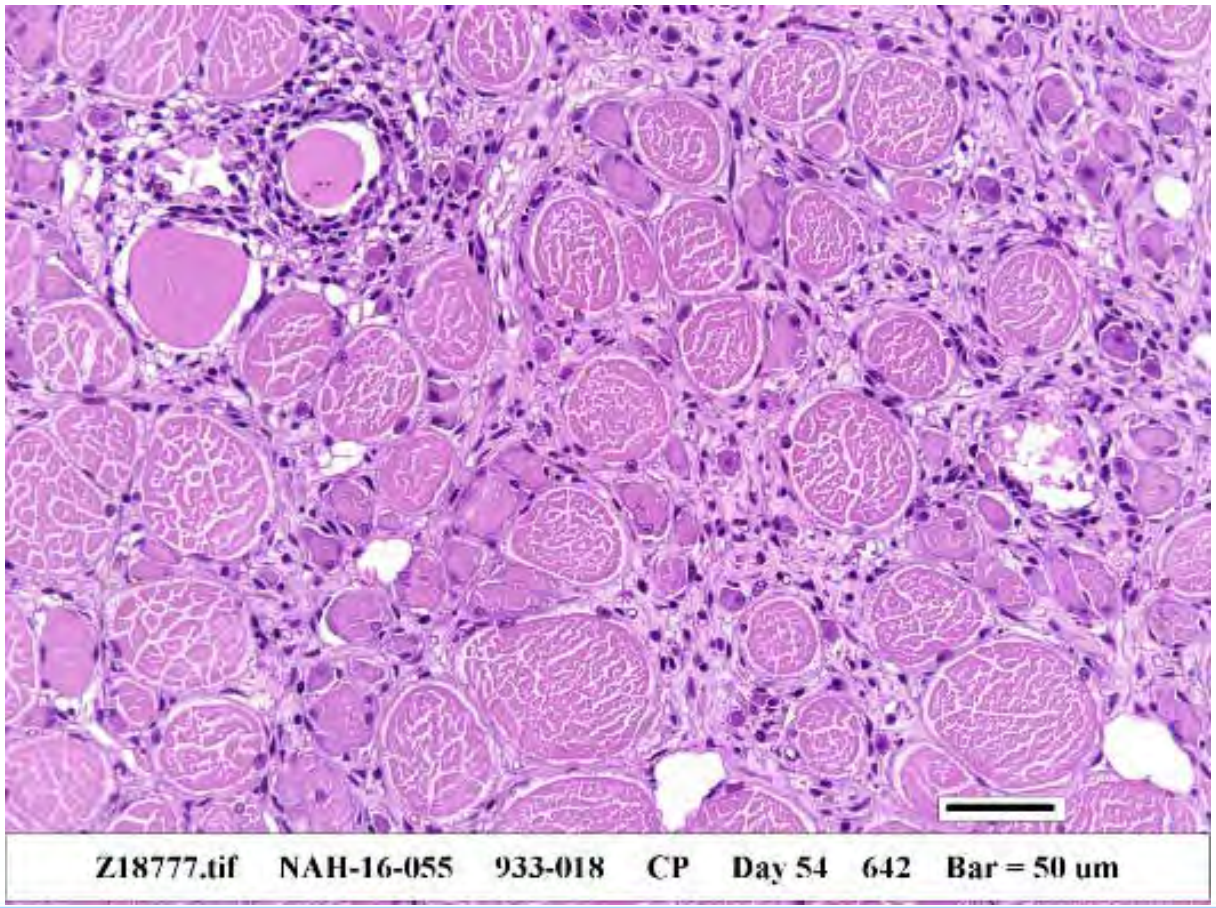
12 month DOI data – Heart histopatholgy scores



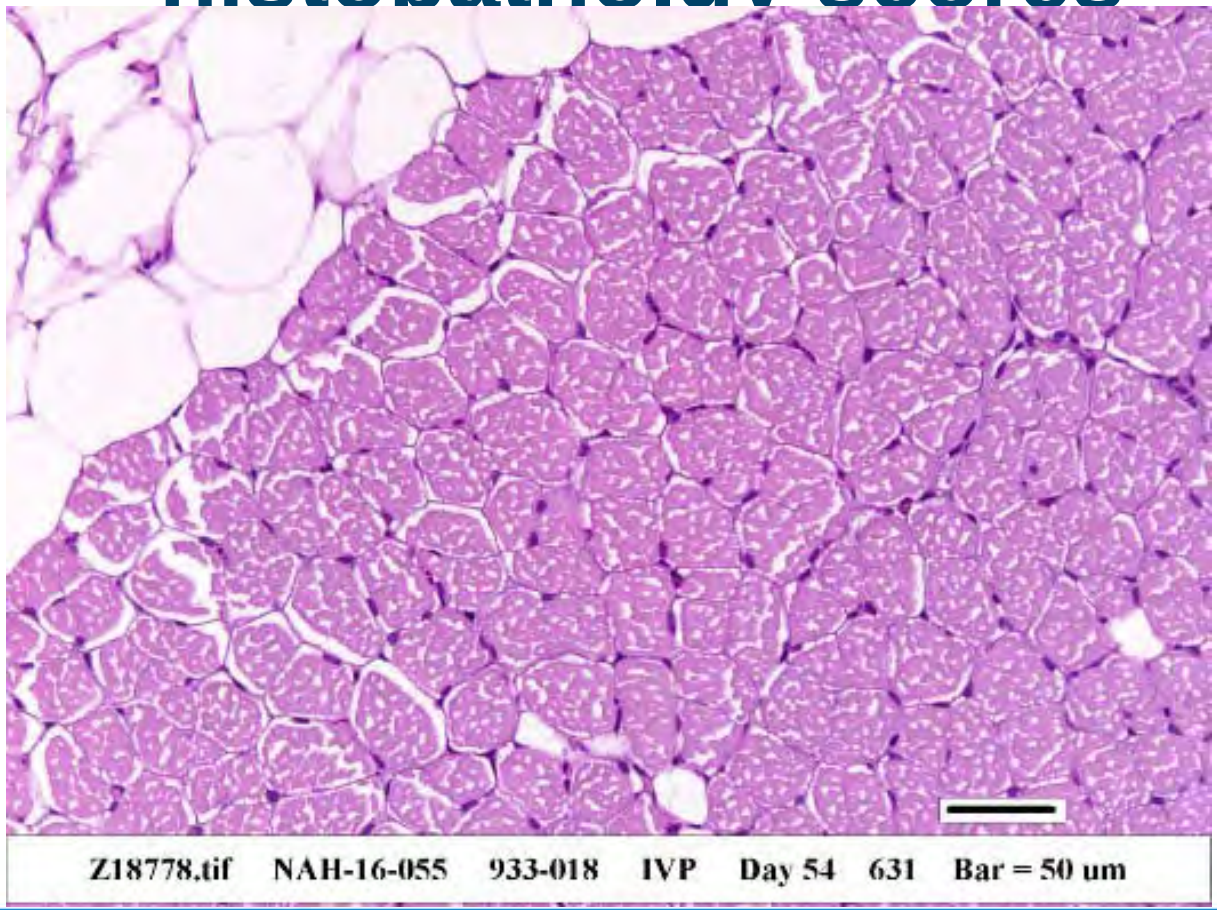


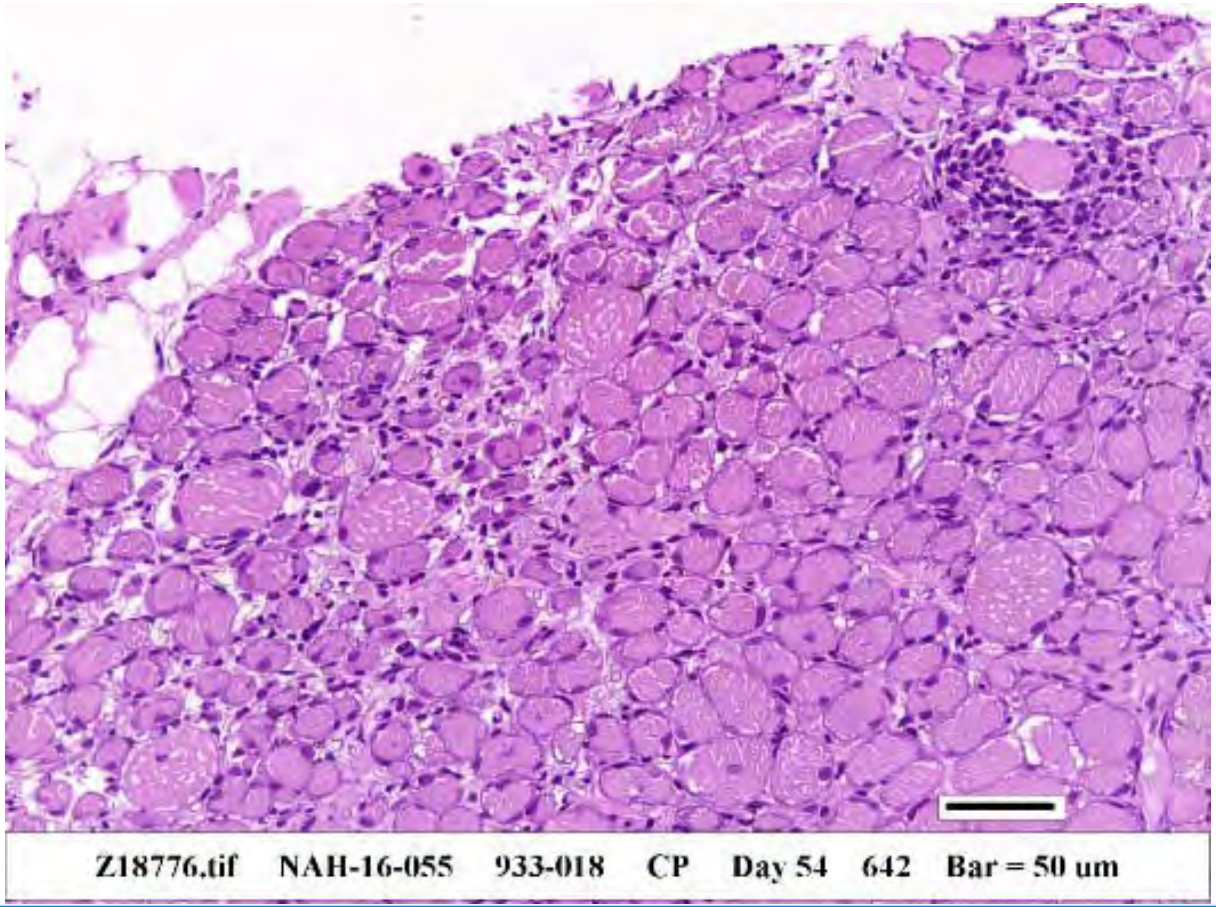
12 month DOI data – Red Muscle histopatholgy scores





12 month DOI data – White Muscle histopathology scores





Summary of findings

- **CLYNAV's duration of immunity was demonstrated following immunization and SAV3 infection challenges in seawater at 6, 9.5 and 12-months post-vaccination:**
  - Significantly **greater relative weight gain** in the CLYNAV group compared to the Control group (saline) after 84 (± 5) days post challenge (DPC; p≤0.05 (6 month) or p≤0.01(9.5 & 12-month)
  - Significantly **higher survival** in the CLYNAV group compared to the Control group through 84 (±5) DPC (p≤ 0.01; if mortality occurs, which was not the case with larger fish at 12-month challenge)

- Additionally, microscopic pathologies were evaluated under this study using an index scoring system and scores were compared between groups (data not presented). Findings were:
  - Significantly **lower microscopic pathology index scores** for pancreas, heart, red skeletal muscle in the CLYNAV group compared to the Saline Control group at time points 21 (± 2), 54 (± 2) and 84 (± 5) DPC, white skeletal muscle 54 (± 2) and 84 (± 5) DPC for 12-month challenge *
  - No difference in microscopic pathology index score for pancreas, heart, red and white skeletal muscle in CLYNAV group compared to Not Vaccinated Not Challenged Group (NVNC) for all time points for the 12-month challenge**

- This GCP study provided information that allowed EMA to grant CLYNAV an extended duration of immunity (DOI) in CLYNAV SPC***

*Statistically significant at each of the three sampled time points: pancreas (all p≤0.01); heart (p≤0.01, p≤0.01, p≤0.05); red muscle (p≤0.05, p≤0.01, p≤0.01); white skeletal muscle (all p≤0.01; ANOVA test).
**(ANOVA test)
***https://www.ema.europa.eu/en/documents/product-information/clynav-epar-product-information_en.pdf

Thank you for your attention!

Forever Salmon