



Veterinærinstituttet
Norwegian Veterinary Institute



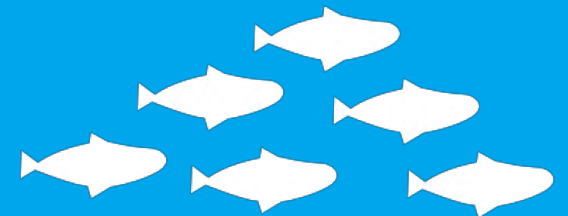
Norwegian University of Life Sciences
Faculty of Veterinary Medicine
Department of Paraclinical Sciences (PARAFAG)

Early phases of cardiomyopathy syndrome (CMS) pathogenesis

- characterized through various diagnostic methods

TriNation Meeting, Edinburgh, November 2022

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Dep. Aquatic biosecurity
Norwegian Veterinary Institute



Cardiomyopathy syndrome - impact

- Most important infectious cause of mortality in Norwegian aquaculture 2020 and 2021
- 169 sites w. a clinical CMS diagnosis in Norway in 2021 (of ~1000 sites)
- Sudden death due to cardiac rupture
- Mainly larger fish
- Late onset



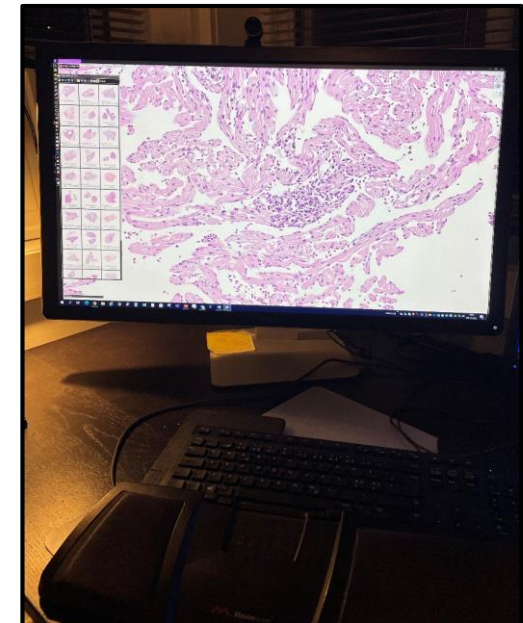
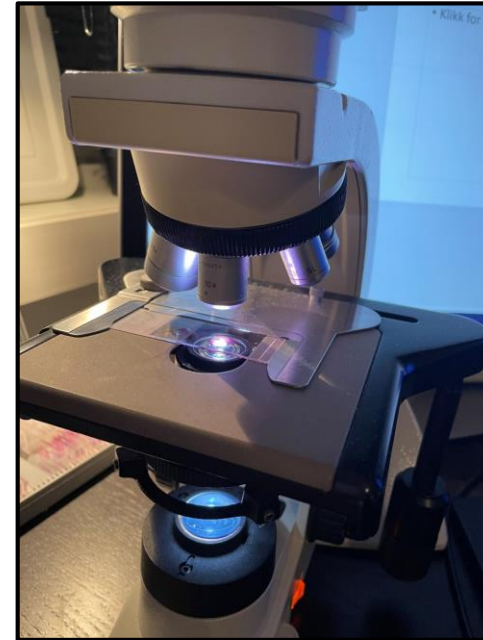
Significant impact



Photos: Per Anton Sæther, Marin Helse

Diagnostic methods

- Histopathology
 - Heart
 - Standard organ set
- Real-time RT-PCR
 - Heart + kidney
- Immunohistochemistry (IHC)
- In situ hybridization (ISH) (i.e. RNAscope®)



RESEARCH ARTICLE

Characterization of early phases of cardiomyopathy syndrome pathogenesis in Atlantic salmon (*Salmo salar* L.) through various diagnostic methods

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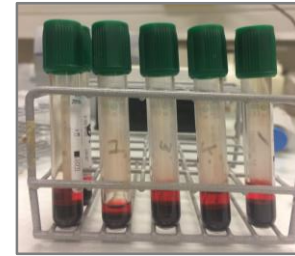
Funding information

PHARMAQ: Funding of the challenge trial

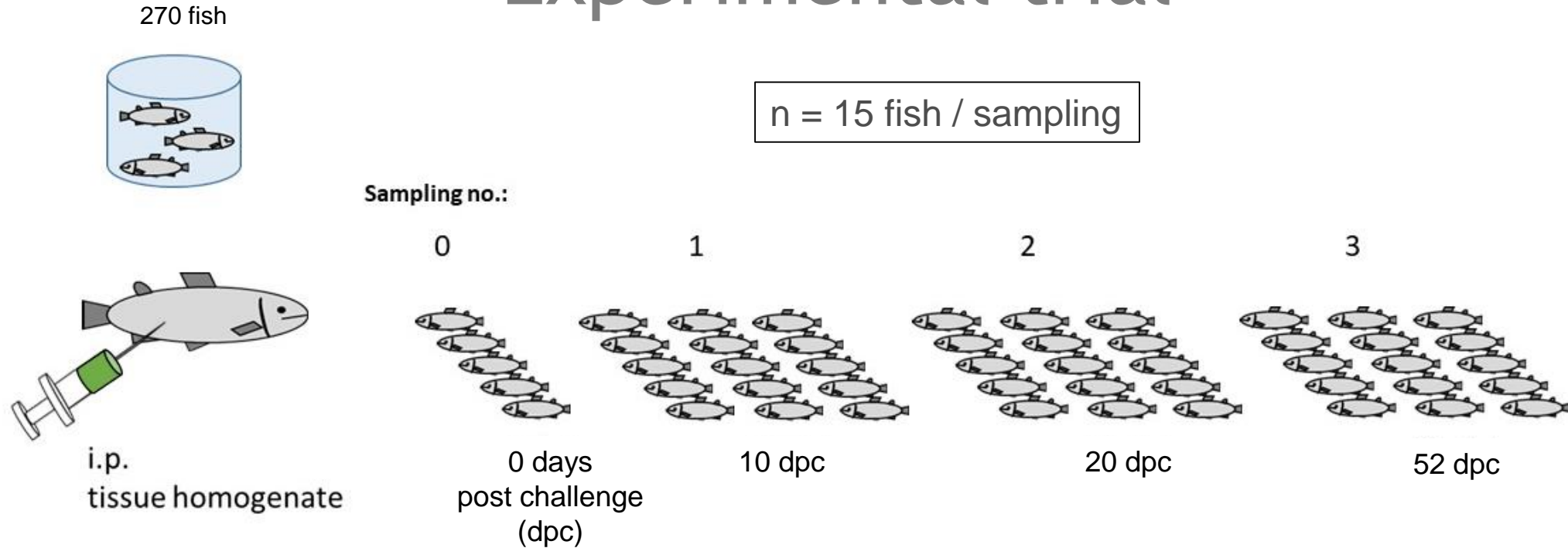
Published June 2022

Specific aims

- Further characterization of CMS pathogenesis
- Analyses of lesions and viral load in
 - Tissues
 - Non-lethal samples: mucus and blood
- Establish a new ISH method (for PMCV RNA detection)
+ compare it to established diagnostic methods:
 - Histopathology
 - Real-time RT-PCR
 - IHC-PMCV



Experimental trial



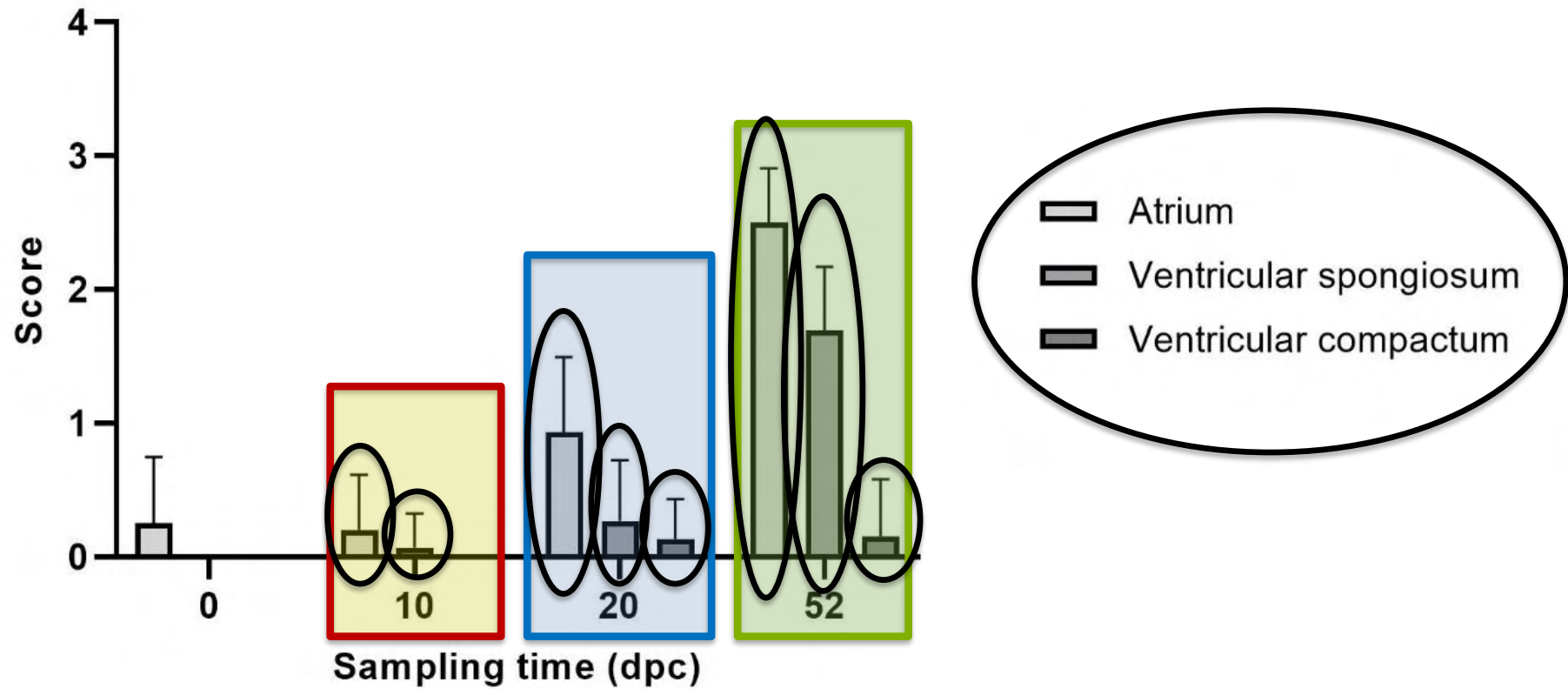
Challenge material:

- 0.1 ml i.p.
- Tissue homogenate (spleen from CMS diseased fish, field outbreak)
 - negative by PCR for SAV, PRV and IPNV
 - Cq-value PMCV = 16.5

Material

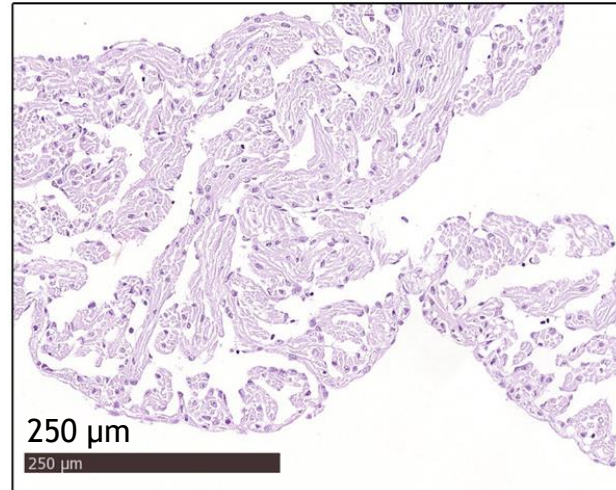
	Formaline	RNAlater	Lysis buffer	
Methode(s)	Histopathology, IHC and ISH	Real-time RT-PCR and sequencing	Real-time RT-PCR	
Material	<ul style="list-style-type: none"> Heart Mid-kidney Skin/muscle 	<ul style="list-style-type: none"> Heart x3 (<i>atrium, spongiosum and compactum</i>) Mid-kidney Skin/muscle 	<ul style="list-style-type: none"> Blood 	Whole blood
				Plasma
				Pelleted blood cells
			<ul style="list-style-type: none"> Mucus 	Pectoral fin Lateral line Anus

Histopathology score of hearts vs. time

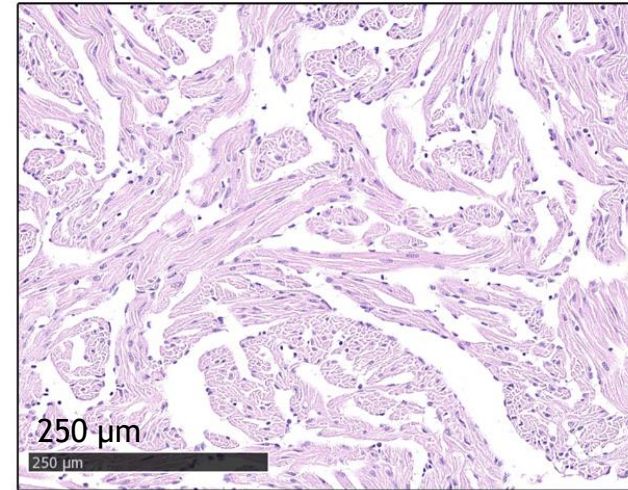


Histopathology - cardiac CMS lesions

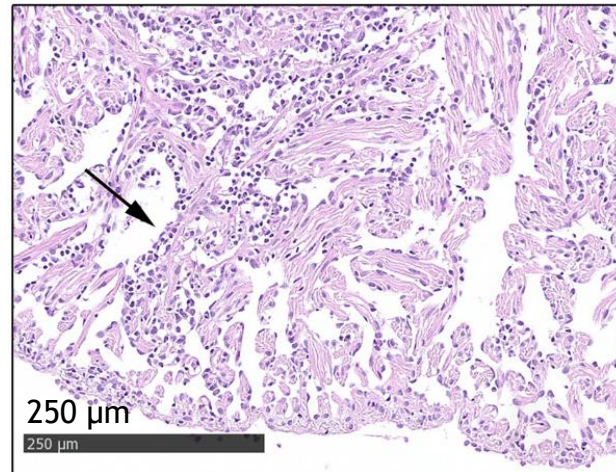
Atrium 0 dpc



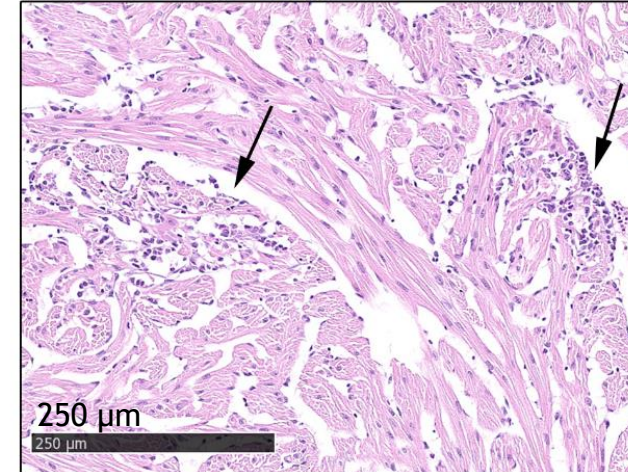
Ventr. spongiosum 0 dpc



Atrium 52 dpc

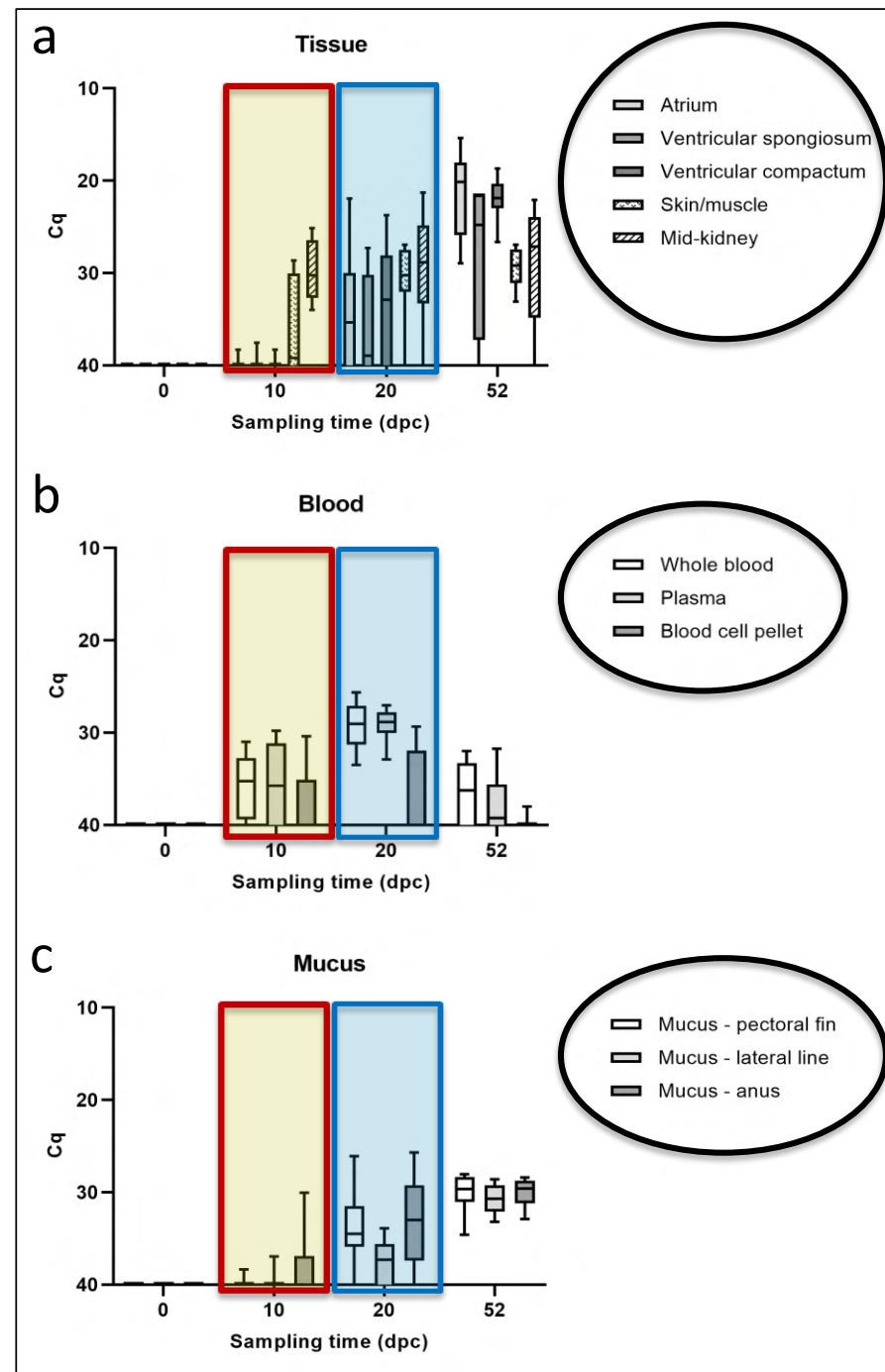


Ventr. spongiosum 52 dpc



Overview

Cq values vs. time



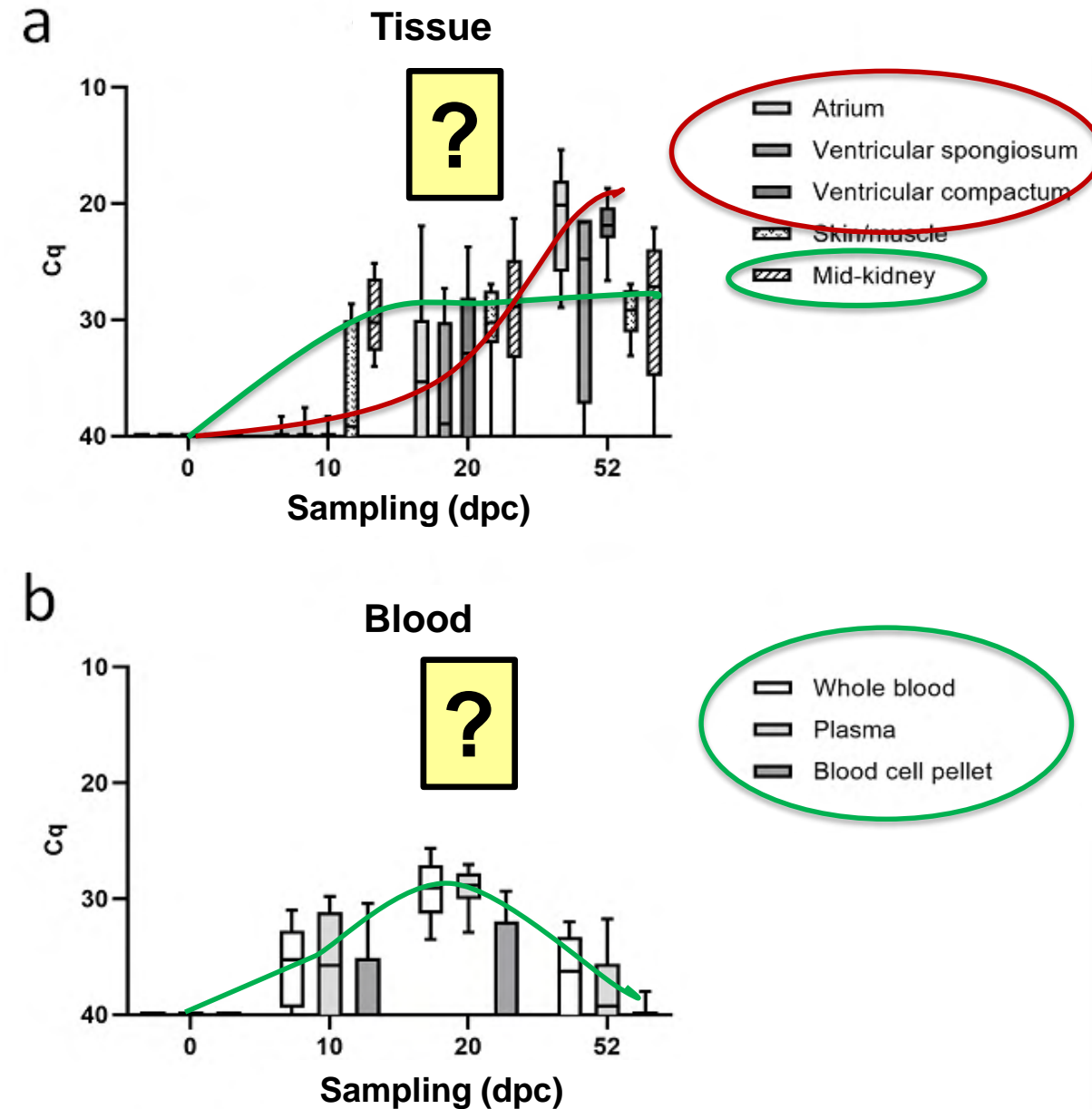
Detection of PMCV RNA by RT-PCR vs. time

Early viremic phase

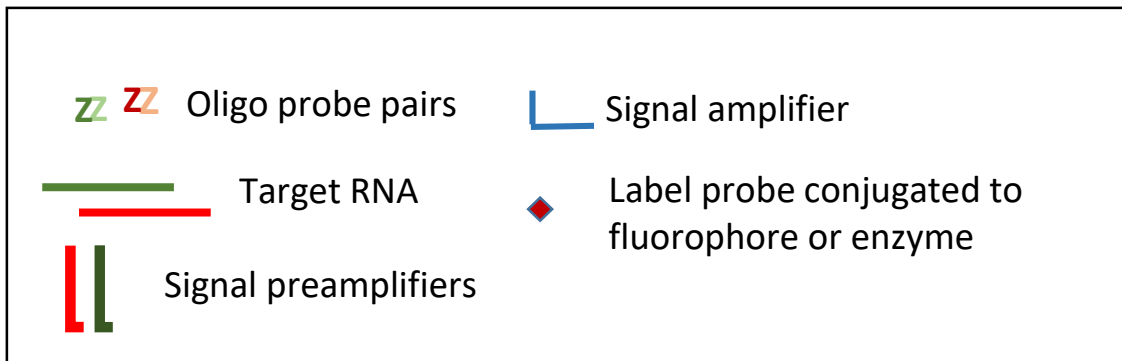
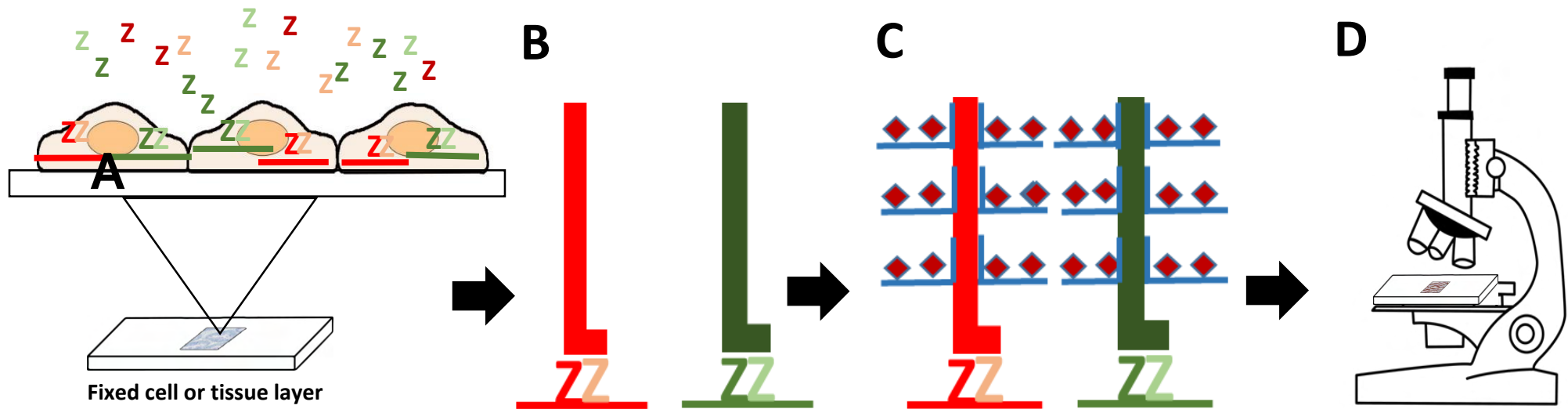
- Blood and kidney

Late phase with cardiac pathology

- Increased level of PMCV RNA in all sampled cardiac tissues

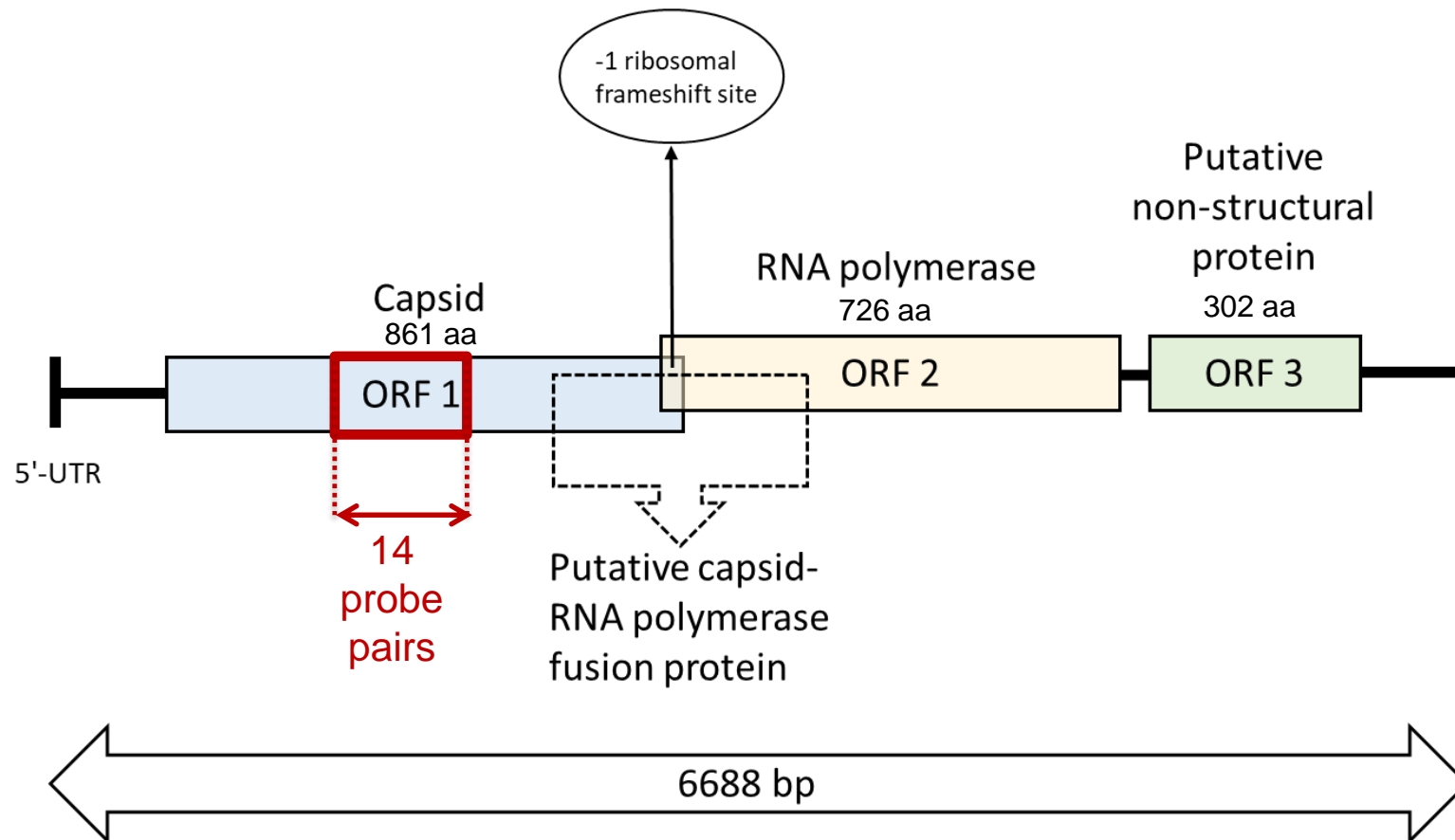


RNAscope® ISH



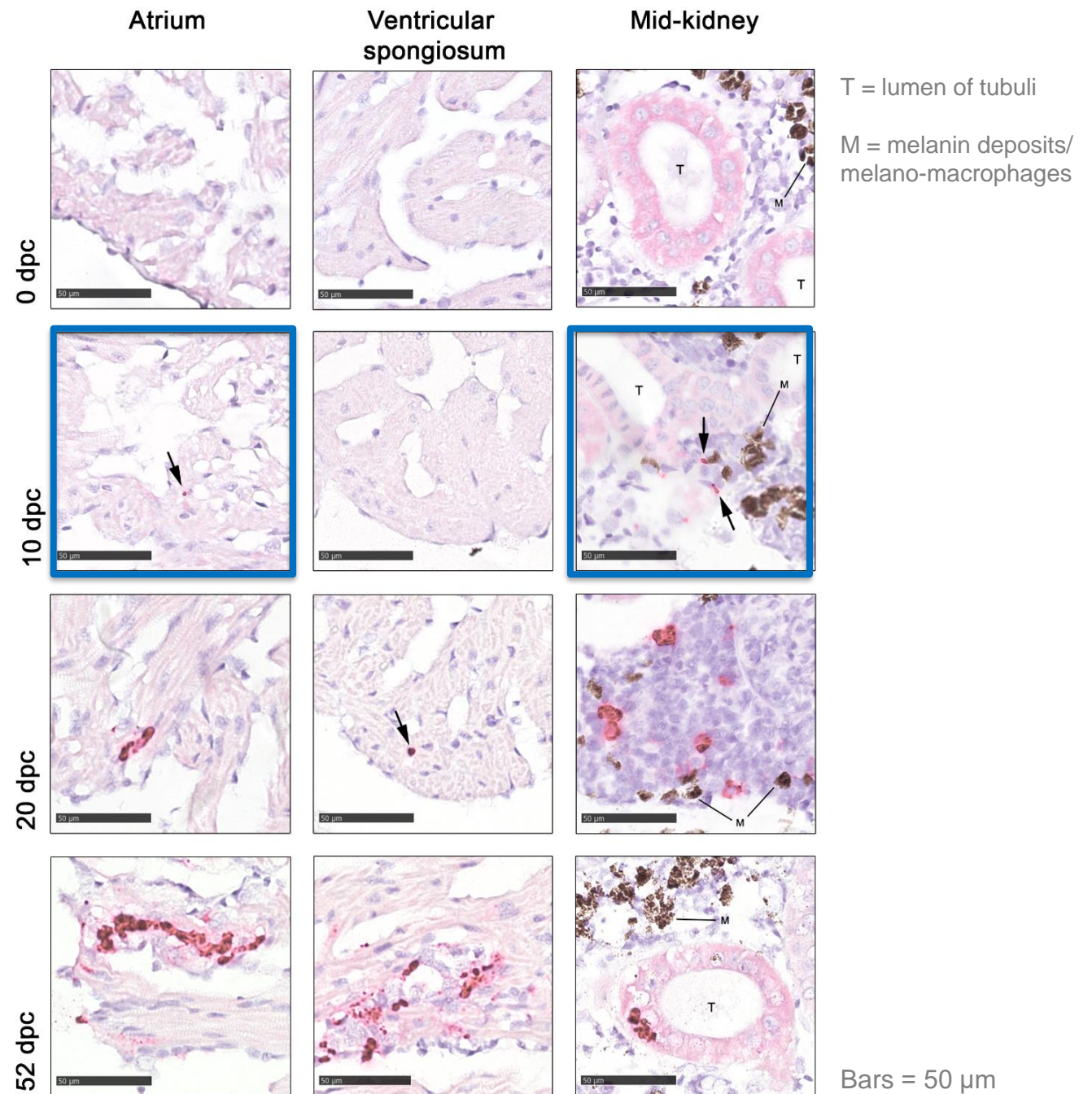
Localisation of probe pairs

RNAscope® PMCV



ISH RNAscope®

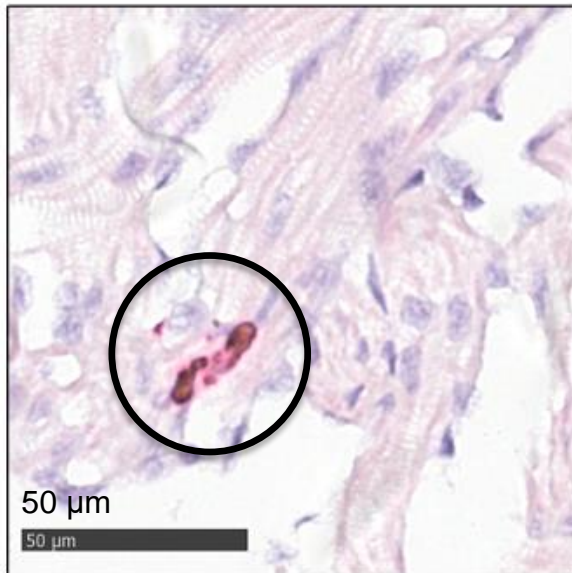
- Positive staining at all sampling points (post 0 dpc)



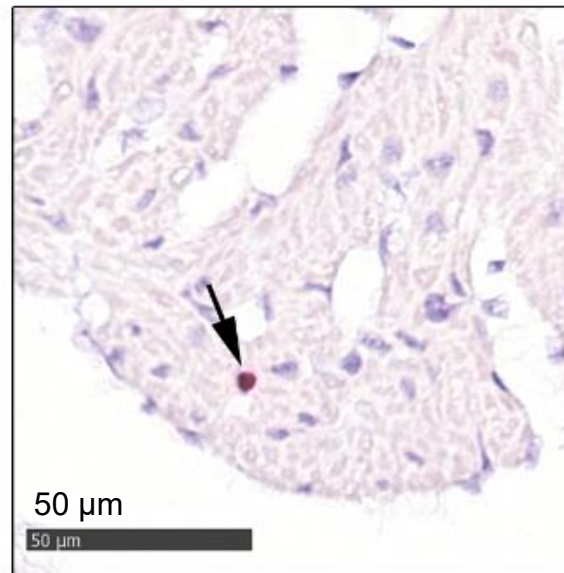
RNAscope® ISH

20 dpc

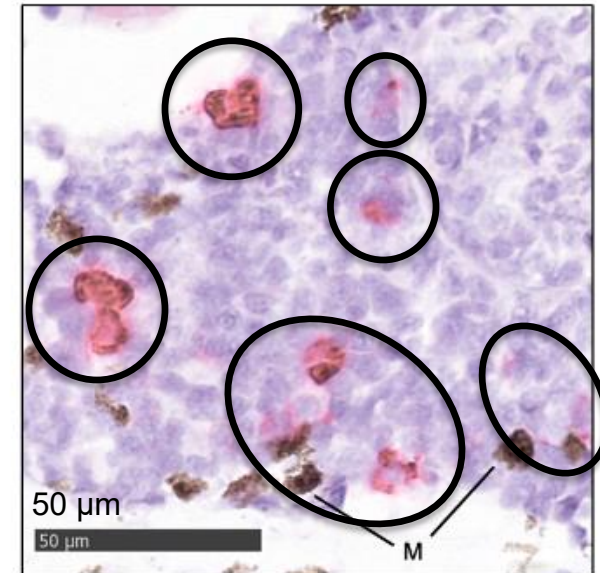
Atrium



Ventricular
spongiosum



Mid-kidney

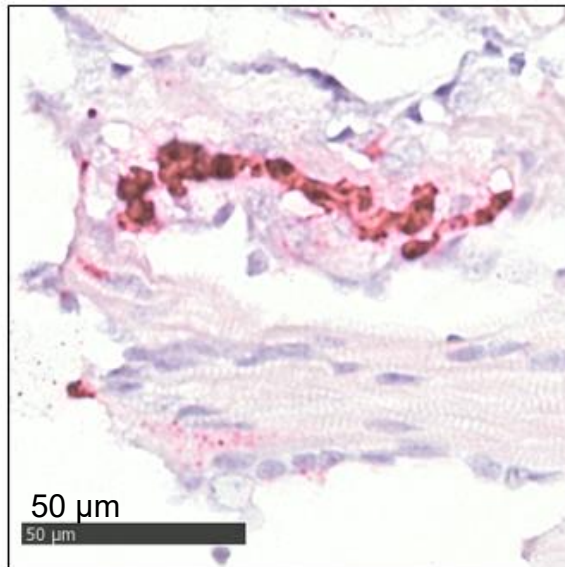


All images 400x magnification

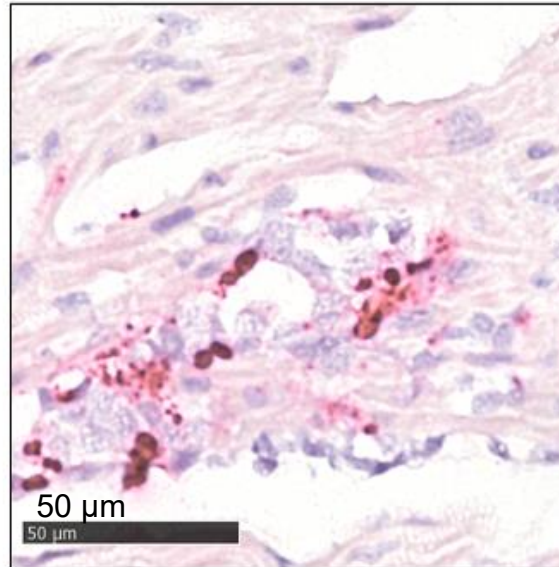
RNAscope® ISH

52 dpc

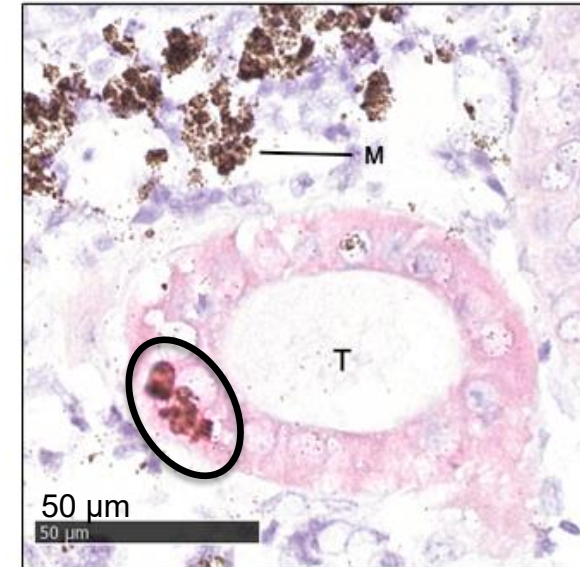
Atrium



Ventricular
spongiosum



Mid-kidney



All images 400x magnification

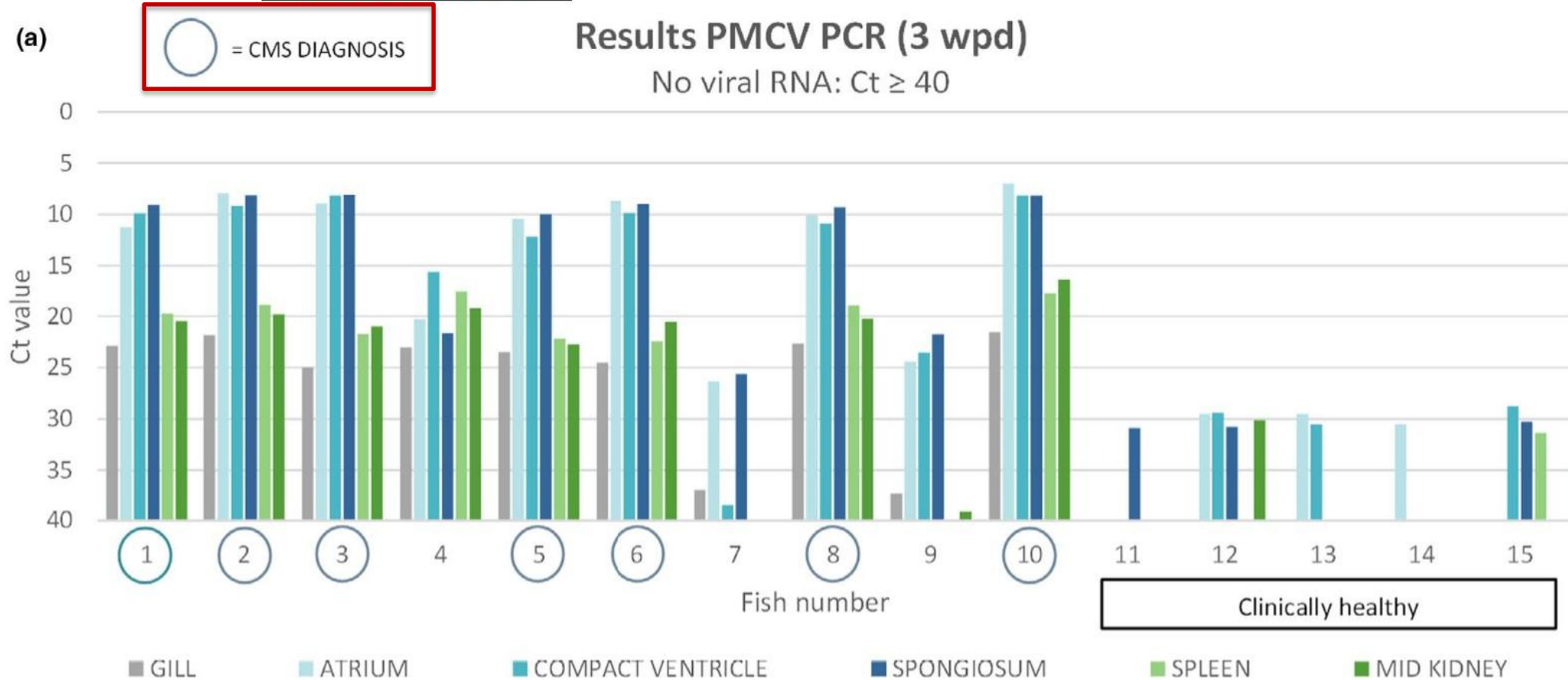
Summary

- The cardiac lesions were in accordance with previous descriptions of CMS
- Indications of a **viremic phase** 10 to 20 dpc, preceding cardiac lesions
- Relatively high amounts of PMCV RNA in mid-kidney 10 to 20 dpc
- **Plasma and/or mid-kidney** candidates for **early detection of PMCV**
- The RNAscope *in situ* hybridisation method: higher sensitivity and robustness compared to the immunohistochemistry method
- **Heart is the organ of choice** for both viral detection and histopathological diagnosis *when cardiac lesions have emerged*

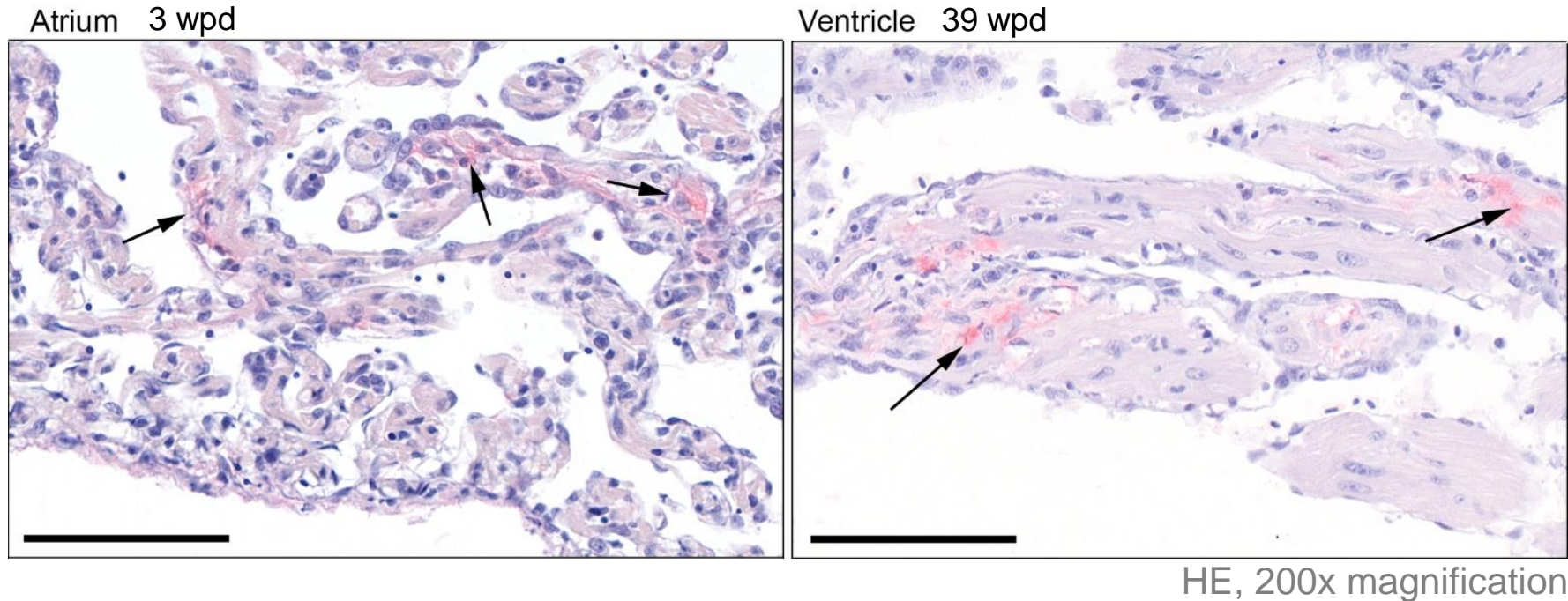
Thanks to

- The co-authors of the paper:
 - Ø. Haugland (Pharmaq), A. B. Mikalsen (NMBU), H. Sindre and H. Tartor (NVI)
- All the contributing laboratory staff at NVI, the Industrial Aquatic Laboratory (ILAB) and Pharmaq

Thank you for your attention!



Immunohistochemistry (IHC) for PMCV



- Antibodies:
 - Polyclonal
 - Targets Δ ORF3 protein