

## Cardiomyopathy syndrome (CMS): Cardiac pathology and cause in field material.

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## Overview

- Introduction & Project description
- Material and methods
- Results
- Conclusions
- Future work

## Introduction: short description CMS

- First described Norway 1985 (Scotland 2000)
- Farmed adult Atlantic salmon in seawater
- Enigmatic syndrome: "hjertesprekk"
- Løvoll *et al* (2010): pyrosequencing
- Haugland *et al* (2011): cultured and identified novel **totivirus** (PMCV)



## Project description

Cardiomyopathy syndrome: A multi-task approach to reduce losses and improve knowledge (2008-2011).

**Our part: Epidemiology and pathogenesis**

Objectives: Risks factors associated CMS (pathology)  
Diagnostic criteria for CMS

Project no. 187301/S40:

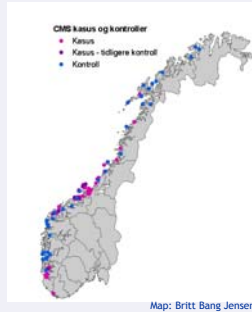


## Materials and methods:

### Case-control (April 2009-May 2010)

86 Norwegian farms

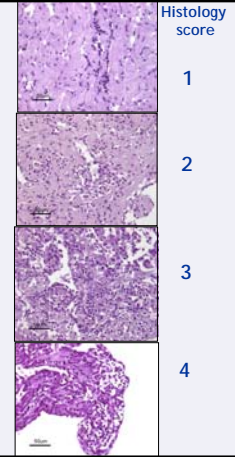
- CASES: CMS diagnosed (NVI) before sampling
- CONTROLS: NO CMS before sampling.
- CONTROLS developed CMS after sampling.



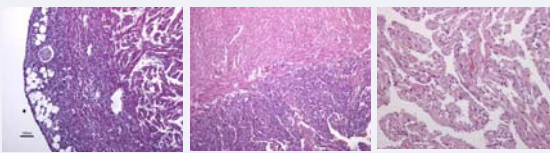
## Histopathology

- 10-30 fish per farm (blind)
- 2300 hearts
- The atrium, spongy and compact layers of the ventricle and epicardium were graded from 0 to 4
- Histology all organs of some fish per farm to confirm the diagnosis.

Individual fish diagnosis & farm diagnosis



**Differential diagnoses:** The heart is a central organ in the organism and plays an important role in the development of several diseases.



2317 hearts classified as: CMS, HSMI, PD  
No Lesions Observed (NLO)  
Myocarditis, mild myocarditis  
Mixed cases (CMS and/or HSMI; CMS and /or PD)  
Other Cardiac Lesions (OCL)

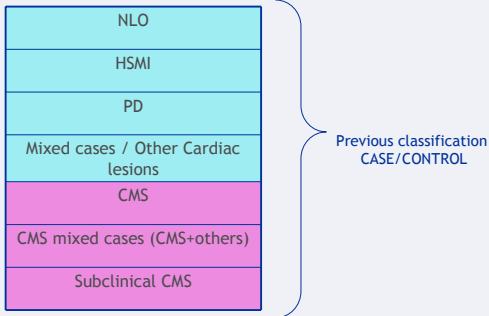
## RT-PCR: confirmatory tool

- One-step real time RT-PCR: PMCV 500 fish (65 farms)

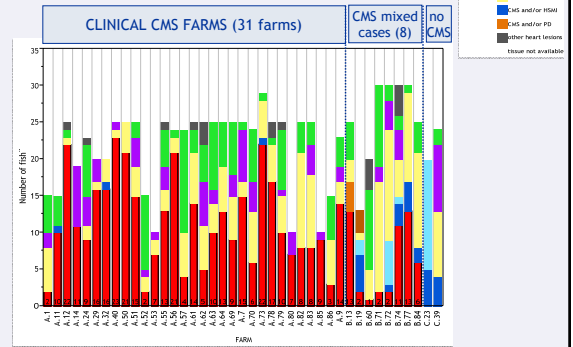
### Ct-levels

- Ct < 25 (high viral load)
- Ct 25-30 (mid viral load)
- Ct 30-40 (low viral load)
- No Ct / Ct > 40 (neg)

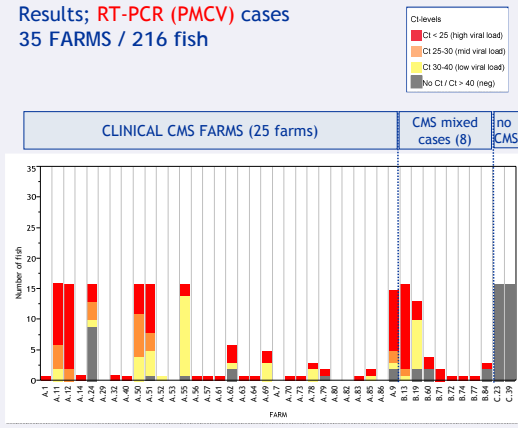
## Histopathology + RT-PCR: FARM DIAGNOSIS 86 farms (89 samplings)



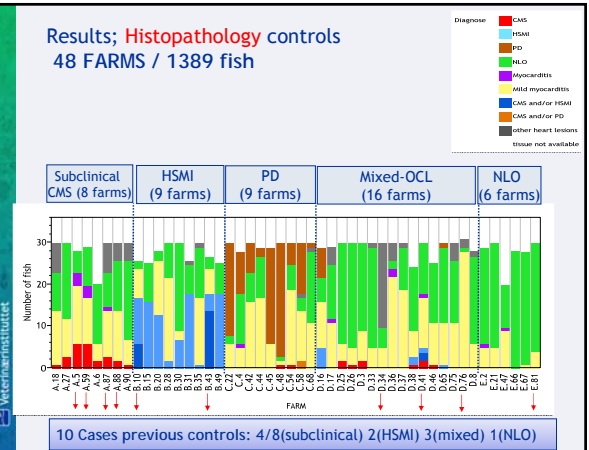
## Results; Histopathology cases 41 FARMS / 927 fish

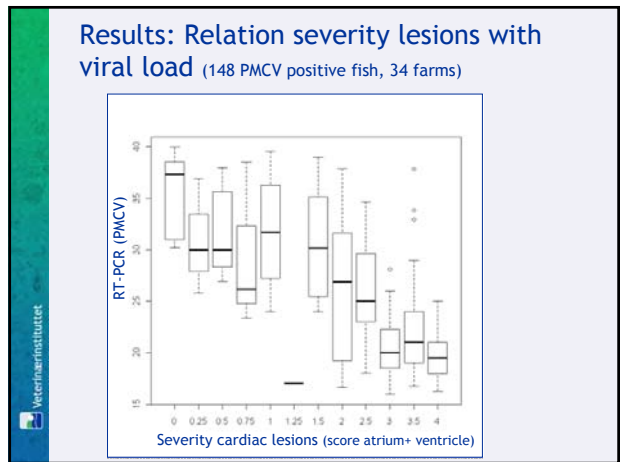
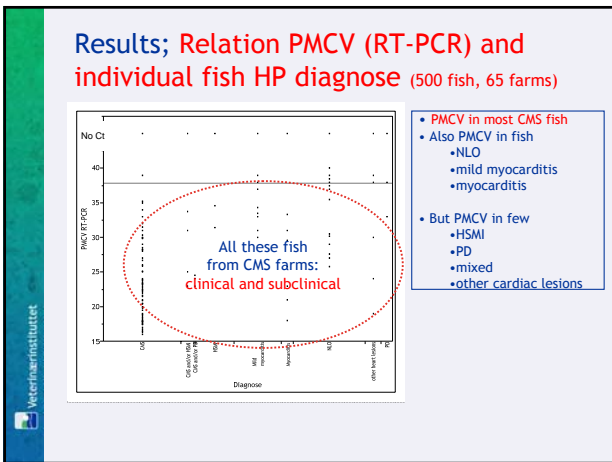
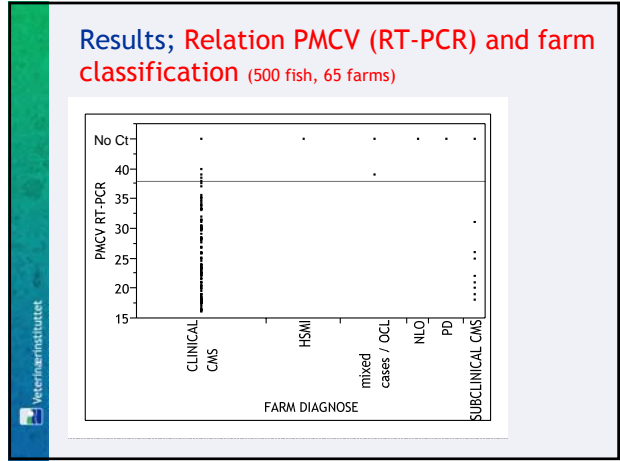
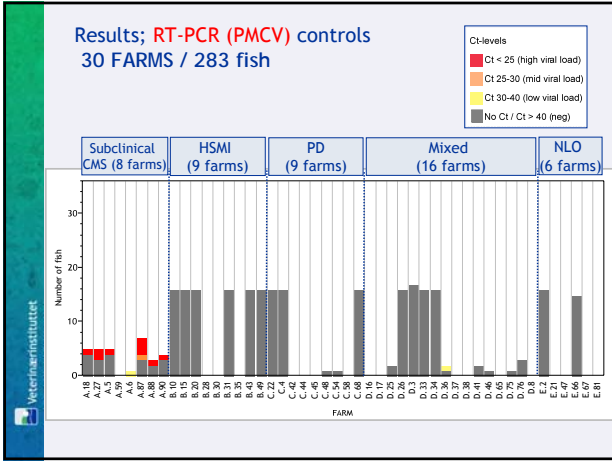


## Results; RT-PCR (PMCV) cases 35 FARMS / 216 fish

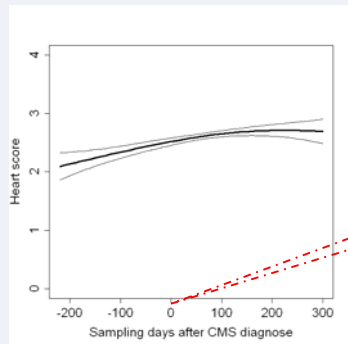


## Results; Histopathology controls 48 FARMS / 1389 fish





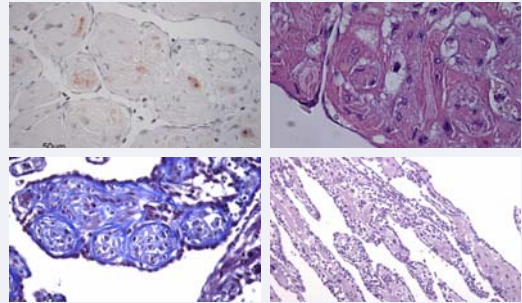
## Results: Severity of cardiac lesions related with the time after confirmation of CMS (49 farms, 1164 fish)



The cardiac lesions INCREASE or leveled of on a high value at least until a year after diagnose

time CMS confirmed

## Results: CMS cases (broodstock) >500 days after first CMS confirmation



## Conclusions

- Diagnosis: histopathology not enough in mixed cases and subclinical CMS.
  - combination HP and PCR more specific diagnosis
- Disease is underdiagnosed: 4/8 subclinical cases not diagnosed.
- Virus and CMS lesions detectable much earlier than disease (4 months earlier)
- Viral persistence (detected after 1,5 year)

## Future work

- Pathogenesis:
    - how the virus infect the fish?
    - how the virus spread in the fish and which organs are infected?
      - RT-PCR and ISH in different organs of field cases
    - what kills the fish?
      - No mortality in challenge trials
      - No problems in young salmon
  - Genetic variation within PMCV?
    - Sequencing: 50 fish from clinical cases are being sequenced.
    - Possibility to sequence fish from subclinical cases.
  - Possibility to validate IHCM (when PMCV Antibody available) in field material
  - Gen bank: all organs from 2300 fish (86 farms) in formalin fixed, paraffine blocks and RNA-later.
- Plenty of material and still a lot to learn but lack of funding !

## Acknowledgements

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