

A scenic view of a fjord at sunset. The sky is filled with dramatic, colorful clouds in shades of blue, purple, and orange. In the foreground, there are several rows of salmon farming cages floating in the water, connected by a line. A small white boat is visible in the middle ground. The background shows rolling hills and mountains under the twilight sky.

Histo cardiac score used in Cardiomyopathy syndrome (CMS), Pancreas disease (PD) and Heart and skeletal muscle inflammation (HSMI)

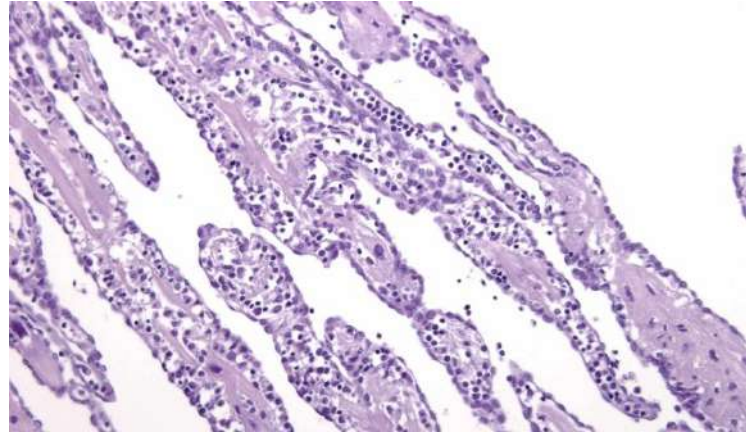
Trination meeting November 2022

Marta Alarcón and Kai Inge Lie

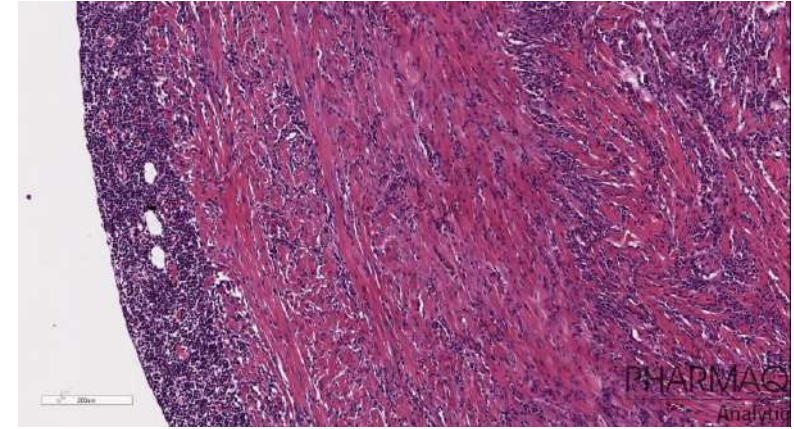


Cardiac histopathology

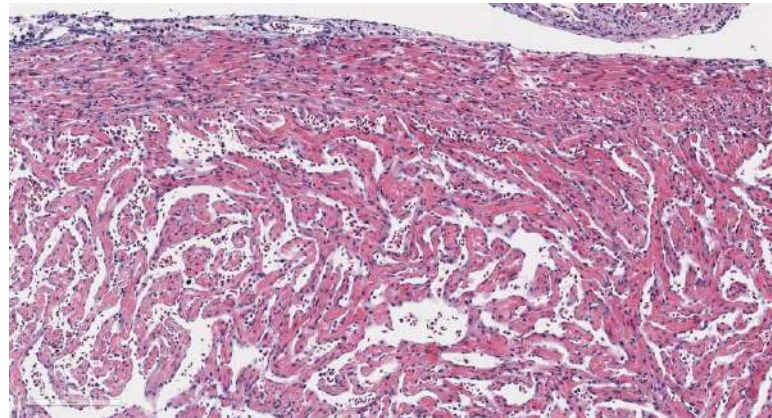
Diagnose and differentiate viral
myocarditis (CMS, HSMI, PD)



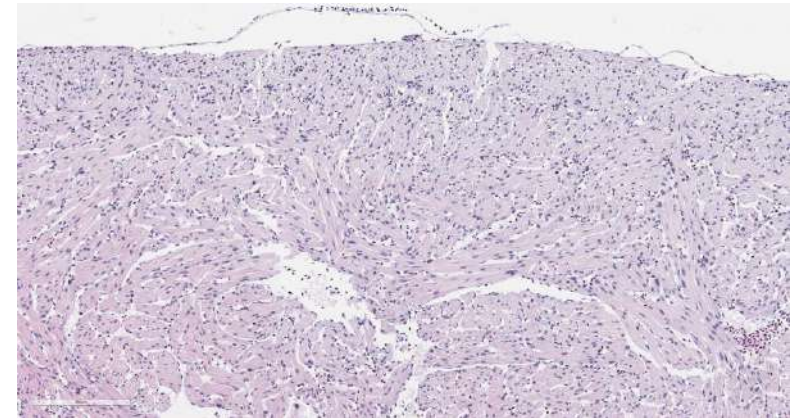
CMS



HSMI



PD



No lesions

Cardiac scoring in research

- Research: Nutrition, genetic and vaccines
- Score: Grading severity of the microscopical lesions
- Important traits of any scoring method:
 - Definable
 - Reproducible
 - Should produce meaningful results

Important to know before start

- It is critical to provide full disclosure to the details of the study to provide the most robust and valid data.
- Important questions to answer before the project start:
 - When should we sample for histopathology?
 - Right time/intervals to be able detect biological differences between groups
 - How should we sample?
 - Consistent and to avoid autolysis
 - How many samples?
 - Include epidemiologist in your study
 - Include baseline samples

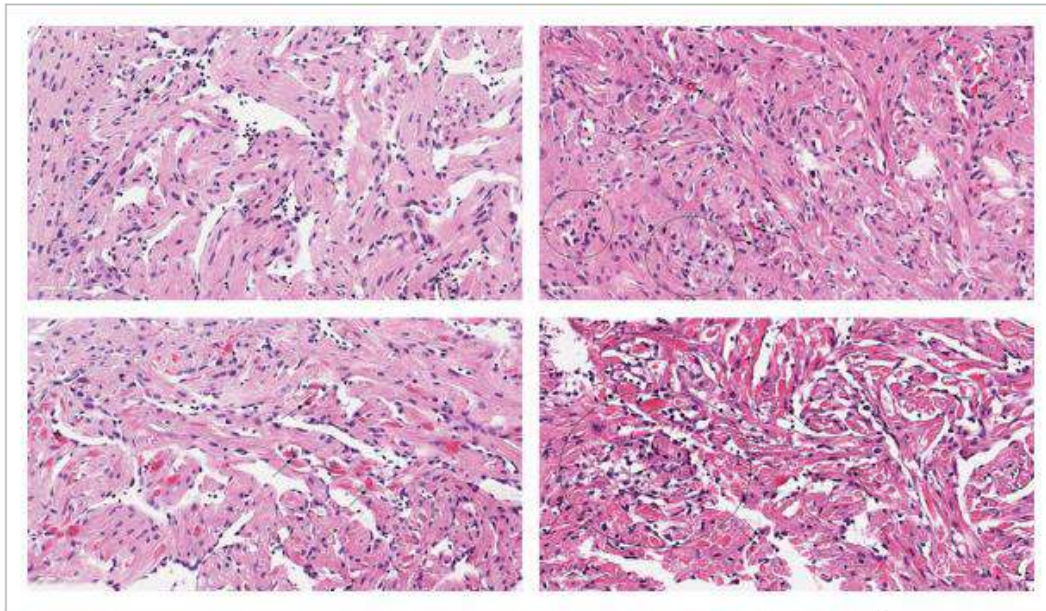
Our experience

- Many thousand hearts scored during the last 8 years from lab and field trials.
- There are several published cardiac histopathological scoring methods but there is currently no universal cardiac score protocol that can be used for all types of projects (There is no “one size fits all” scoring system).
- The scoring method should be tailored to the specific project to be able to detect differences in the pathology between groups if those differences are present.

Histopathological score approaches

- **QUALITATIVE:** Nominal, e.g.: presence or absence of a lesion (binomial), several categories (gender, race, ...)
- **SEMI-QUANTITATIVE: Ordinal**, category showing progression of severity or percentage of affected tissue. No lesions (0), mild (1), moderate (2) and severe (3)
- **QUANTITATIVE:** Interval, Quantification of parameters on a scale between two extremes. Example: Temperature
- **QUANTITATIVE:** Ratio: morphometry (ratio scale include zero). Example: Length

Examples PD



PD: Semiquantitative evaluation of heart (0-4)

Aksnes, I., Braaen, S., Markussen, T., Åkesson, C. P., Villoing, S., & Rimstad, E. (2021). Genetically modified attenuated salmonid alphavirus: A potential strategy for immunization of Atlantic salmon. *Journal of Fish Diseases*, 44, 923– 937.

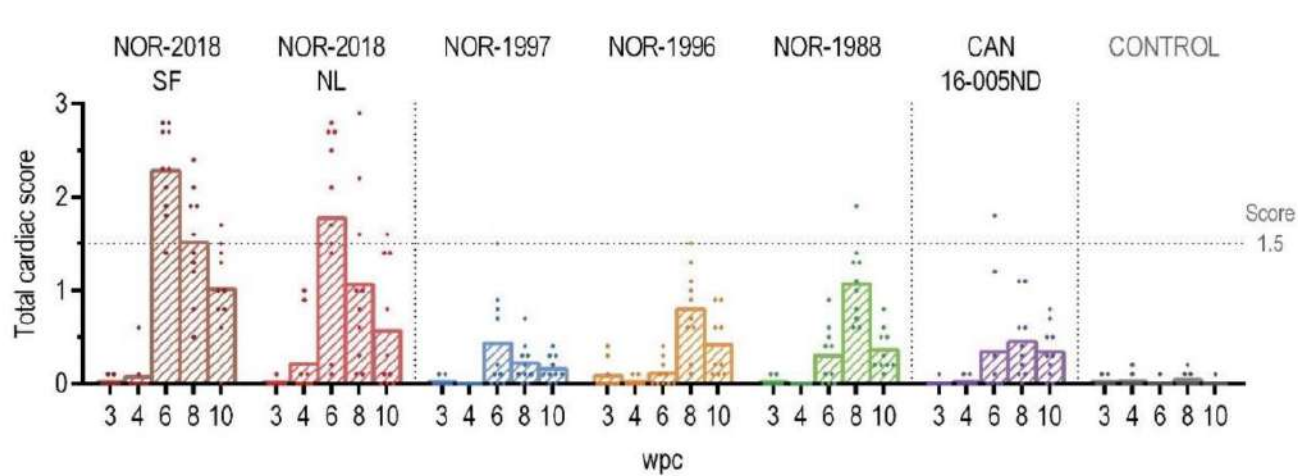
TABLE 3. Semi-quantitative scoring system for evaluating PD/SAV-associated heart lesions on a scale from 0 to 4, where score 0: no lesions; score 1: very mild lesions; score 2: mild lesions; score 3: moderate lesions; score 4: severe lesions

Score	Description
0	No lesions, normal
1	Focal acute myocardial degeneration/necrosis < 7 fibres affected
2	Multifocal myocardial degeneration/necrosis ± inflammation (<15% of ventricle affected)
3	Multifocal myocardial degeneration/necrosis ± inflammation (>15 & <50% of ventricle affected)
4	Severe diffuse myocardial degeneration/necrosis ± inflammation (>50% of ventricle affected)

Examples- HSMI

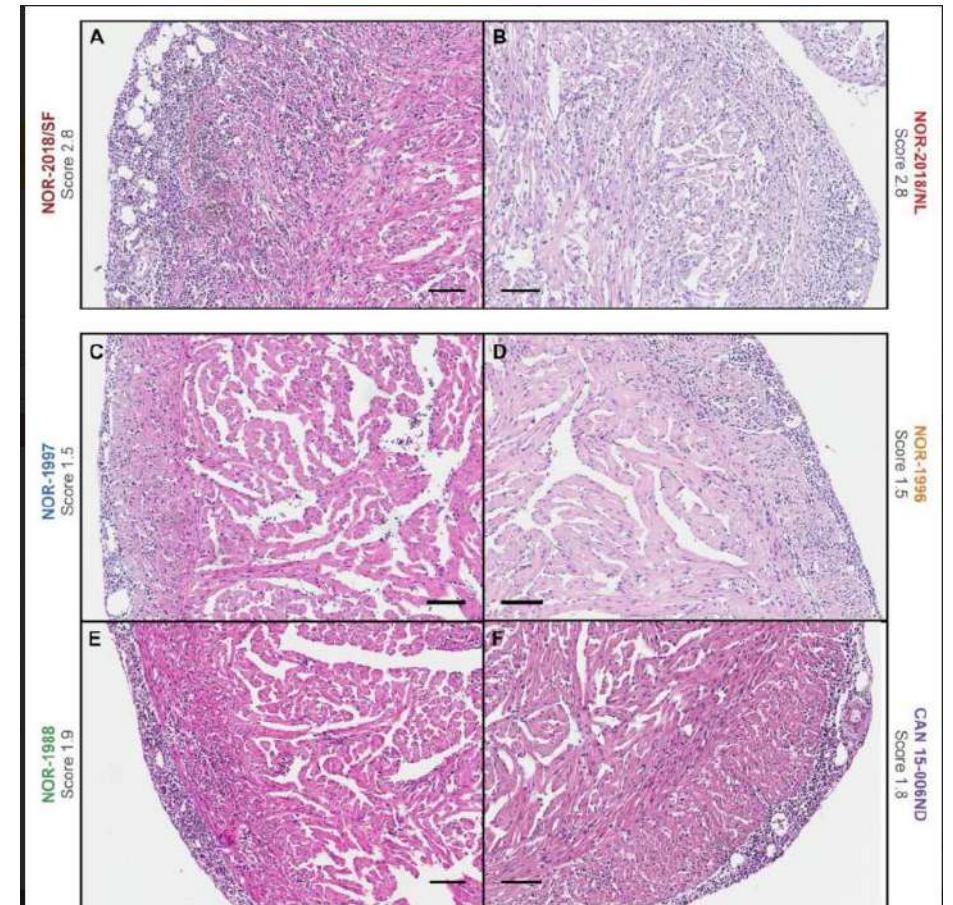
HSMI: Quantitative evaluation heart (0-3)

Wessel, Ø.; Hansen, E.F.; Dahle, M.K.; Alarcon, M.; Vatne, N.A.; Nyman, I.B.; Soleim, K.B.; Dhamotharan, K.; Timmerhaus, G.; Markussen, T.; Lund, M.; Aanes, H.; Devold, M.; Inami, M.; Løvoll, M.; Rimstad, E. Piscine Orthoreovirus-1 Isolates Differ in Their Ability to Induce Heart and Skeletal Muscle Inflammation in Atlantic Salmon (*Salmo salar*). *Pathogens* 2020, 9, 1050. <https://doi.org/10.3390/pathogens9121050>



B

Strain wpc (mean score ±SD)	NOR-1997 6 wpc (0.4 ±0.5)	NOR-1996 8 wpc (0.80 ±0.6)	NOR-1988 8 wpc (1.1 ±0.4)	CAN 16-005ND 8 wpc (0.5 ±0.4)
NOR-2018/SF 6 wpc (2.3 ±0.5)	*** < 0.001	*** < 0.001	*** < 0.001	*** < 0.001
NOR-2018/NL 6 wpc (1.8 ±1.0)	** 0.003	* 0.019	* 0.045	** 0.005



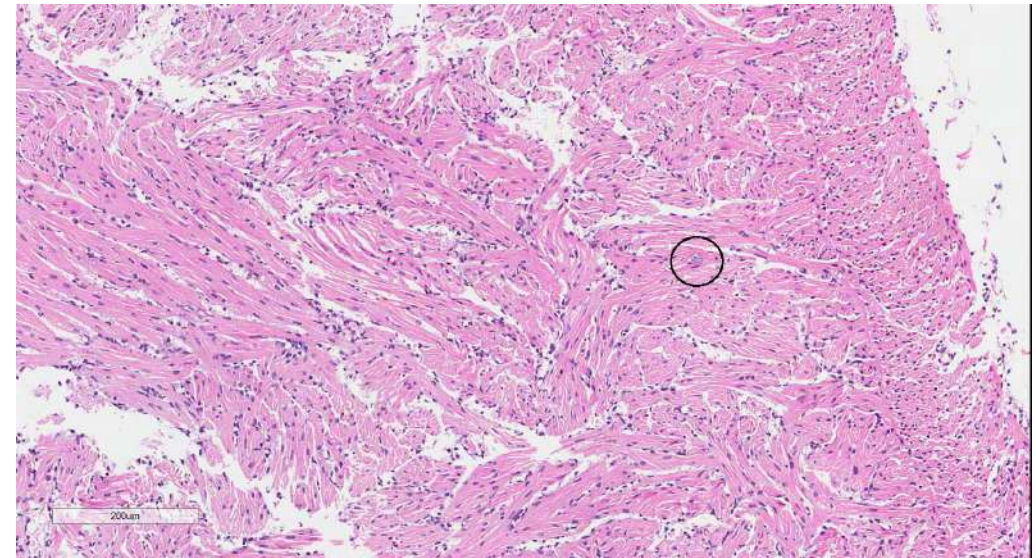
Example: CMS trial

- Duration of experiment not stated
- CMS, cohabitation trial
- Breeding study
- 1091 animals (atlantic salmon)
- Grouping/family of fish not stated
- CMS scoring method: Semiquantitative evaluation of atrium and cardiac ventricle (0-4). (Each grade is defined on basis of severity/extension of inflammation).

Score	Atrium	Ventricle
0	43	22
1	297	268
2	494	727
3	178	72
4	77	2

Pitfalls-

- Bacteria and HSMI challenge study (co-infections)
- PRV-infected fish in PMCV challenge study (co-infections)
- PD score: different type of lesions shortly after viral infection (necrosis, inflammation) than several weeks after infection (signs to regeneration/ repair)



Slide	Scoring 1		Scoring 2	
	Atrium Path A	Ventricle Path A	Atrium Path A	Ventricle Path A
1	1	1	1	1
2	3	1	3	2
3	1	1	1	1
4	2	1	2	0
5	1	1	1	1
6	2	2	2	1
7	2	2	2	1
8	2	2	2	2
9	1	1	1	1
10	0	1	0	0
11	2	1	2	1
12	3	1	3	2
13	1	1	1	0
14	0	1	0	1
15	1	1	1	0
16	4	2	4	2
17	1	1	1	1
18	2	2	2	2
19	2	1	2	1
20	0	0	0	0
21	1	1	1	1
22	0	1	0	1
23	0	0	0	1
24	3	2	3	1
25	2	2	2	1
26	1	1	2	1
27	2	0	2	0
28	0	1	0	1
29	0	0	0	0
30	1	1	1	1
31		1		1
32	0	0	0	0
33	1	1	1	1
34	2	2	2	2
35	2	2	2	2
36	1	1	0	1
37		1		2
38	3	1	3	2
39	2	2	2	2
40	1	1	1	1
41	1	1	1	1
42	2	1	2	1
43	4	2	4	2
44	2	2	2	2
45	3	2	3	2
46	2	1	2	1
47		1		1
48	1	1	0	1
49	3	2	2	2
50	1	0	1	0
51	3	2	3	2
52	1	1	1	1
53	3	2	3	2
54	2	2	1	0
55	2	1	1	1
56	3	2	3	2
57	2	2	2	2
58	1	1	1	1
59	3	2	3	2
60	2	1	1	1
61	2	1	2	1

Example: CMS Intra-observer variability

Cohens kappa

- **Atrium:**
 - First scoring vs Second scoring: 0.944337811900192
(almost perfect agreement)
- **Ventricle:**
 - First scoring vs Second scoring: 1.0
(perfect agreement)

Validation work HSMI: inter observer variation

Heart-ID	Path A		Path B		Path C	
	Epicardium	Ventricle	Epicardium	Ventricle	Epicardium	Ventricle
1	1	3	2	3	1	3
2	3	3	3	3	2	4
3	3	4	3	4	4	3
4	3	3	3	3	3	2
5	3	4	3	4	4	4
6	2	3	3	3	3	3
7	3	3	3	3	3	3
8	2	1	2	2	2	1
9	1	3	2	3	1	3
10	2	3	3	4	3	3
11	2	3	2	2	3	3
12	1	4	2	3	2	3
13	2	3	2	3	2	3
14	2	3	2	3	1	3
15	4	3	4	3	4	3
16	1	2	2	2	1	3
17	2	3	3	3	3	3
18	3	4	3	4	2	4
19	2	4	2	3	2	4
20	2	2	2	2	2	3
21	0	0	1	0	1	0
22	1	2	2	2	2	3
23	2	1	2	2	2	3
24	1	0	1	0	1	0
25	2	3	2	3	2	3
26	1	2	2	1	1	2
27	2	4	3	4	2	4
28	2	3	2	3	1	3
29	0	1	1	0	1	1
30	1	3	2	3	2	3
31	2	3	2	3	2	2
32	2	2	2	3	3	3
33	1	3	1	3	1	2
34	2	4	2	3	3	3
35	2	2	2	2	2	2
36	2	3	2	2	3	3
37	2	3	2	3	3	3
38	2	3	2	3	2	3
39	1	2	1	1	1	2
40	2	3	2	3	2	3
41	1	2	2	1	1	2

Cohens kappa:

Epicardium:

Path A vs Path B: 0.7352342158859471 (moderate agreement)

Path A vs Path C: 0.69 (moderate agreement)

Path C vs Path B: 0.681592039800995 (moderate agreement)

Ventricle:

Path A vs Path B: 0.8475967174677609 (strong agreement)

Path A vs Path C: 0.800531914893617 (strong agreement)

Path C vs Path B: 0.7727272727272727 (moderate agreement)

Interpretation of Cohen's kappa.

Value of Kappa	Level of Agreement	% of Data that are Reliable
0–.20	None	0–4%
.21–.39	Minimal	4–15%
.40–.59	Weak	15–35%
.60–.79	Moderate	35–63%
.80–.90	Strong	64–81%
Above .90	Almost Perfect	82–100%



[Biochem Med \(Zagreb\)](#). 2012 Oct; 22(3): 276–282.
Published online 2012 Oct 15.

PMCID: PMC3900052
PMID: [23092060](#)

Interrater reliability: the kappa statistic

[Mary L. McHugh](#)

Summary (Take home message)

- Study design
- Sampling
- Scoring method
- Histopathological evaluation
- Statistical analyses
- Publication



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