



Norges miljø- og  
biovitenskapelige  
universitet



# Development of CMS challenge models

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# About VESO

- Pharmacy and head office in Oslo  
Vaccines, pharmaceuticals and disinfectants
- Research facility at Vikan – customized & standardized lab trials
  - Veso Vikan hatchery
  - AVS Chile Colaco - SRS & sea lice challenge trials
- Field trials – two salmon farming licenses for R&D

Bacteria	Virus	Parasites
<i>Aeromonas salmonicida</i>	IPNV (IPN)	Sea lice
<i>Vibrio sp.</i>	ISAV (ISA)	Amoeba
<i>Yersinia ruckeri</i>	SAV/ SPDV (PD)	Costia
<i>Moritella viscosa</i>	PRV (HSMI)	
<i>Piscirickettsia salmonis</i>	PMCV (CMS)	

# Cardiomyopathy syndrome



- First diagnosed in Norway, 1985
- Scotland, Ireland, Canada
- Causative agent: Piscine myocarditis virus (PMCV), dsRNA virus
- Affects Atlantic salmon at their second year at sea or shortly after sea transfer
- Sudden mortality
- No vaccine

# Cardiomyopathy syndrome pathology and diagnostics



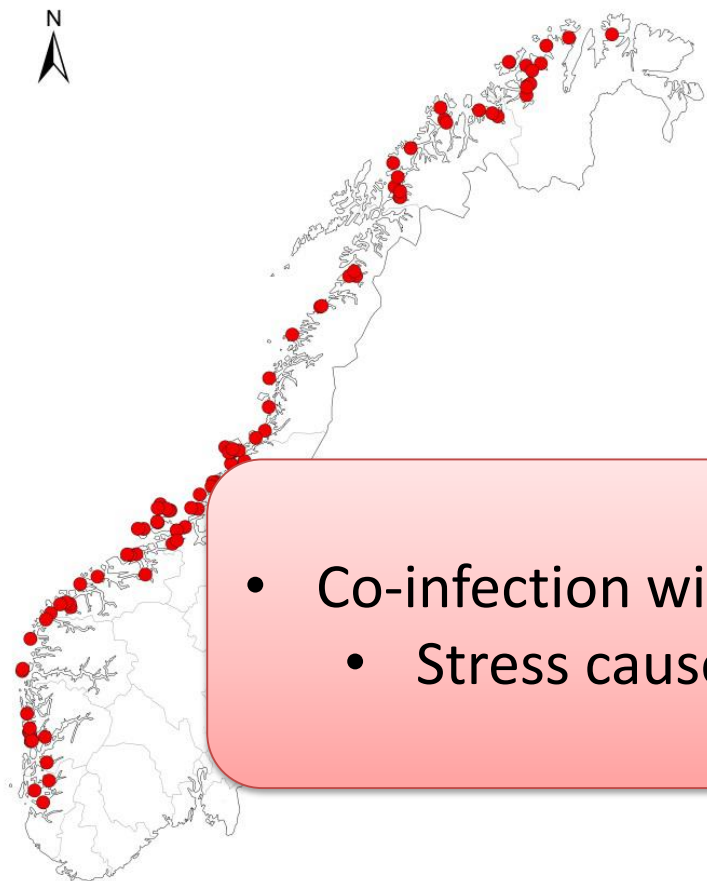
## External

- Ventral skin haemorrhages
- Raised scales due to oedema

Histopathology in heart  
RT-qPCR

## Internal

- Histopathological changes in heart
  - multifocal pattern of the affected tissue of the atrium
  - Inflammation & severe myocardial damage, almost no intact cells in the atrium and spongy layer of the ventricle
- Ascites, blood clots in pericardial cavity, discoloration of liver



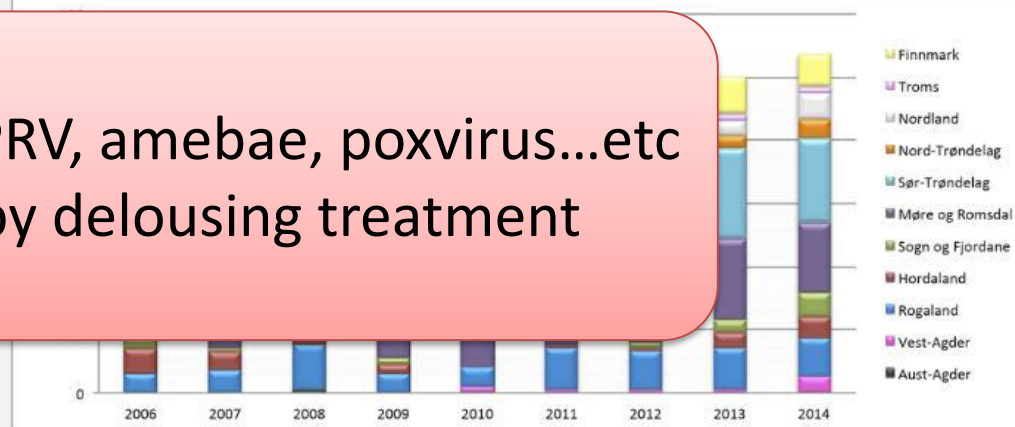
© Veterinærinstituttet 2017

0 75 150 300 Kilometer

## Situation in Norway

- 105 cases in 2015
- Economic losses
- Both wild and farmed

- Co-infection with PRV, amebae, poxvirus...etc
  - Stress caused by delousing treatment



Fylkesvis fordeling av antall lokaliteter registrert med kardiomyopatisyndrom (CMS) i perioden 2006-2014

# Materials and methods



- Smoltified Atlantic salmon, unvaccinated, specific pathogen free
- 50 - 200 grams at challenge
- 12°C seawater or brackish water, L:D=24:0
- Tissue-homogenate from field outbreak  
PMCV+/PRV- confirmed by real-time RT-qPCR
- Absorbed with anti-PRV antibody
- Treated with gentamicin
- Sampling: Histology and PCR

# Pilot trials



## Material from field outbreak

- Pilot 1: i.p. challenge

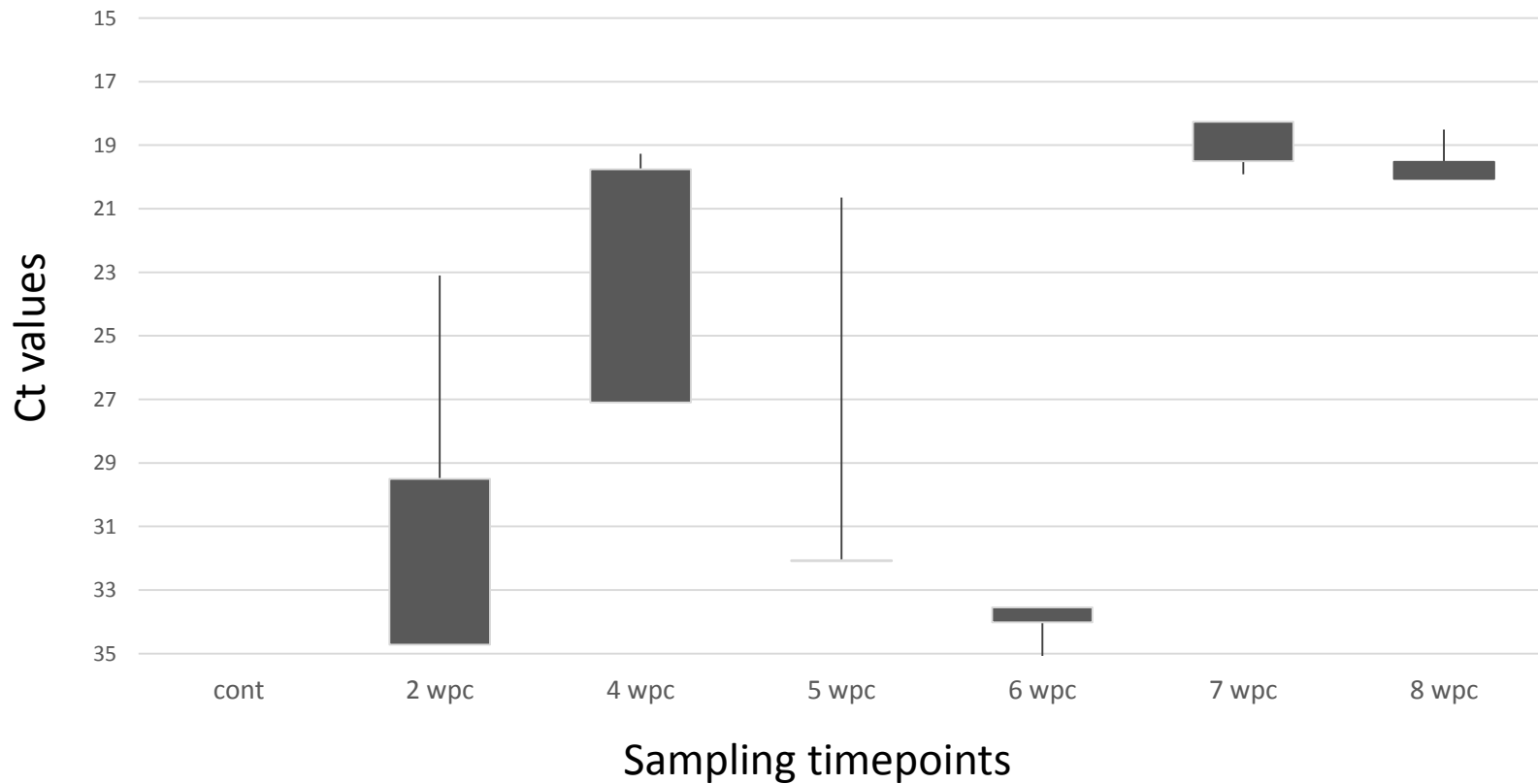
Pilot 1.	Cont.	6 wpc	7 wpc	8 wpc
PMCV+ (SYBR)	0/3	3/3	3/3	3/3
PRV+ (probe)	0/3	2/3	2/3	0/3

- Pilot 2: i.p. challenge

Pilot 2.	Cont.	2 wpc	4 wpc	5 wpc	6 wpc	7 wpc	8 wpc
PMCV+ (SYBR)	0/4	1/4	4/4	2/4	0/4	4/4	4/4
PRV+ (probe)	0/4	0/4	0/4	0/4	0/4	0/4	0/4

# Pilot 2. results

## Ct values after challenge





# Cohabitation challenge i.m. or i.p. as shedders

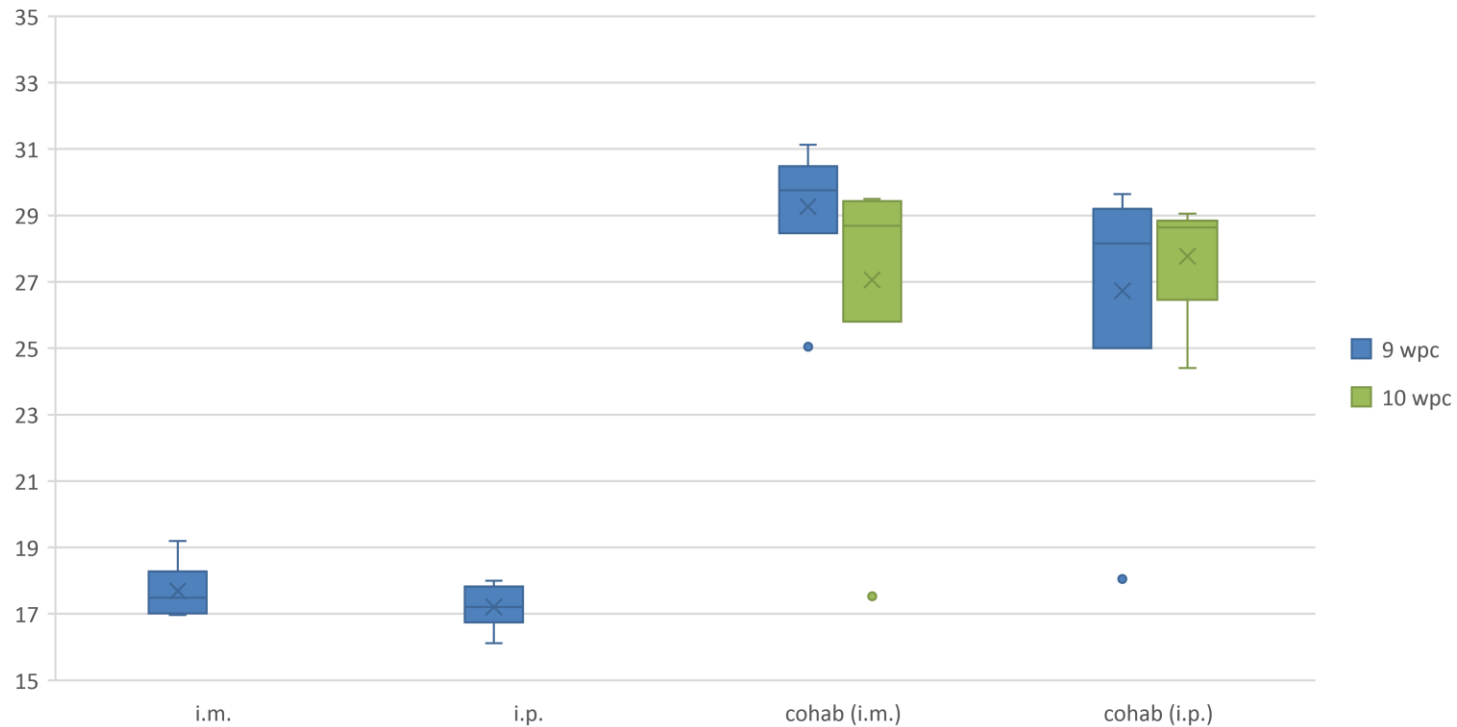


Challenge 3. i.p./i.m. coma		Cont.	9 wpc	10 wpc
PMCV+ (SYBR)	i.m.	0/6	6/6	-
	Cohab	0/6	<b>3/6</b>	<b>3/6</b>
	i.p.	0/6	6/6	-
	Cohab.	0/6	<b>2/6</b>	<b>2/6</b>

Challenge material from  
pilot 2, 7 wpc pooled

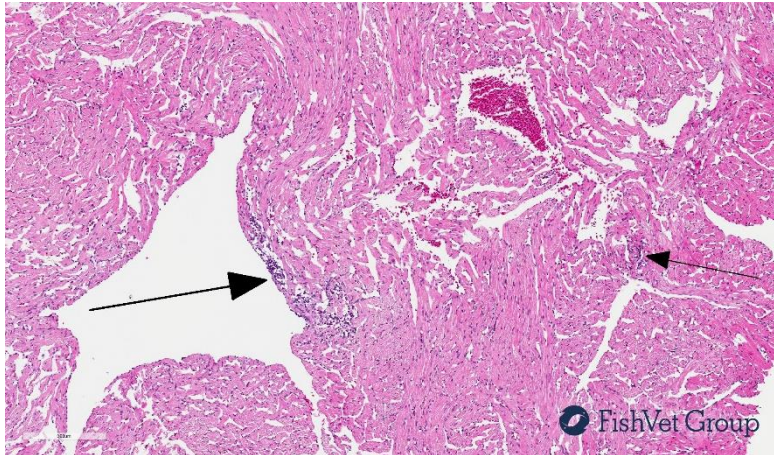
Cohabitants became PMCV+ after 9 – 10 weeks post challenge  
No clinical sign

# Cohabitation challenge Ct values in different groups



# Cohabitation challenge

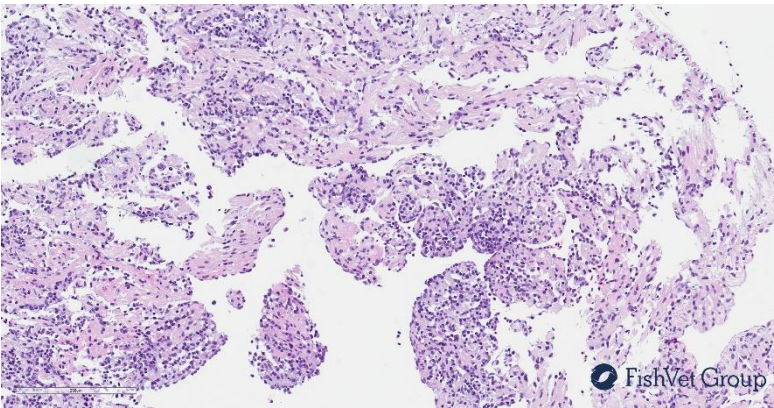
## Histopathology in heart



### i.m. challenged fish

- Inflammation in spongiosum

**Score 1**



### i.p. challenged fish

- Inflammation in spongiosum/epicardium

**Score 1.5**

# Long-term cohabitation challenge

12 i.p. injected fish as shedders

36 untreated fish as cohabitants

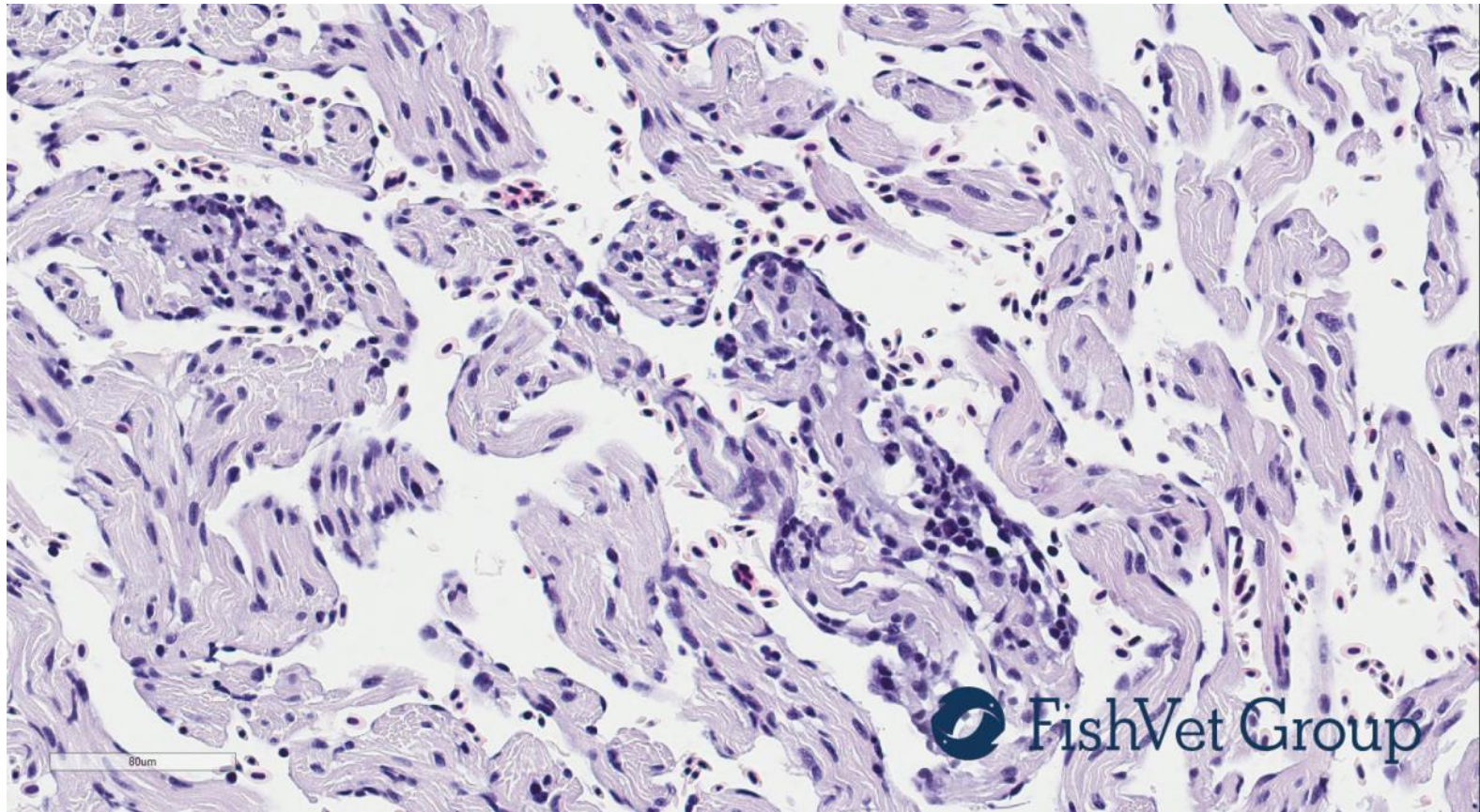
Challenge 4. Cohabitants	Cont.	9 wpc	10 wpc	12 wpc	24 wpc
PMCV+ (probe)	0/3	1/3	1/3	1/3	13/24
Ct values	-	30.9	27.12	22.99	L 20.52 H 34.62 A 23.78
Average +					
PRV+ (probe)	0/3	0/3	0/3	0/3	0/24

← Score 1  
← Score 0.25



# Cohabitant histopathology

24 weeks Ct 20.25, histo score 1



# Cardiomyopathy syndrome in the lab



## ~~External~~

- ~~• Ventral skin haemorrhages~~
- ~~• Raised oedema~~

## ~~Internal~~

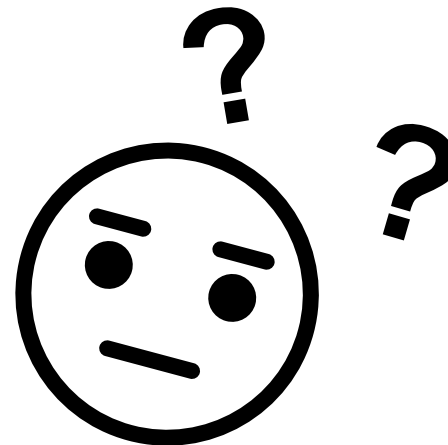
- ~~• Histology
  - ~~– myocardium, clearly separated~~
  - ~~– Inflammation & severe degeneration, almost no intact cells in the atrium and spongy layer of the ventricle~~~~
- ~~• Ascites, blood clots in pericardial cavity, discoloration of liver~~

*No mortality!*

No mortality so far...

What happens in the field?

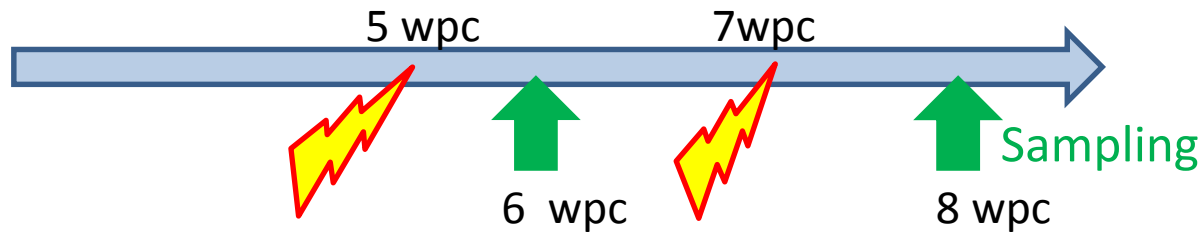
- Co-infection
- Delousing stress
- Bigger fish



# Stress treatment



	Treatment	i.p.	cohab
Tank A	No	50	0
Tank B	Yes	13	50



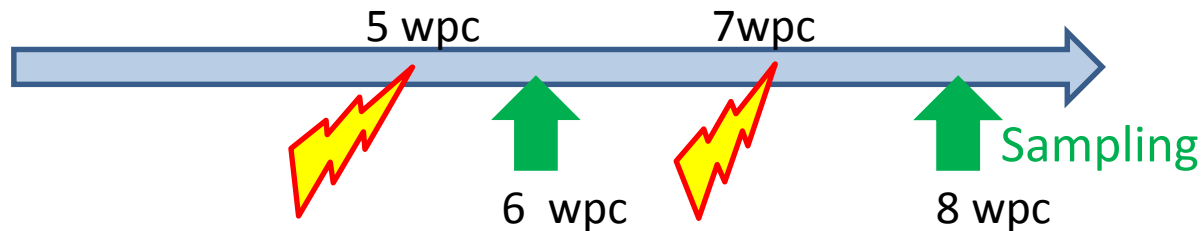
Stress treatment: Exposure to low oxygen for 30 min, 3 times a week



# Stress treatment Results



	Treatment	i.p.	cohab	Ct 6 wpc i.p.	Ct 8 wpc i.p.	Histo score
Tank A	No	50	0	21.14	25.13	0.25
Tank B	Yes	13	50	20.73	22.37	0.5 – 0.75



Stress treatment: Exposure to low oxygen for 30 min, 3 times a week

No cohabitants became PMCV positive  
after stress treatment

# Conclusions



- Horizontal transmission of disease in controlled lab settings
- PMCV positive cohabitants after 9 – 24 weeks, 30 – 50%
- **No mortality**
- **Good correlation between Ct values and histo score**
- Similar trend in field cases
- **PRV-free = Symptoms caused purely by PMCV**
- Stress treatment: did not help transmission but higher histo score