



Identifikasjon og karakterisering av virus assosiert med CMS

Øyvind Haugland¹, Aase B. Mikalsen^{1¶}, Pål Nilsen^{2¶}, Karine Lindmo^{2¶}, Beate J. Thu¹, Trygve M. Eliassen², Norbert Roos³, Marit Rode², Øystein Evensen^{1*}

¹Norwegian School of Veterinary Science, Department of Basic Sciences and Aquatic Medicine

²PHARMAQ AS

³Department of Molecular Biosciences, University of Oslo

¶ - equal contribution

*corresponding author





Kardiomyopati (CMS) – en velkjent sykdom

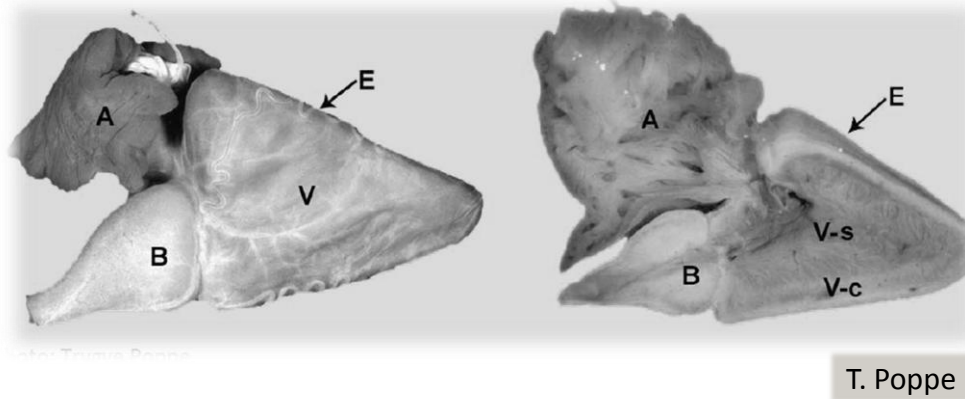
- Først beskrevet som sykdom i Norge i 1985 (Ferguson et al., 1990; Amin and Trasti, 1988).
- Senere beskrevet også i Skottland og Færøyene (Poppe and Sande, 1994; Rodger and Turnbull, 2000)
- Mistanke om CMS i Canada (Brocklebank and Raverty, 2002)
- Chile???
- Rammer atlantisk laks i sen sjøfase
- Fisken kan dø uten å ha hatt kliniske tegn til sykdom
- Obduksjonsfunn: Hjertetamponade med blod i hjertesekken og moderat til uttalt ascites
- Tilsvarende forandringer påvist i villaks



Fiskehelse og fisesykdommer
Foto: Trygve Poppe



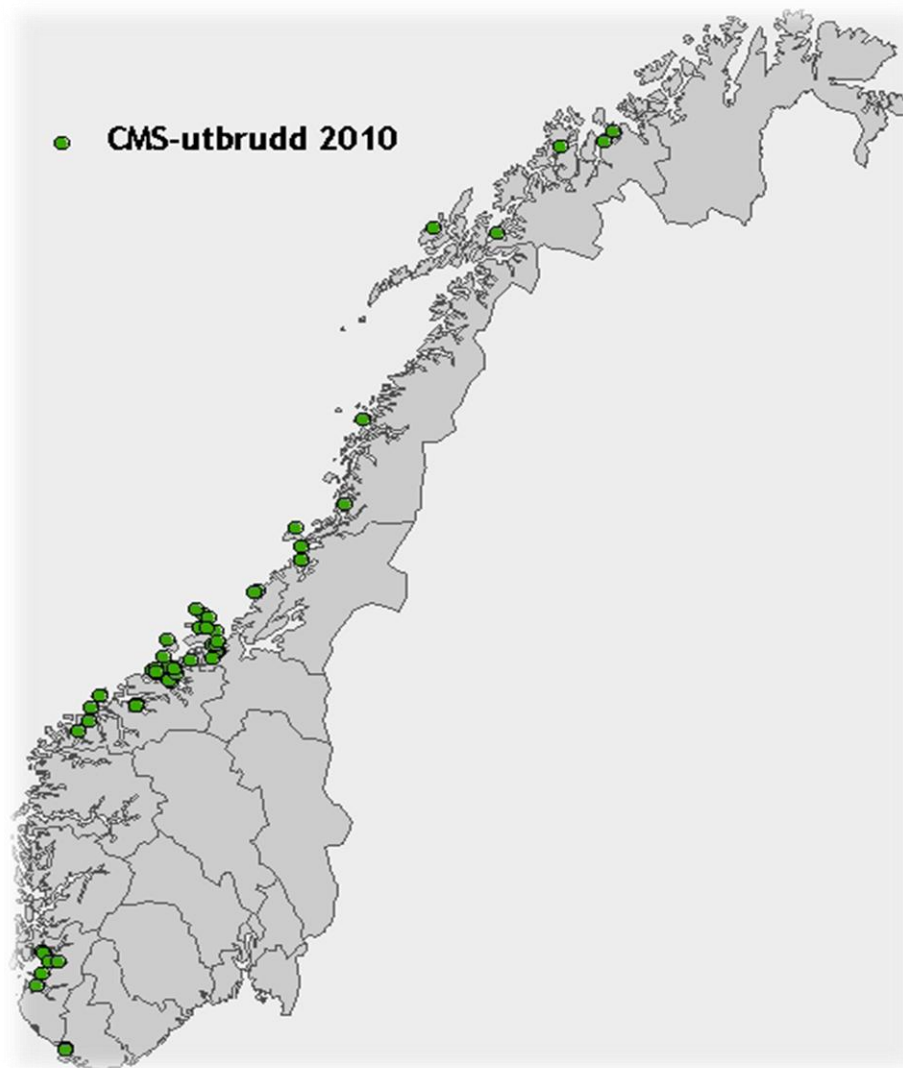
Laksens hjerte



- A: Atrium
- B: Bulbus arteriosus
- V: Ventricle
 - V-s, spongy ventricle
 - V-c, compact ventricle
- E: Epicard



- 53 utbrudd rapportert i Norge i 2010
- Jevnt antall utbrudd siste 10 år
- Spredt i hele landet



Kilde: Veterinærinstituttet



Hva skyldes CMS?

- Det var to hypoteser da vi startet i 2006:

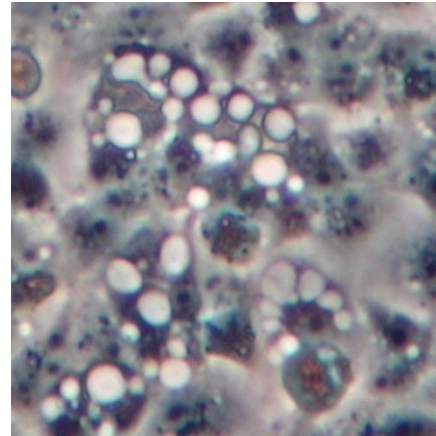
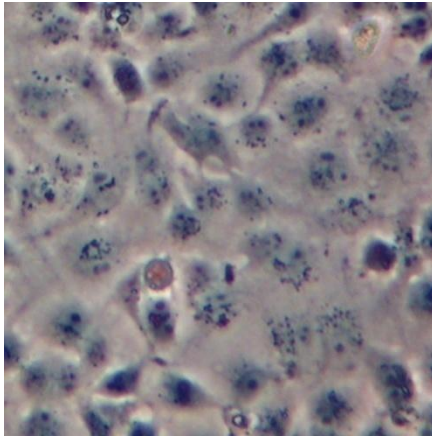
1. Infeksiøs sykdom

2. Produksjonslidelse

Vi studerte hypotese 1. Infeksiøs sykdom



In vitro dyrkning av "CMS-virus"



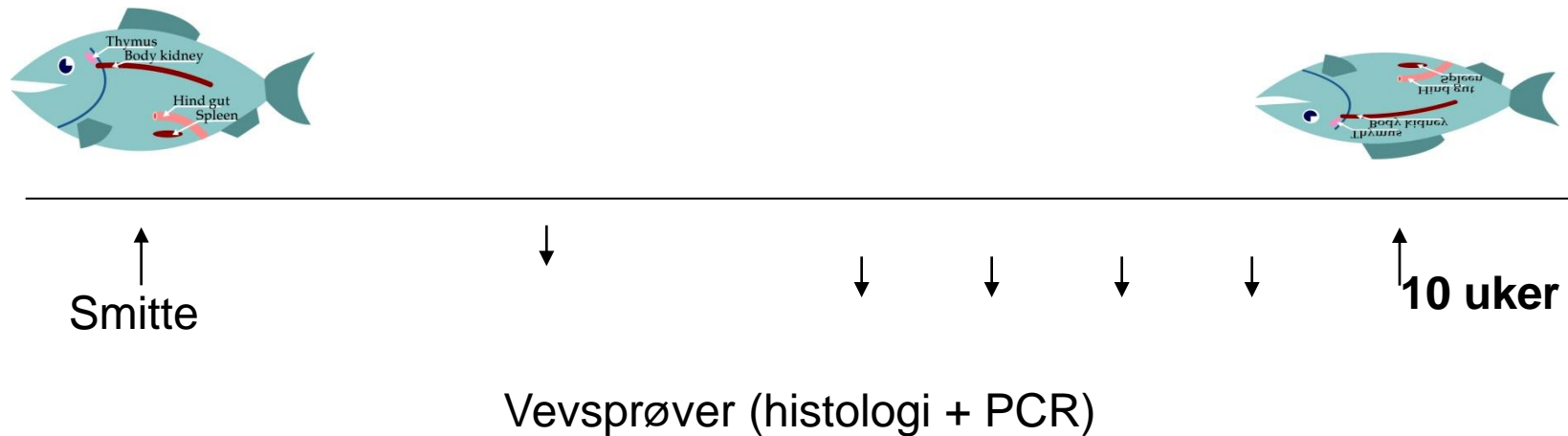
10-14 dager etter inokulering

- CPE synlig ca 7 dager etter inokulering, cytoplasmatiske vakuoler med variabel størrelse
- Passering av supernatant ga CPE nye cellekulturer
- Ingen CPE ble observert etter inokulering med hjerte fra kontrollfisk
- 3-4 passasjer mulig



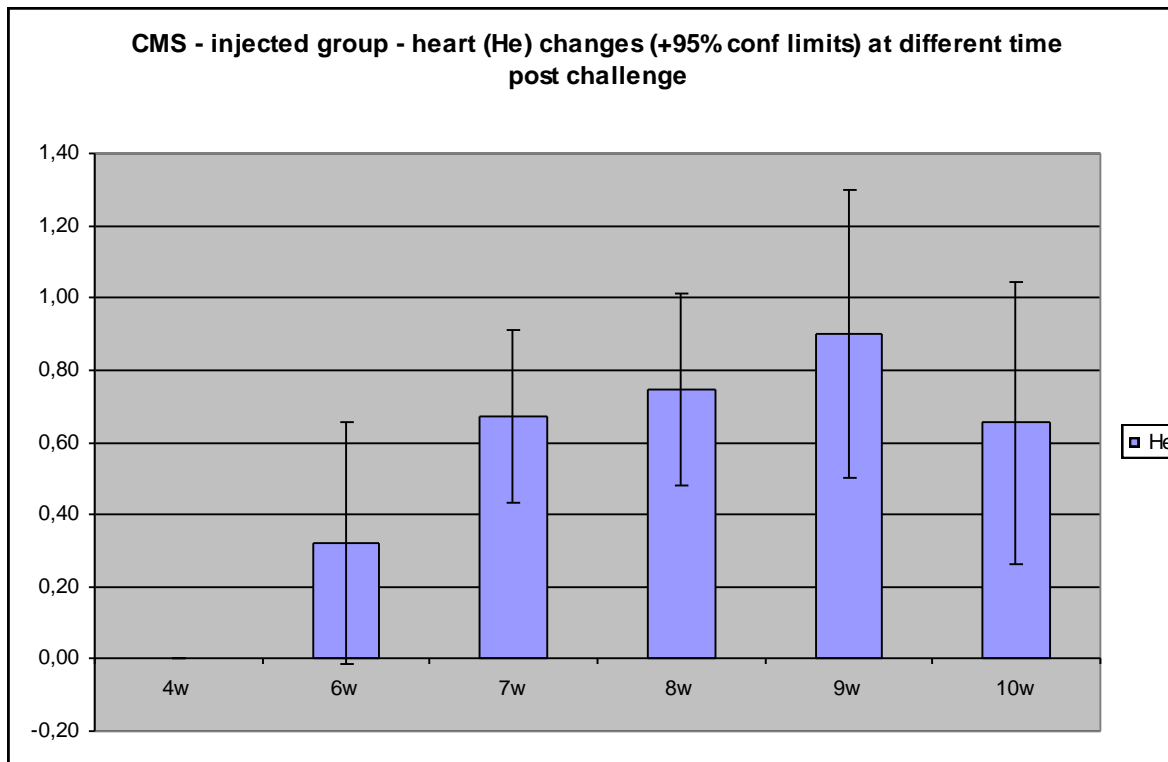
Hva skjer når vi smitter fisk med materiale fra cellekultur med CPE?

- Sampling 4,6,7,8,9 og 10 uker etter smitte
- Monitorere histopatologiske forandringer
- Studere immunrespons ved Real Time PCR



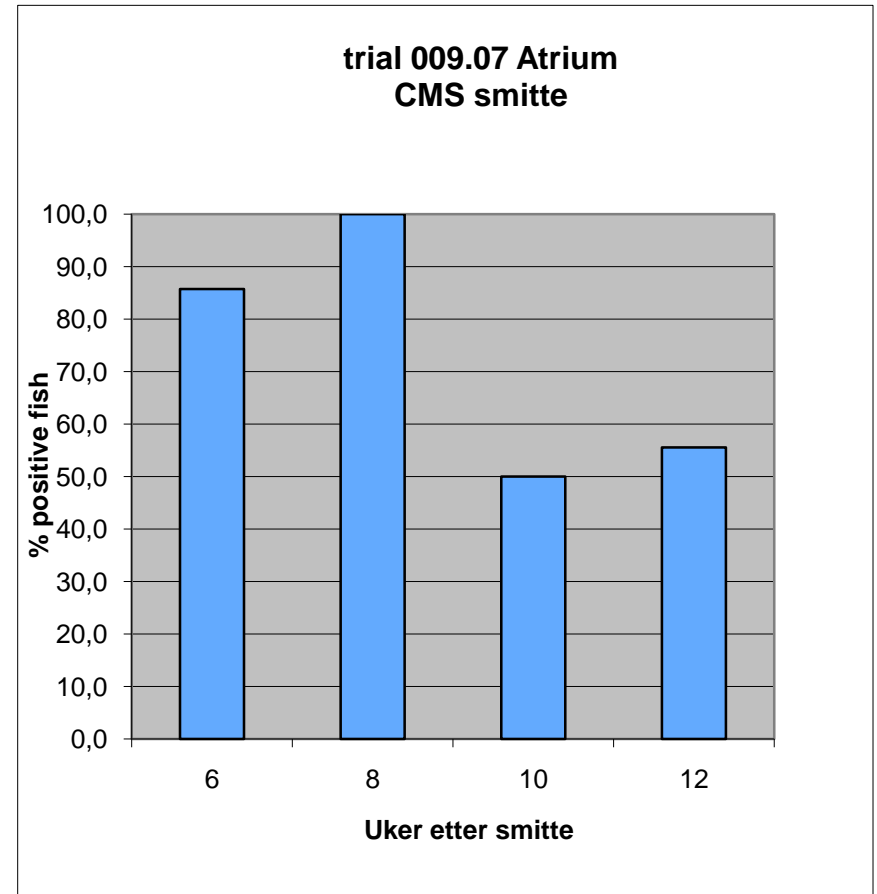
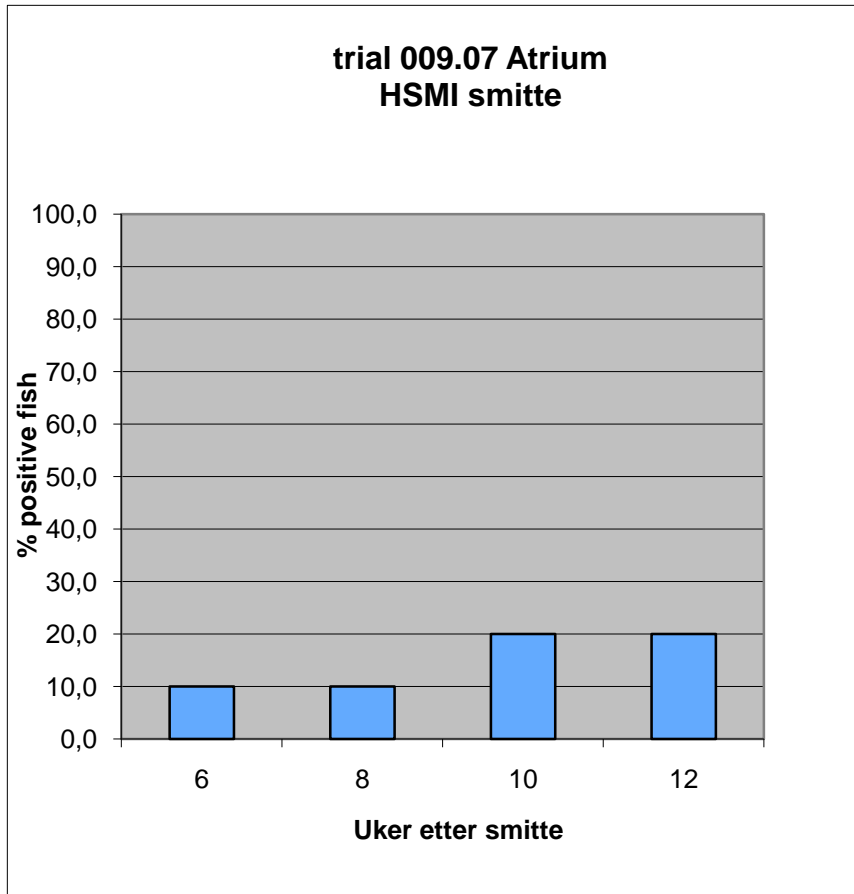


Histologi-score, CMSVsmitte





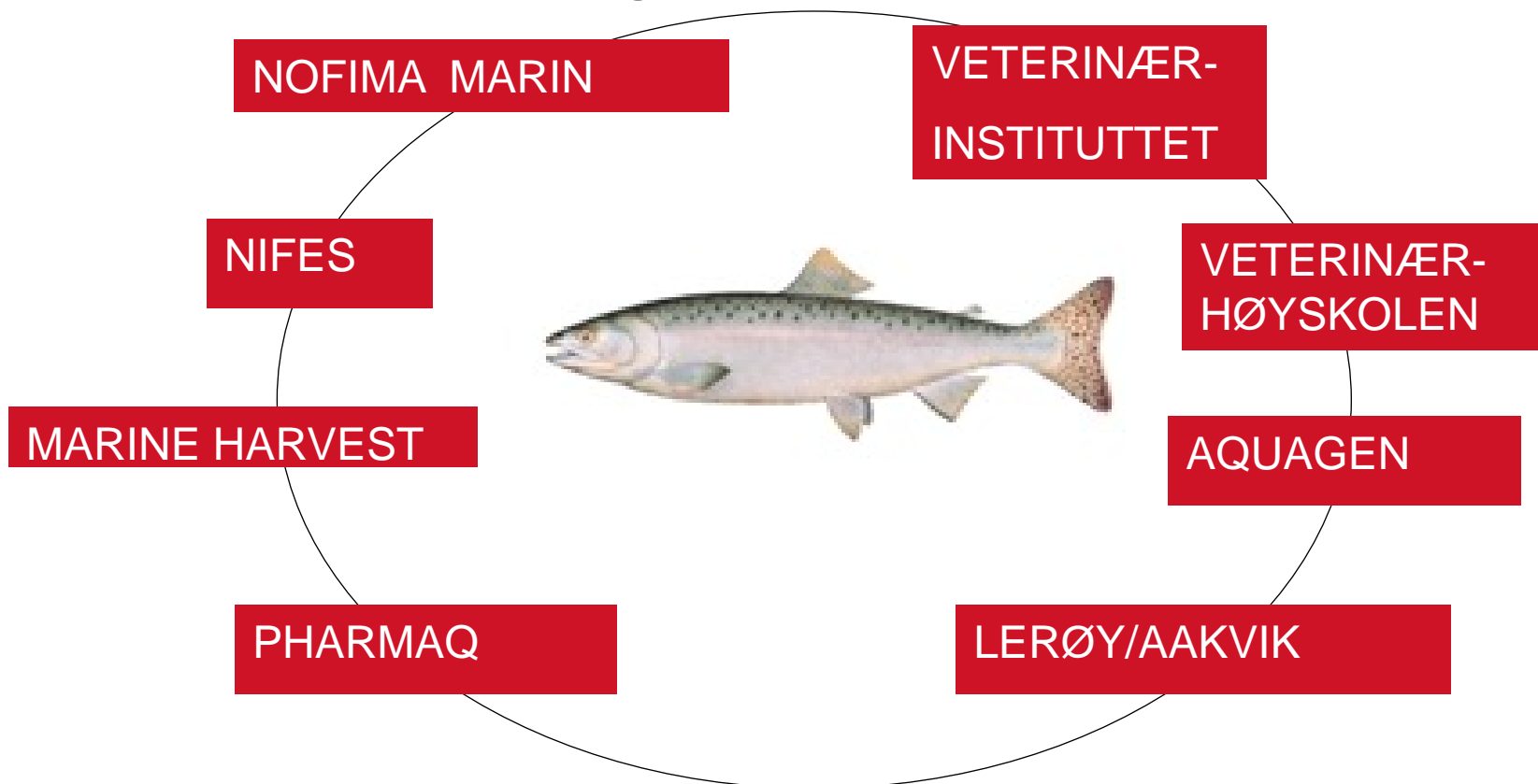
Er HSMB og CMS to ulike sykdommer?





NFR project 187301/S40: 2008-2011

Cardiomyopathy syndrome: a multi-task approach to reduce losses & improve knowledge





Andre miljø var også aktive

Vol. 87: 235–242, 2009
doi: 10.3354/dao02129

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published December 3



Comparative experimental transmission of cardiomyopathy syndrome (CMS) in Atlantic salmon *Salmo salar*

D. W. Bruno*, P. A. Noguera

Marine Scotland, Marine Laboratory, PO Box 101, 375 Victoria Road, Aberdeen A

ABSTRACT: Cardiomyopathy syndrome (CMS) has been recorded in wild Atlantic salmon *Salmo salar*. Characteristic heart lesions primarily involving the myocardium were observed in natural outbreaks with associated mortality. To date, no experimental trials have been conducted in the laboratory. The present study reports on the first successful experiment of CMS in Atlantic salmon in Scotland, with full development of the histopathology described for the syndrome. Tissue homogenates of CMS-infected fish indicative of lesions from Scottish and Norwegian natural outbreaks, respectively, were injected into Atlantic salmon post-smolts. Lesion development was faster in the fish group receiving the Norwegian homogenate, but equivalent at the end time point of the experiment. The study demonstrated that the reported lesion in the fish group receiving the Norwegian homogenate, and that tissue homogenate from severely affected fish contain the transmissible agent.

Vol. 87: 225–234, 2009
doi: 10.3354/dao02123

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published December 3



Experimental transmission of cardiomyopathy syndrome (CMS) in Atlantic salmon *Salmo salar*

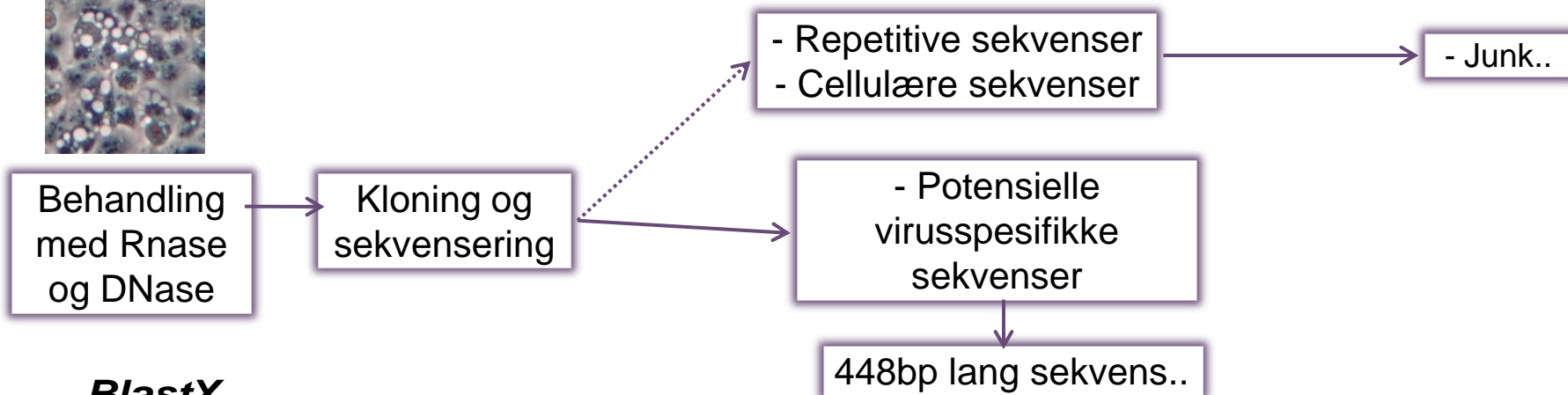
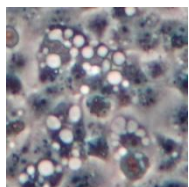
C. Fritsvold^{1,*}, R. T. Kongtorp¹, T. Taksdal¹, I. Ørpetveit¹, M. Heum¹, T. T. Poppe²

¹National Veterinary Institute, PO Box 750 Sentrum, 0108 Oslo, Norway
²Norwegian School of Veterinary Science, PO Box 8146, 0653 Oslo, Norway

ABSTRACT: Cardiomyopathy syndrome (CMS) is a disease of unknown aetiology, having significant economic impact as it primarily affects large, farmed Atlantic salmon *Salmo salar* L. in seawater, close to harvest. In the present study, we have demonstrated that CMS is a transmissible disease under experimental conditions. Histopathological lesions consistent with CMS were induced in Atlantic salmon post-smolts after injection of tissue homogenate from farmed fish diagnosed with CMS. Six weeks post-injection (p.i.), experimental fish started developing focal to multi-focal lesions in the atrial endo- and myocardium, with subsequent progression to the ventricle. This proceeded into severe endocarditis and subsequent myocarditis with mononuclear cell infiltration of the atrium and, to a lesser degree, the spongy layer of the ventricle. These lesions were consistent with histopathological findings in field outbreaks of CMS. From Week 33 p.i., lesions also appeared in the compact myocardium, with focal epicarditis adjacent to focal myocardial lesions. In conclusion, these results indicate that CMS has an infectious aetiology and should be treated as a potentially contagious disease.



Kloning og karakterisering av PMCV-genomet



BlastX

Sequences producing significant alignments:

		(Bits)	Value
ref NP_620070.1 	gag-pol fusion protein [Giardia lamblia virus]	49.7	1e-04
gb AAB01579.1 	gag [Giardia lamblia virus]	49.7	1e-04
gb AAM77694.1 	gag [Giardia lamblia virus]	49.7	1e-04
gb ABB36743.1 	gag [Giardia lamblia virus]	49.7	1e-04
ref YP_001109580.1 	RNA-dependent RNA polymerase [Botryotinia fuckeliana totivirus]	38	0.050
ref YP_122352.1 	putative RNA dependent RNA polymerase [Sphaeropsis sapinea RNA virus 1]	33	0.15
gb ABX79995.1 	putative RNA-dependent RNA polymerase [Leishmania RNA virus]	31	0.25
ref NP_047558.1 	RNA dependent RNA polymerase [Black raspberry cryptic virus]	29	0.33
ref YP_001649206.1 	RNA-dependent RNA polymerase [Black raspberry cryptic virus]	24	0.56
ref NP_041191.1 	RNA dependent RNA polymerase [Leishmania RNA virus]	35.8	1.6
ref YP_094312.1 	hypothetical protein lpg0258 [Legionella pneumophila]	35.0	2.8
ref YP_001249676.1 	hypothetical protein LPC_0335 [Legionella pneumophila]	34.7	3.6

Svake treff på rare virus...

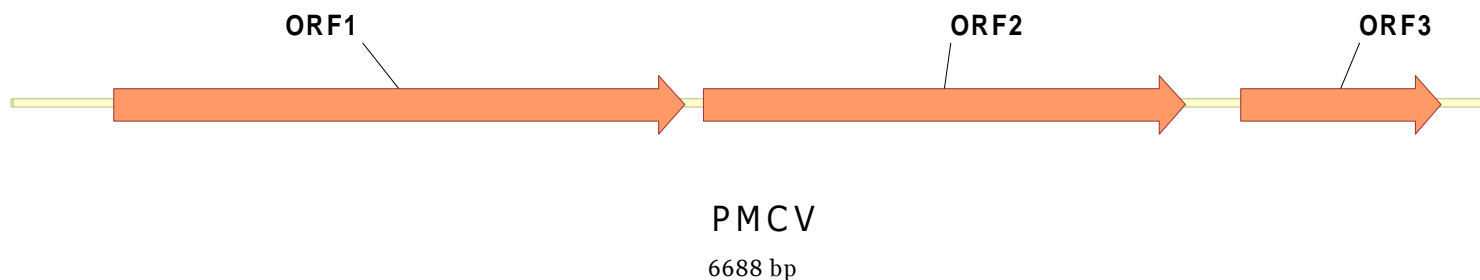
- Giardia lamblia virus
- Botryotinia fuckeliana totivirus
- Sphaeropsis sapinea RNA virus 1
- Leishmania RNA virus
- Black raspberry cryptic virus



Kloning og karakterisering av PMCV-genomet

Primere ble designet basert på flere sekvenser og brukt for å screene

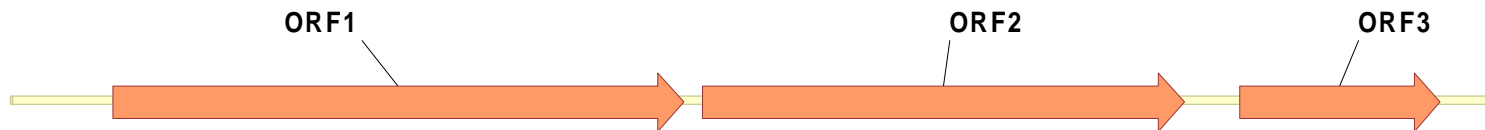
- Cellekulturer inokulert med ulike fiskevirus
 - Vevsprøver fra smitteforsøk
 - Feltprøver fra anlegg med og uten CMS
-
- **En del jobbtimer senere hadde vi hele genomet...**





Kloning og karakterisering av PMCV-genomet

- Sekvensanalyse viste at det er tre åpne leserammer i genomet
- Signifikant sekvenshomologi til andre kjente virussekvenser sees bare i ORF2 som er den RNA-avhengige RNA-polymerasen
- Fordi viruset forårsaker en nekrotiserende myokarditt i fisk har vi foreslått navnet piscine myocarditis virus (PMCV)

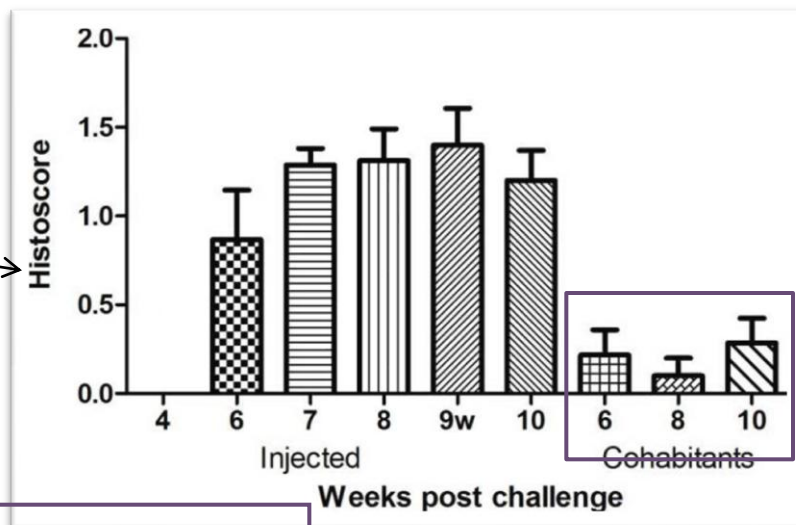


PMCV
6688 bp

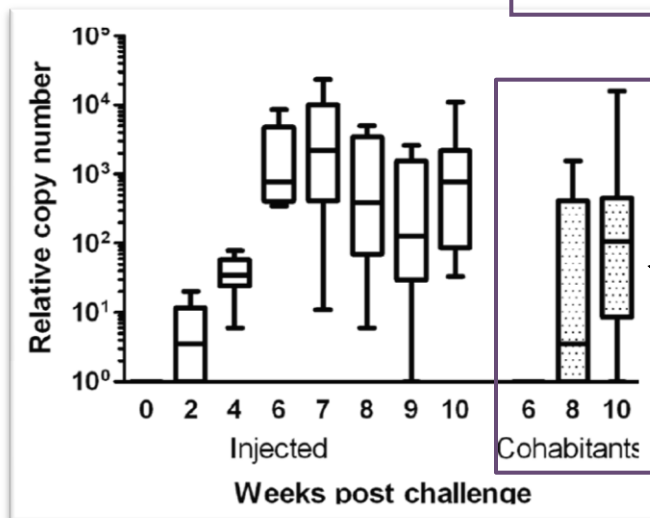


Hva skjer i et PMCV smitteforsøk?

Histologiscore på hjertevev fra smitteforsøk



Horizontal overføring

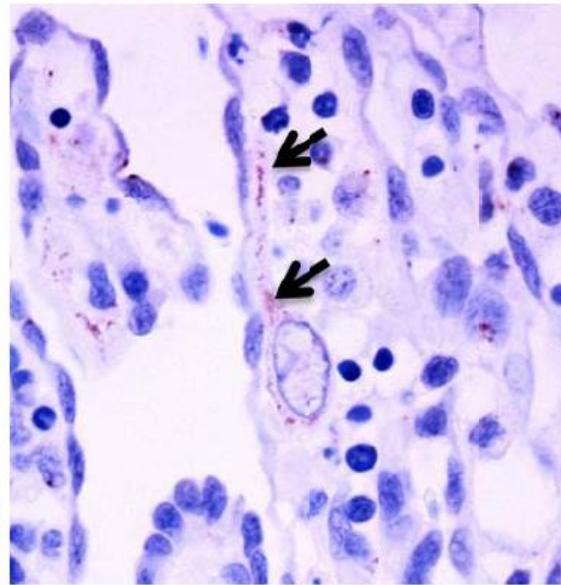
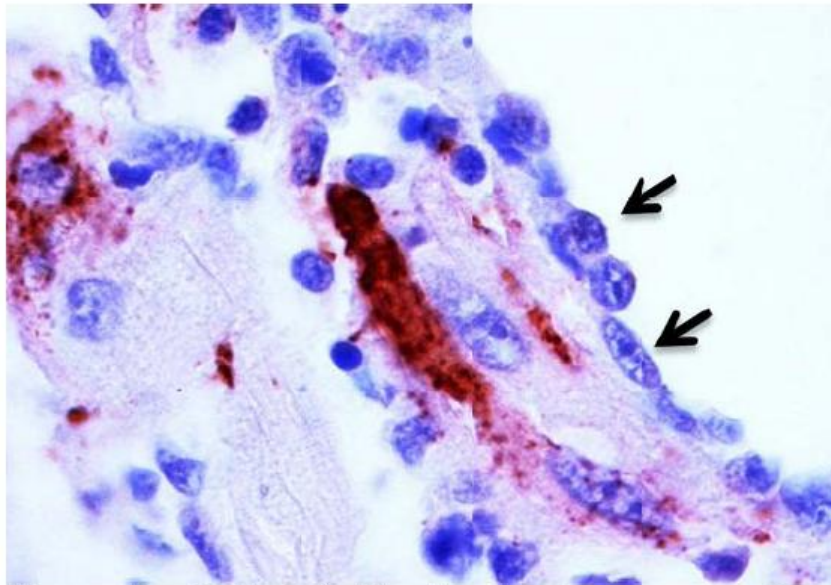


RT-qPCR analyse: PMCV nivå i hjerteprøver (ventrikkel) fra samme forsøk



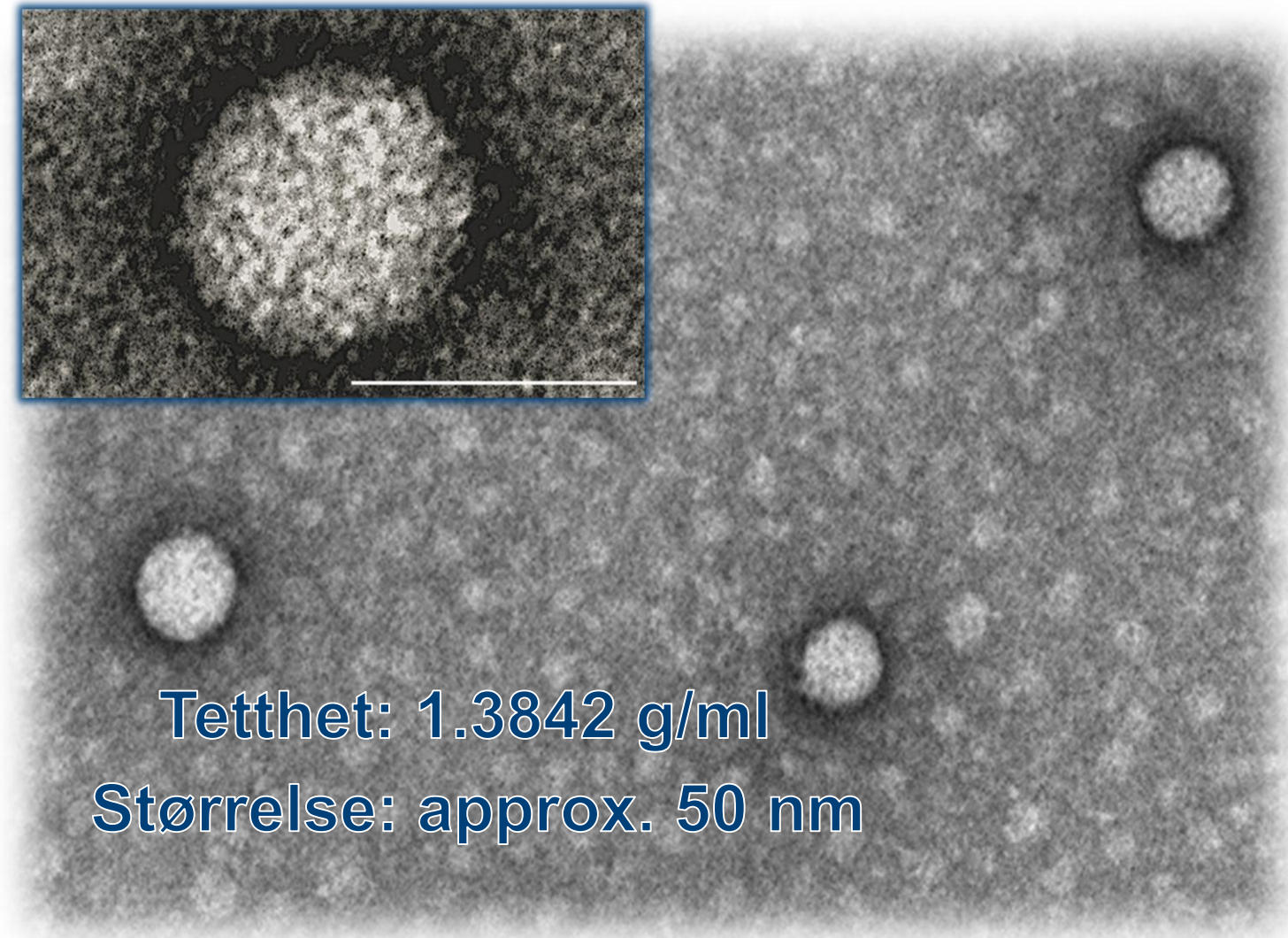
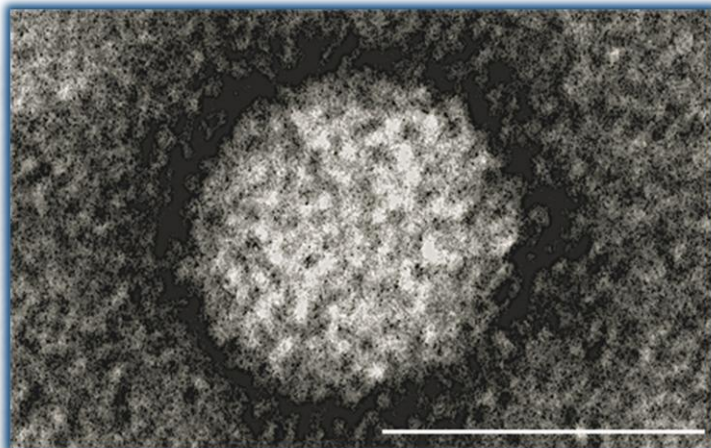
Hvor i hjertet finnes PMCV?

- Finner virus i hjertets muskelceller (myocytter)
- Omkringliggende betennelses- og endotelceller er negative





Hvordan ser PMCV ut?

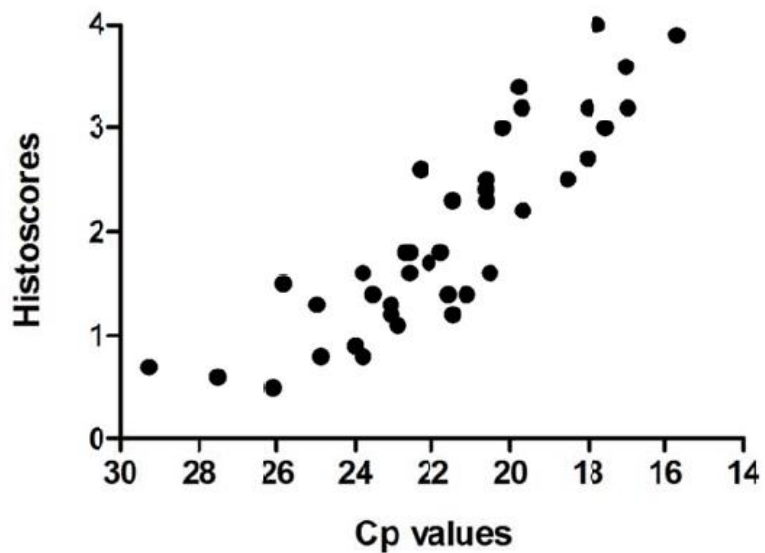


Tetthet: 1.3842 g/ml
Størrelse: approx. 50 nm



Er det korrelasjon mellom vevsskader og PMCV-nivå?

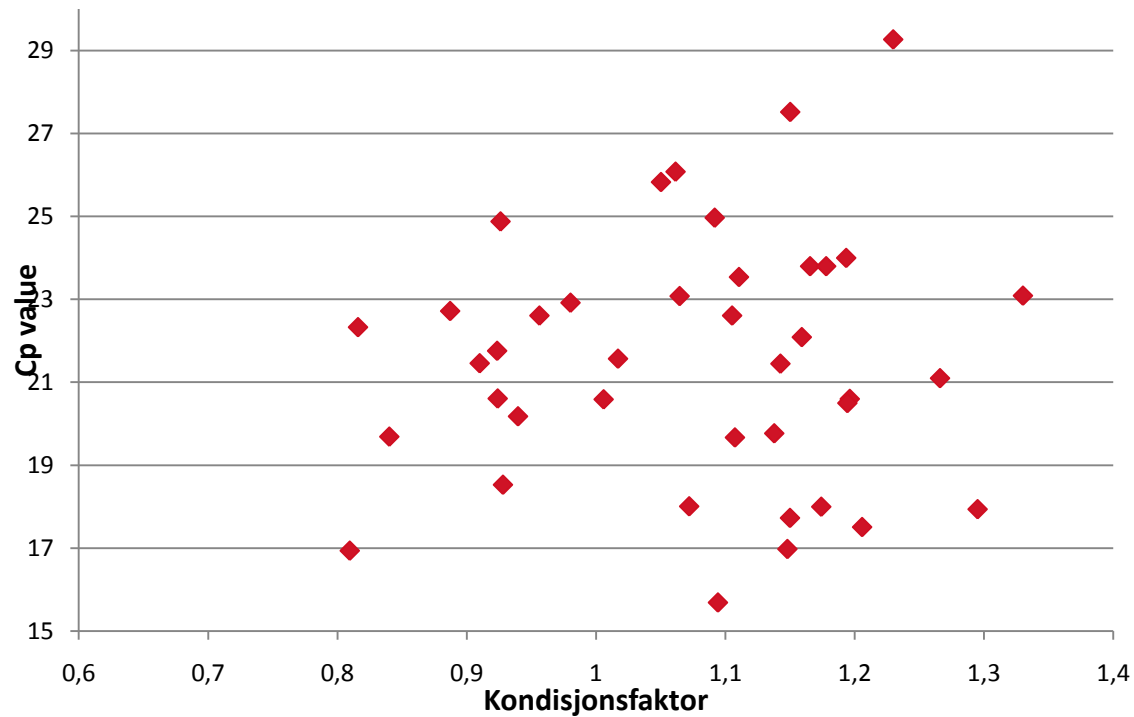
- Positiv korrelasjon mellom histologiscore og PMCV-nivå i hjertevev



Takk til Dr. Olav Breck og Eirik Hoel, Marine Harvest for innsamling av prøver



Er det korrelasjon mellom kondisjonsfaktor og PMCV-nivå?



- Ingen korrelasjon mellom kondisjonsfaktor og PMCV-nivå i hjertet



Cardiomyopathy Syndrome of Atlantic Salmon (*Salmo salar* L.) Is Caused by a Double-Stranded RNA Virus of the *Totiviridae* Family[∇]

Øyvind Haugland,¹ Aase B. Mikalsen,^{1¶} Pål Nilsen,^{2¶} Karine Lindmo,^{2¶} Beate J. Thu,¹ Trygve M. Eliassen,² Norbert Roos,³ Marit Rode,² and Øystein Evensen^{1*}

Norwegian School of Veterinary Science, Department of Basic Sciences and Aquatic Medicine, P.O. Box 8146 Dep., N-0033 Oslo, Norway¹; Pharmaq AS, P.O. Box 267, N-0213 Oslo, Norway²; and Department of Molecular Biosciences, University of Oslo, P.O. Box 1041, Blindern, Oslo, Norway³

Received 12 October 2010/Accepted 4 March 2011

Cardiomyopathy syndrome (CMS) of farmed and wild Atlantic salmon (*Salmo salar* L.) is a disease of yet unknown etiology characterized by a necrotizing myocarditis involving the atrium and the spongy part of the heart ventricle. Here, we report the identification of a double-stranded RNA virus likely belonging to the family *Totiviridae* as the causative agent of the disease. The proposed name of the virus is piscine myocarditis virus (PMCV). On the basis of the RNA-dependent RNA polymerase (RdRp) sequence, PMCV grouped with *Giardia lamblia* virus and infectious myonecrosis virus of penaeid shrimp. The genome size of PMCV is 6,688 bp, with three open reading frames (ORFs). ORF1 likely encodes the major capsid protein, while ORF2 encodes the RdRp, possibly expressed as a fusion protein with the ORF1 product. ORF3 seems to be translated as a separate protein not described for any previous members of the family *Totiviridae*. Following experimental challenge with cell culture-grown virus, histopathological changes are observed in heart tissue by 6 weeks postchallenge (p.c.), with peak severity by 9 weeks p.c. Viral genome levels detected by real-time reverse transcription (RT)-PCR peak earlier at 6 to 7 weeks p.c. The virus genome is detected by *in situ* hybridization in degenerate cardiomyocytes from clinical cases of CMS. Virus genome levels in the hearts from clinical field cases correlate well with the severity of histopathological changes in heart tissue. The identification of the causative agent for CMS is important for improved disease surveillance and disease control and will serve as a basis for vaccine development against the disease.



Oppsummering

- Det kausale agens for CMS er et ikke-segmentert, dobbelt- trådet RNA virus
- Fordi viruset forårsaker en nekrotiserende myokarditt i fisk har vi foreslått navnet piscine myocarditis virus (PMCV)
- Basert på RdRp-sekvensen er det i slekt med Gardiavirus i virusfamilien Totiviridae
- Bare RdRp-proteinet viser noen homologi til kjente sekvenser



Når kausalt agens er kjent – hva da?

- Diagnostikk
 - Sikrere/enklere diagnostikk
- Sykdomsovervåkning
 - Vet når fiskepopulasjonen er smittet
 - Kan ta avgjørelser basert på kunnskap for eksempel ang slakt
- Identifisere mulig smittereservoar
 - Funnet i villfisk (Wiik-Nielsen et al, Frisk Fisk 2011)
- Vaksineutvikling
 - Kjenner nå de aktuelle antigenene
 - Muliggjør målrettet vaksineutvikling



We make aquaculture progress

PHARMAQ

