

Does PMCV primarily infect salmon?

Funding: FHF, prosjekt #901671

Participants:

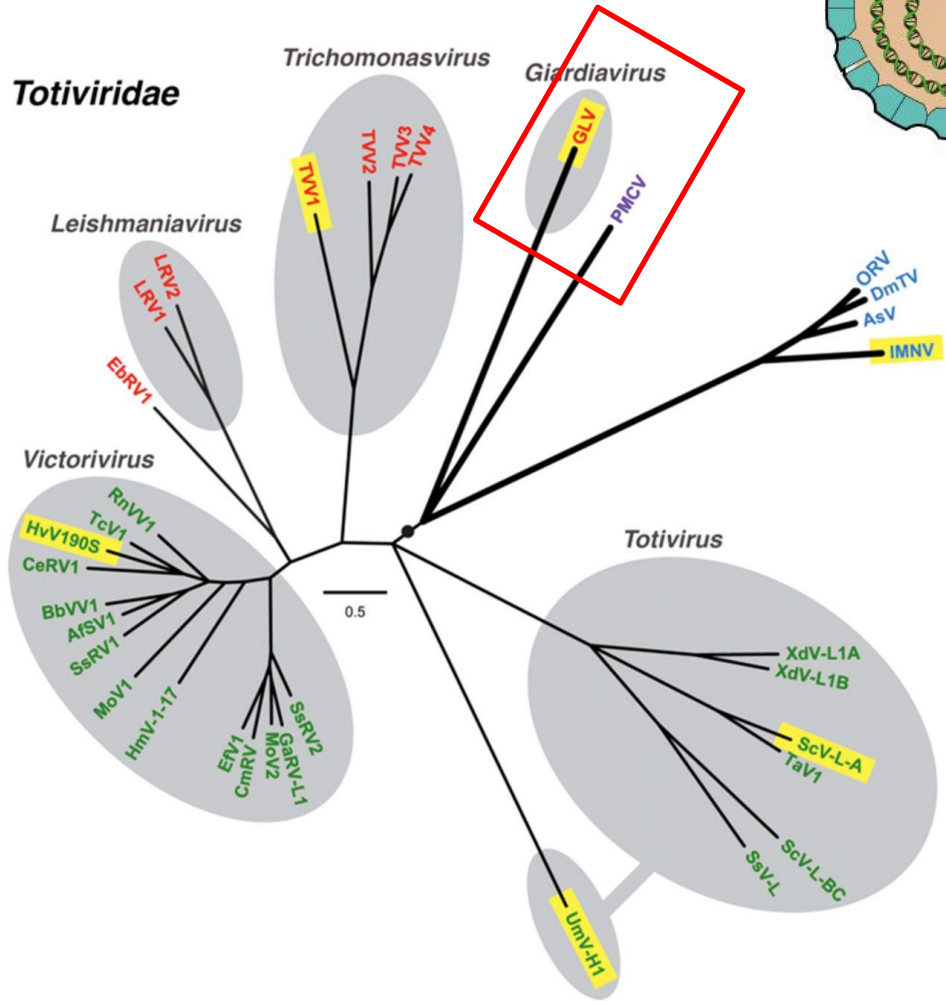
Norwegian University of Life Sciences

Pharmaq Analytic

Nofima

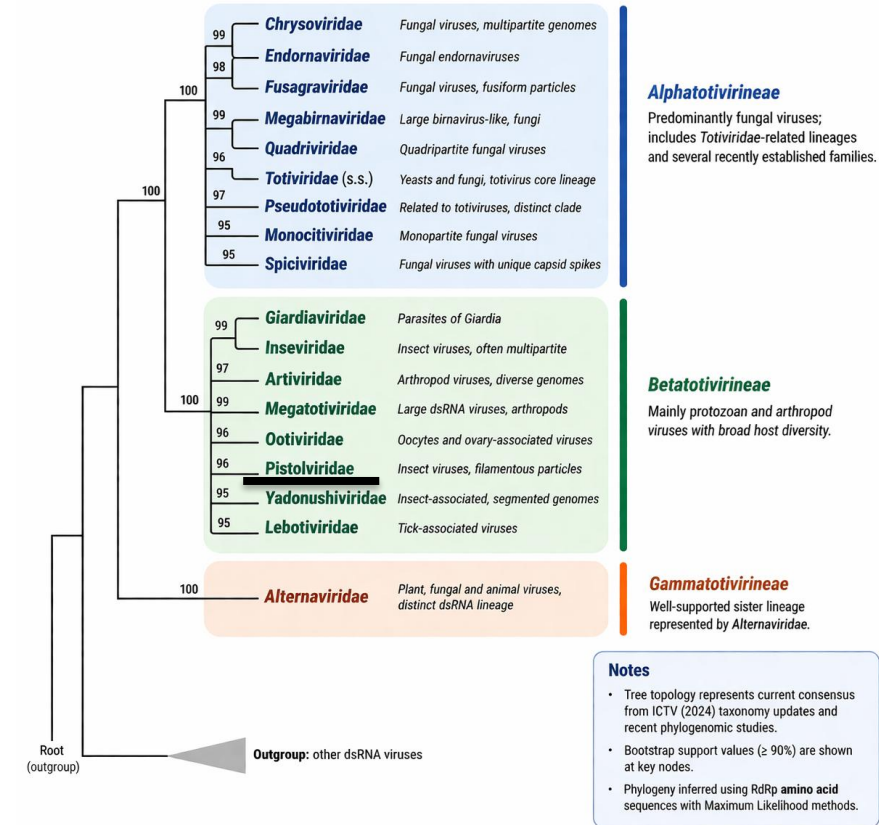
Faroese Food and Veterinary Authority

Leader: Espen Rimstad, NMBU

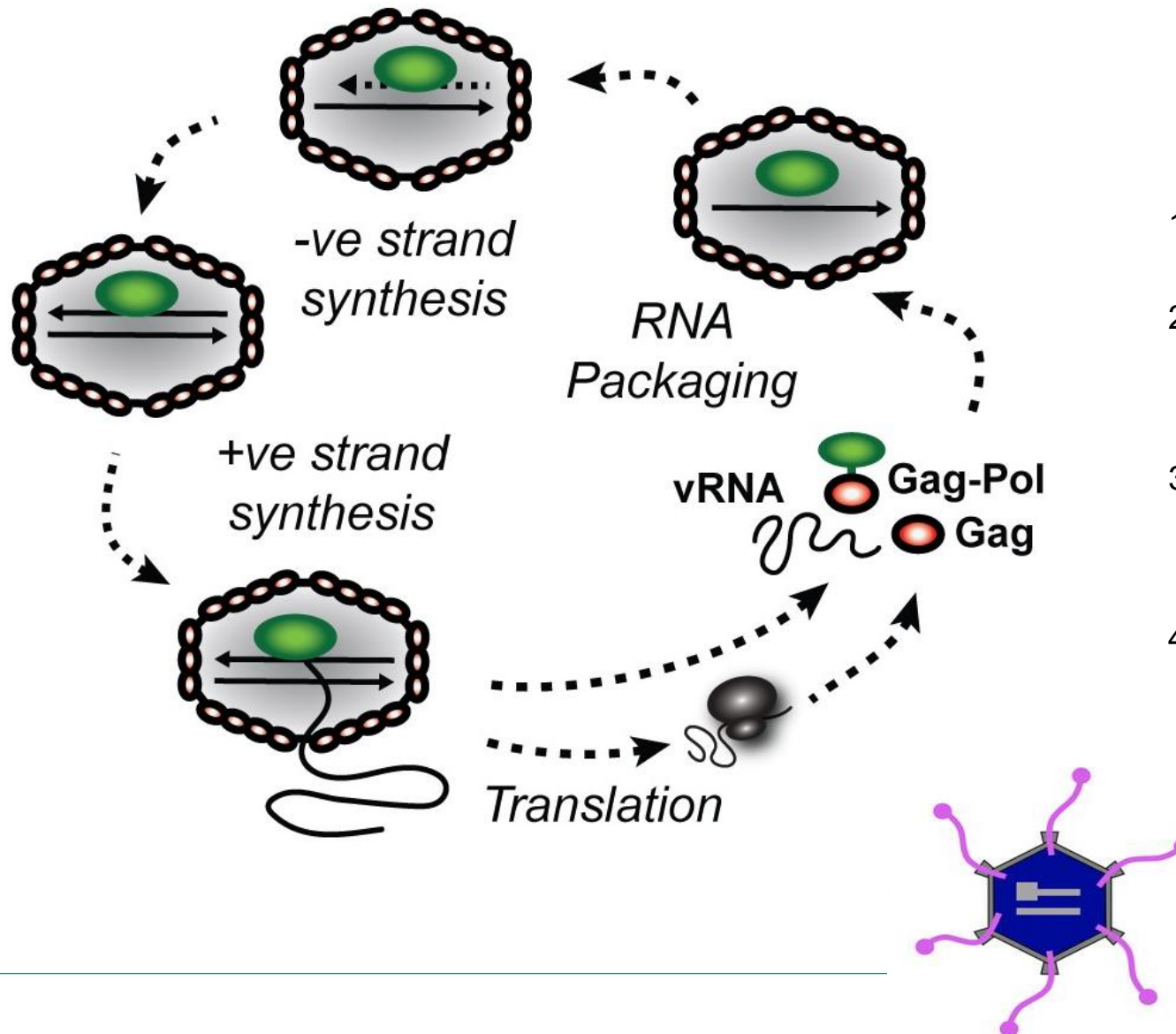
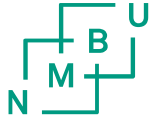


Ghabrivirales: Updated Phylogenetic Tree

Inferred from RNA-dependent RNA polymerase (RdRp) amino acid sequences



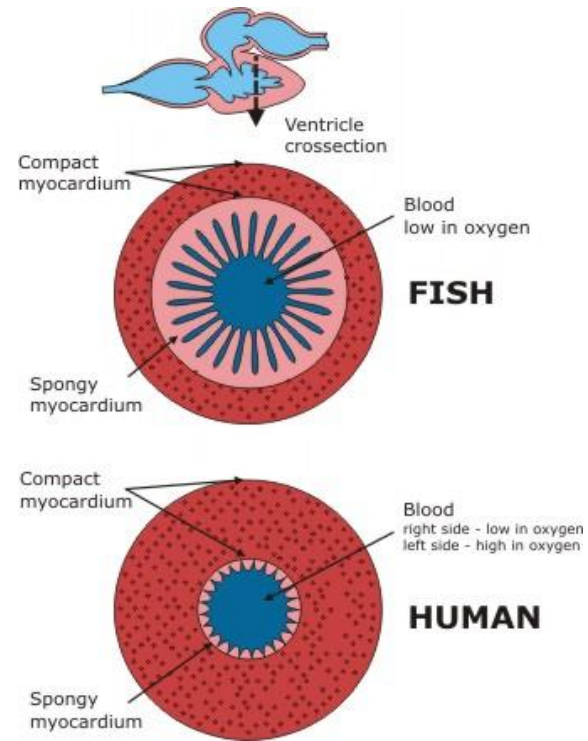
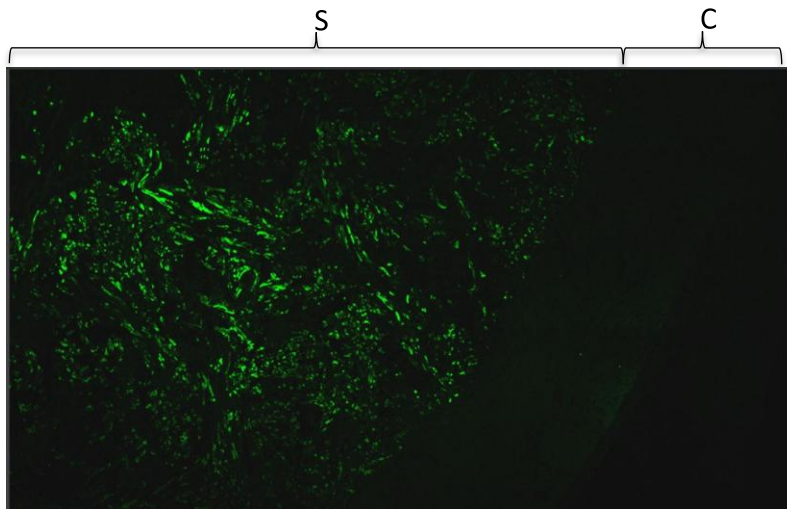
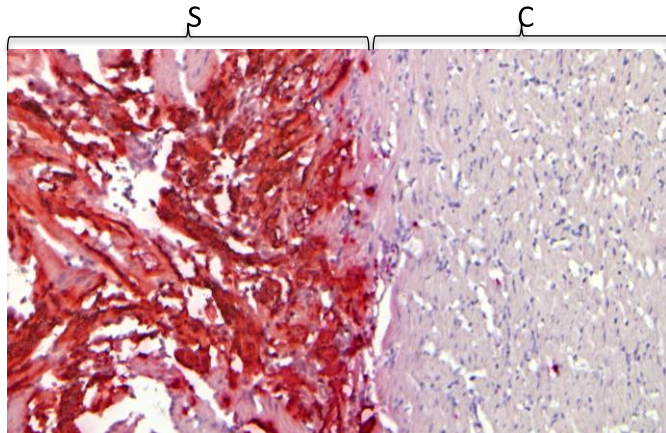
Replication of dsRNA in a vertebrate cell



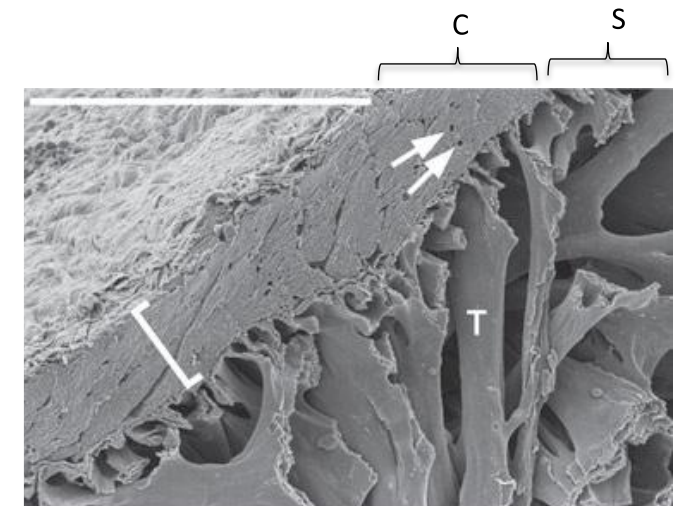
1. dsRNA is never released into the cytoplasm
2. mRNA (identical to the + strand of the dsRNA genome) is emitted from the capsid.
3. mRNA is translated and packaged before new dsRNA is made.
4. There are thus 2 types of viral RNA in the cell, dsRNA – genomic, +ssRNA = viral mRNA

Viral RNA in heart

- CMS occurs usually towards the end of the production cycle
- In the heart ventricle, PMCV RNA is limited to the spongiosum, and to a very small extent found in the compactum

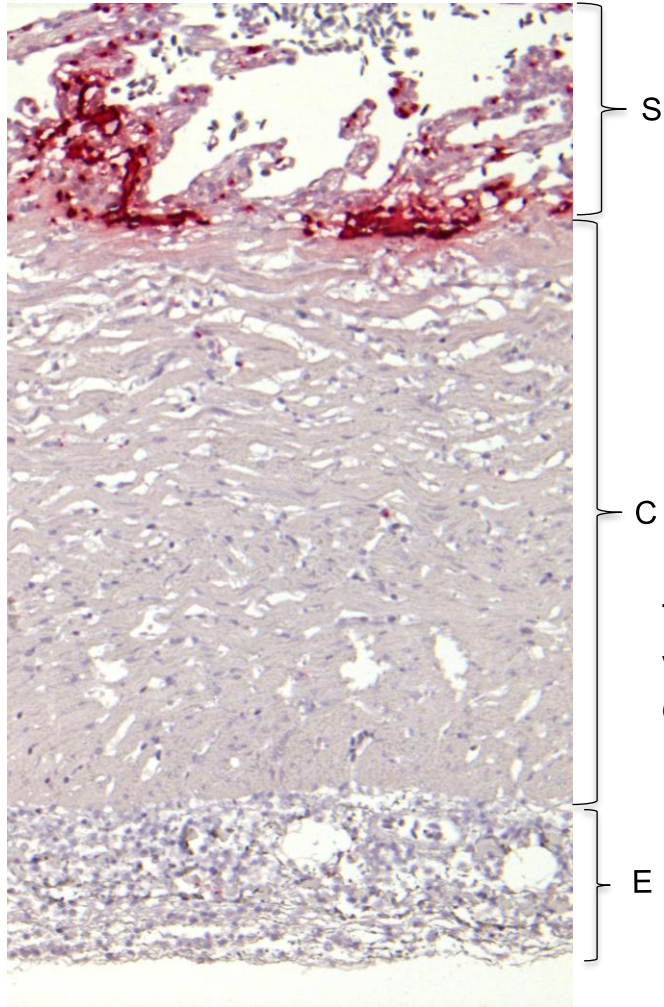


Source: T.Poppe



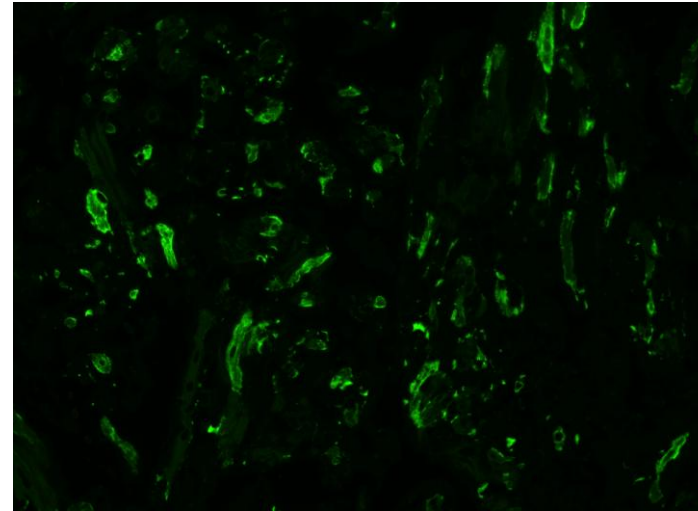
Pieperhoff et al. J. Anatomy 2009

Viral RNA in heart



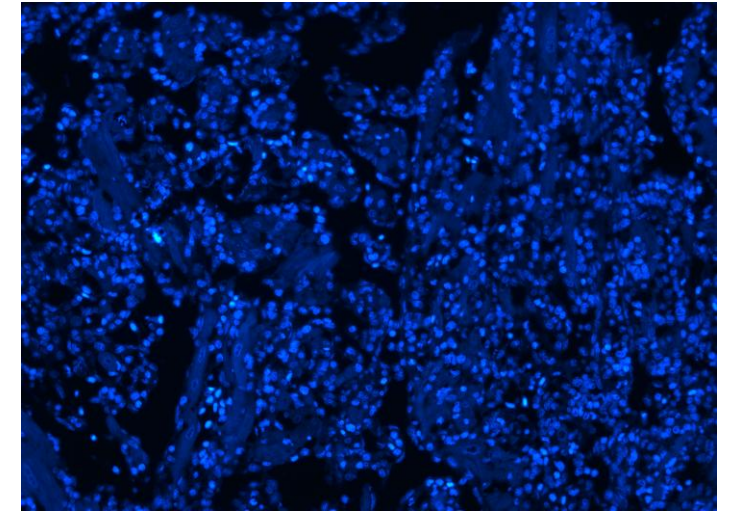
There is no viral RNA in compactum

Staining of PMCV



S

Same view, staining of all cells



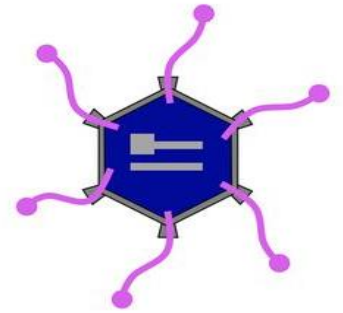
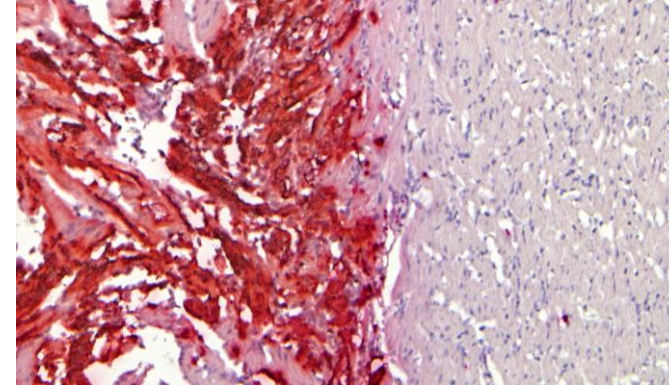
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There is no viral RNA in blood cells

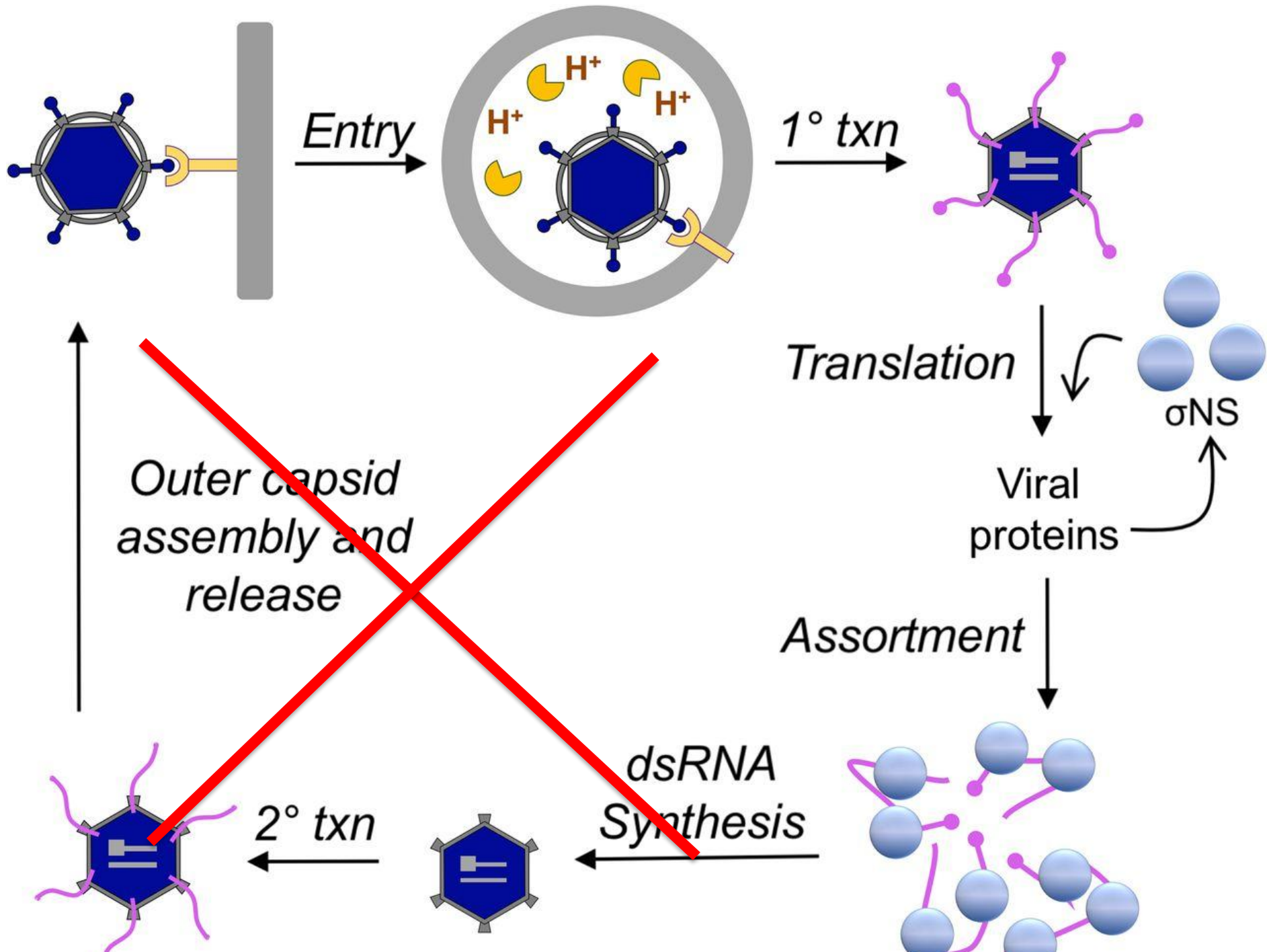
Detection of PMCV dsRNA and mRNA using RT-qPCR

	PMCV RNA heated = ssRNA + dsRNA	Relative ssRNA of total RNA
Heart 1	17.05	88%
Heart 2	13.36	100%
Heart 3	14.19	81%
Heart 4	12.79	100%
Heart 5	17.70	52%

Kidney 1	21.66	7%
Kidney 2	20.20	20%
Kidney 3	20.26	6%
Kidney 4	21.74	9%
Kidney 5	19.00	5%

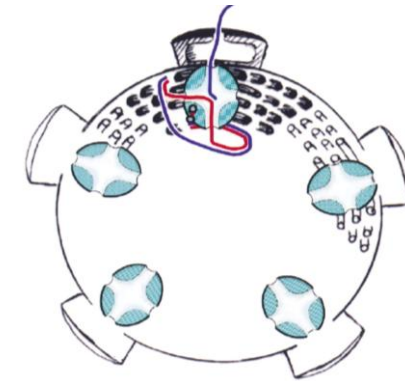
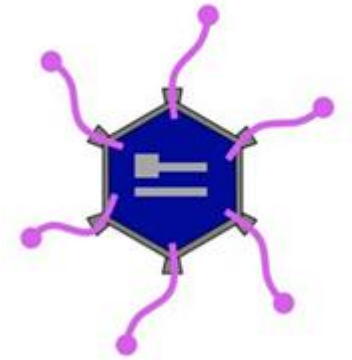


PMCV produces large amounts of viral mRNA in the heart
Only minor production of viral particles in the heart!

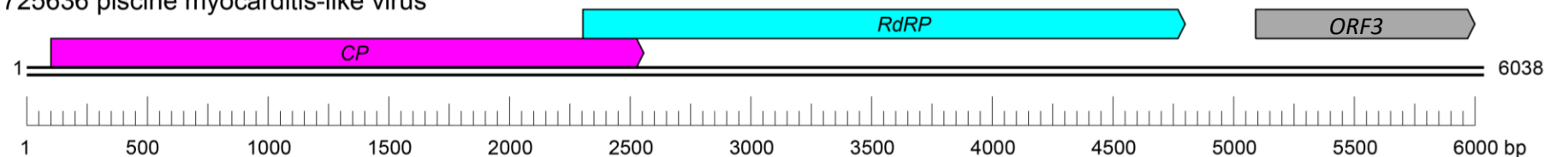


Different expression levels between the parts of the genome?

	ORF 2	ORF 3	ΔCq	Relative amount of ORF3 ssRNA versus ORF2
Heart 2	12.08	15.36	-3.28	10%
Heart 3	12.97	17.94	-4.97	3%
Heart 4	11.34	16.60	-5.26	3%
Heart 5	18.31	21.72	-3.41	9%
Average				6.25%
Kidney 2	22.91	24.23	-1.32	40%
Kidney 3	23.84	25.43	-1.59	33%
Kidney 4	24.68	27.10	-2.42	19%
Kidney 5	23.13	24.71	-1.58	33%
Average				31.25%



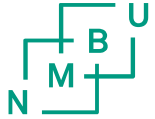
KT725636 piscine myocarditis-like virus



Conclusions from studies of heart ventricle:

- Infection restricted to spongiosum *There is no indication of infection of blood cells or compactum*
- Spread within heart ventricle: *There is no indication of spread of virus (we tested for viral RNA) from spongiosum to compactum.*
- Viral RNA genomic dsRNA versus mRNA: *There is relatively little production of viral dsRNA in heart, which is essential for viral particles!*
- The genome of PMCV: *There are differences in expression levels in the viral genome, with less ORF3 RNA in samples with much mRNA.*
- *Together this indicates that salmon heart is a blind alley for production of new virus particles.*

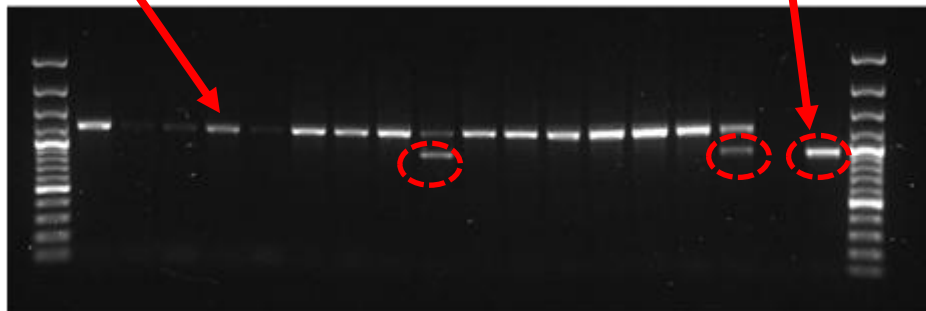
Other host organisms for PMCV? Are there fungi or protists in heart/kidney tissue?



PCR for conserved riboRNA regions in the genome, sequencing of product. "Barcoding". Protozoa/fungi.
Most came out negative....

PCR product with the wrong size – sequencing showed it was salmon DNA

PCR product with correct size – sequencing showed that it was fungal DNA



Primersett	Navn		Primer sekvens	PCR prod (bp)
5	Trypanosomer	TRYP-Fw	CCAWACAACAAACATATGATGCTGC	650
		TRYP-Rw	TCCHGATATGGTWTTKCCYCG	
6	Apicomplexa	APIC-Fw	GAAACTGCGAATGGCTCATT	1500
		APIC-Rw	CTTGCGCCTACTAGGCATTC	
7	Mikrosporidier	CM-V5F	GATTAGANACCNNGTAGTTC	200
		CM-V5R	TAANCAGCACAMTCCACTC	
8	Myxozoa	Myxo_617F	CGCGCAAATTACCCAMTCCA	900
		Myxo_2313R_all	CGTTACCGGAATRRCTGACAG	
9	Ichtyoph	NS1	GTAGTCATATGCTTGCTC	1770
		NS8	TCCGCAGGTTACCWACGGA	
10	Ichtyophonus	vc7F	GTCTGTAAGTGGTACGGCAGTTTC	75
		vc5R	TCCCGAACTCAGTAGACACTCAA	
11	Sopp ITS	ITS1	TCCGTAGGTGAACCTGCGG	Ca 600
		ITS2	GCTGCGTTCTTCCATCGATGC	
12	Sopp ITS	ITS3	GCATCGATGAAGAACGCAGC	Ca 600
		ITS4	TCCTCCGCTTATTGATATGC	
13	Sopp 28S	NL1	GCATATCAATAAGCGGAGGAAAAGGGT	Ca 600
		NL4	CCGTGTTTCAAGACGG	
14	Sopp, LSU	LROR	ACCCGCTGAACTTAAGC	ca 1000
		LR5	TCCTGAGGGAAACTTCG	

Other organs where PMCV RNA is present?

Fish No.	Heart	Gills	Kidney	Spleen	Liver	Muscle	Skin Scrape	Pyloric Ceca	Mid-Gut	Hind-Gut
1	-	-	-	-	-	-	35.9	-	-	-
2	24.5	26.0	22.9	21.9	29.9	27.4	26.4	27.5	25.7	27.5
3	-	-	-	-	-	-	-	-	-	-
4	16.4	28.6	22.5	22.7	26.7	26.8	28.4	26.3	25.3	26.3
5	36.9	-	35.0	-	-	-	37.4	-	-	-
6	-	-	-	-	-	-	-	-	-	-
7	24.4	26.4	22.9	22.9	29.5	28.1	29.9	27.4	22.2	27.1
8	22.9	28.8	21.9	21.8	27.1	27.8	29.6	29.6	29.1	30.5
9	16.9	28.0	24.5	24.9	30.5	28.4	28.7	28.9	28.3	27.7
10	26.2	26.3	23.9	25.1	30.8	28.0	27.3	29.5	26.5	27.6
Ave	21.9	27.3	23.1	23.2	29.1	27.8	28.4	28.2	26.2	27.8

(Cq values from samples collected from a farm with detection PMCV two months earlier, but no clinical CMS)

PMCV in skin/mucus

Louse counting - anesthesia tanks*

	CMS positive farms	CMS negative farm (gill swabs)
Sideline swab/scrape (mucus)	58/58	0/10
Water	18/18	0/5

*Samples from M.M. Dahl, Faeroes

Fish #	Not heat treated (ssRNA)	Heat treated (ssRNA + dsRNA)	ΔCq	Ratio PMCV ssRNA versus total PMCV RNA (%)
1	22,9	22,2	0.7	62
2	23,0	22,0	1.0	50
3	27,6	22,8	4.8	4
4	28,5	23,1	5.4	2
5	27,5	22,1	5.4	2
6	28,1	22,5	5.6	2
7	34.5	29.9	4.6	4
8	36.0	30.2	5.8	2
9	28,8	23,9	4.9	3
10	32.4	28.3	4.1	6

PMCV in skin/mucus

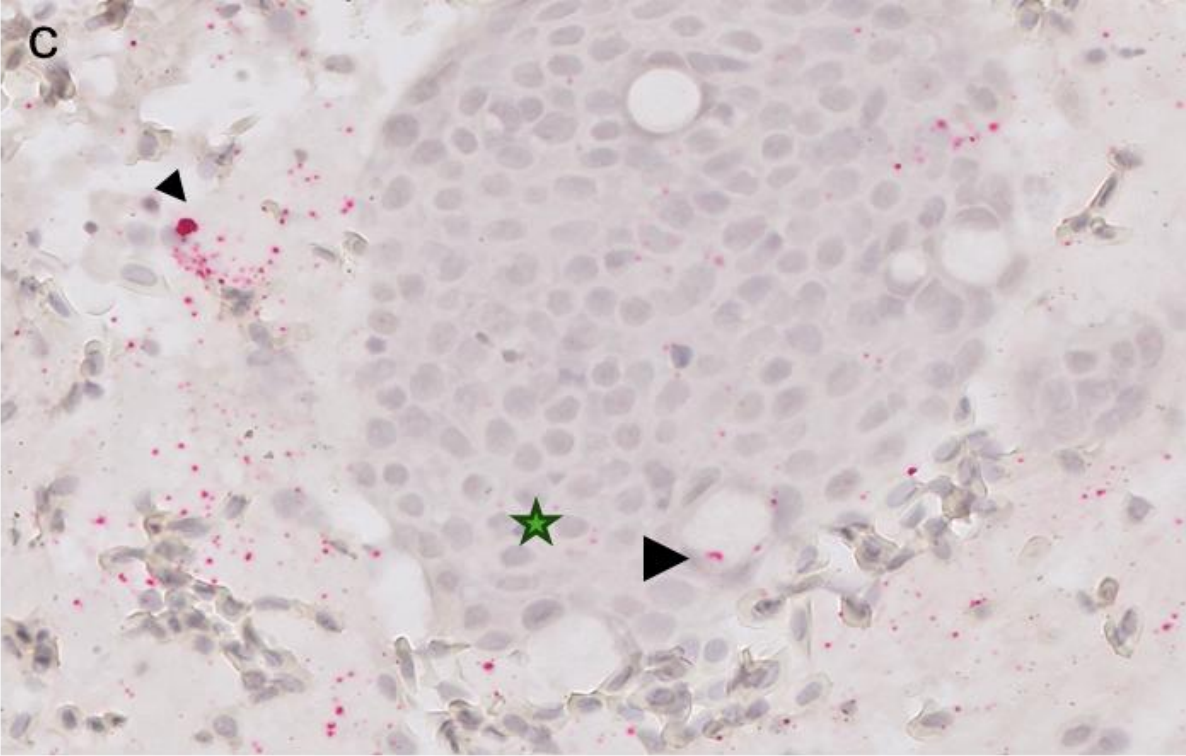
Any particular part of the skin where the virus is concentrated?

Fish	Swab	Scrape from skin		
	Gills	Midline	Basis of thoracal fin	Anal opening
1	26.84	26.78	26.24	27.54
2	27.22	26.97	23.96	27.27
3	30.23	31.26	25.19	30.30
4	29.02	25.29	24.48	25.82
5	24.61	23.90	22.36	24.17
Ave	27.58	26.84	24.45	27.02

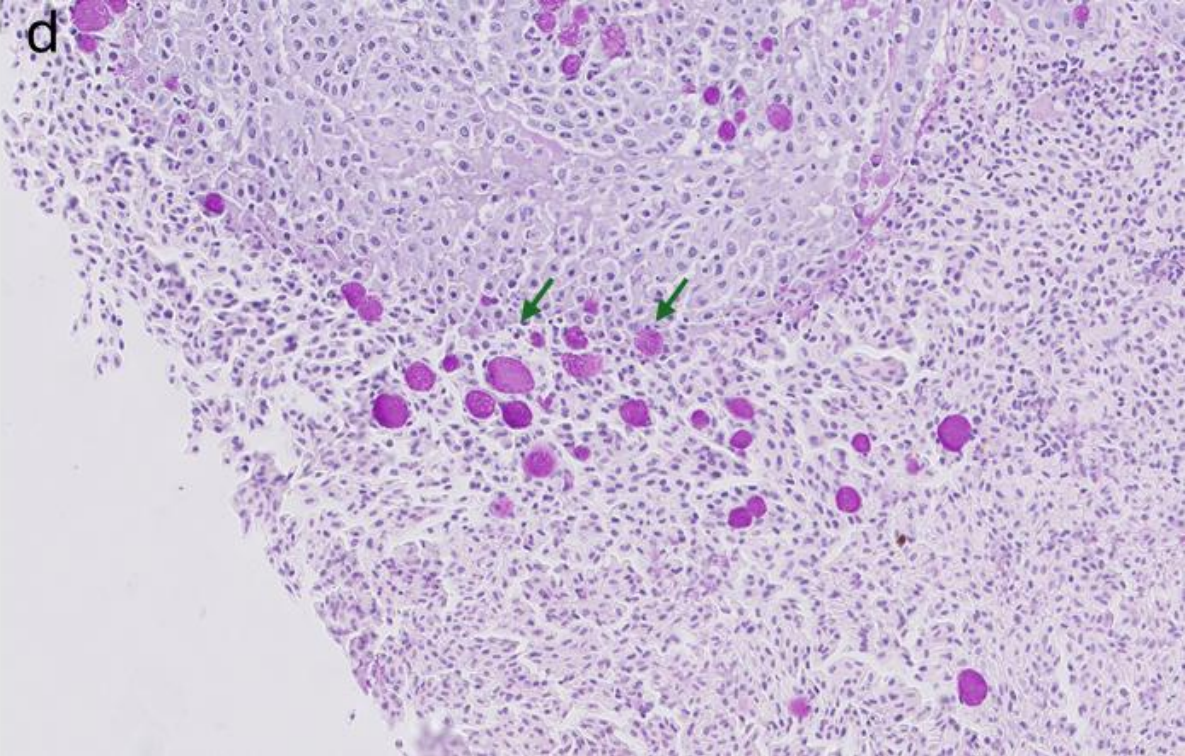
Samples from a PMCV positive population, no clinical CMS

No indication of concentration of viral RNA in gills or around anal opening. They do not stand out as point of shedding.

RNAScope, skin scrape/swab. **Detection of PMCV.** Goblet cells

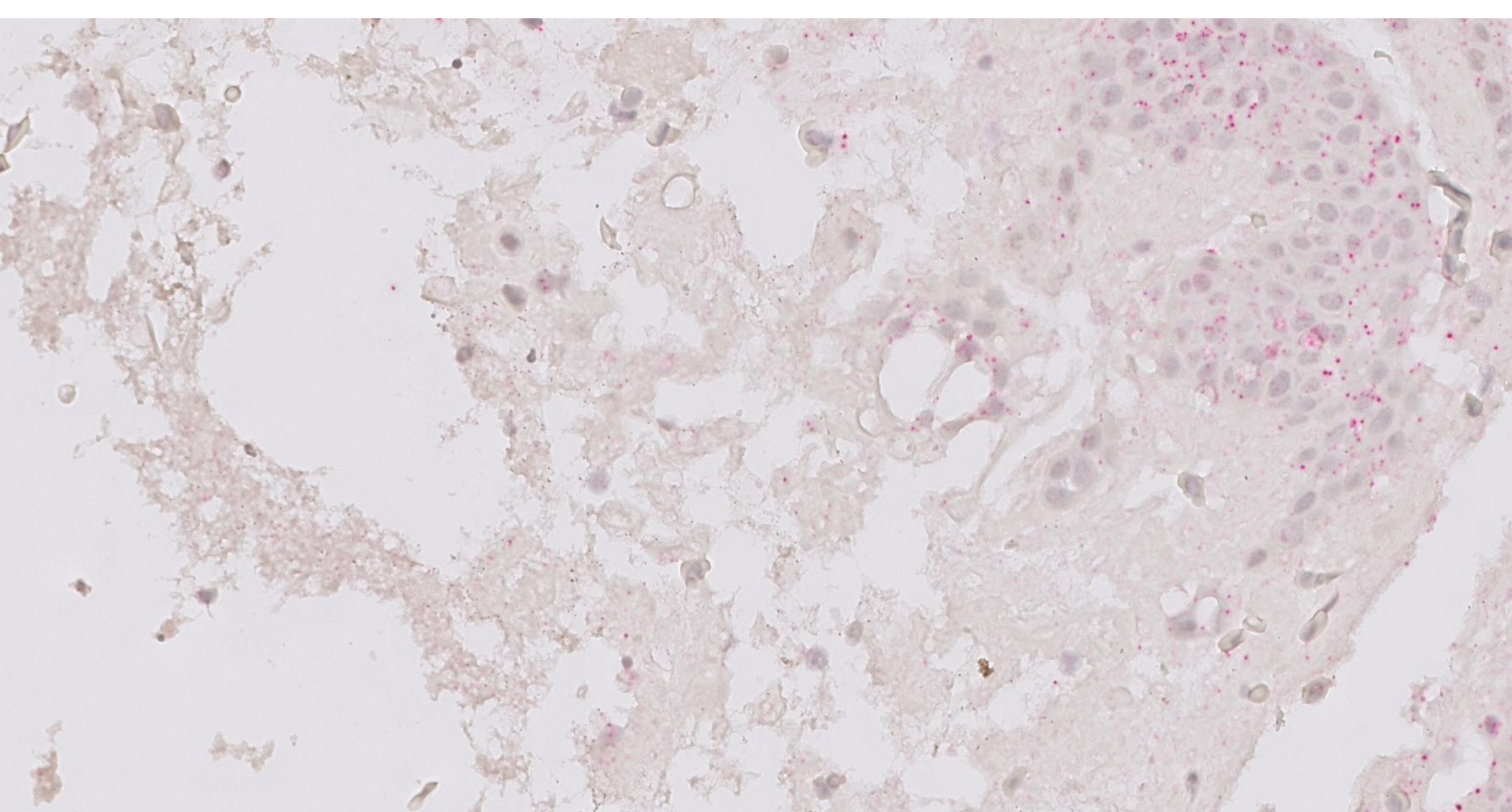


40x



PAS staining

- There are large amounts of PMCV in skin mucus (use non-lethal testing for PMCV!)
- Skin is the organ with most positive samples
- PMCV replicates in cells in the skin
- We find PMCV, apparently, free virus (i.e. not cell-associated) in skin mucus (biosecurity measures for anesthetic tanks, crowding during delousing operations etc.!))



PPIB, positive controll for detecion of salmon RNA 40X

Does PMCV primarily infect salmon?

No

The end

