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Epidemiologisk studie av Kardiomyopati-syndrom (CMS): Spredning, risikofaktorer og sykdomsforløp i norsk lakseoppdrett (CMS-Epi)

Veterinærinstituttet
Norwegian Veterinary Institute

Sluttrapport: «Epidemiologisk studie av Kardiomyopatisyndrom (CMS): Spredning, risikofaktorer og sykdomsforløp i norsk lakseoppdrett (CMS-Epi)»(901118)

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1. Sammendrag (Norsk og engelsk)

CMS er et økende problem. Både i Norge, der det rammer tidligere, oftere og hardere enn før, men også i andre lakseproduserende land rundt Atlanterhavet. I Fiskehelserapporten for 2018, fremheves CMS som den viktigste infeksiøse sykdommen i norsk lakseoppdrett etter lakselus. Det er derfor enda mer aktuelt med løsninger på begrensing og kontroll av sykdommen.
Prosjektet «Epidemiologisk studie av Kardiomyopatisyndrom (CMS): Spredning, risikofaktorer og sykdomsforløp i norsk lakseoppdrett» (CMS-Epi) ble satt i gang for å søke å gi svar spørsmål om når fisken smittes med PMCV, hvor lenge den kan ha virus i seg, hvilke faktorer som påvirker utvikling til klinisk utbrudd og hva som kan gjøres for å bryte smittekjeden eller begrense skadene ved CMS.
Prosjektet ble satt i gang november 2015, og avsluttet ved utgangen av 2018. Prosjektet var organisert i fem arbeidspakker, som er koblet opp mot delmål. Prosjektgruppen besto av Veterinærinstituttet som var ansvarlig for gjennomførelse av alle arbeidspakker, samt Pharmaq Analytiq som var ansvarlig for utførelse av laboratorieanalyser. Styregruppen besto av representanter fra fire (etter hvert fem) ulike oppdrettsselskap, og disse har vært aktivt involvert i planlegging og utførelse av studiene, samt diskusjon av resultatene.
Ett av funnene i prosjektet er en mulig sammenheng mellom utbrudd av CMS og hva som skjer i settefiskfasen. Fra felt har det blitt rapportert at det ses store forskjeller i hvilke fiskegrupper som blir berørt under et CMS-utbrudd på samme lokalitet, og at det ofte er fisk fra de samme settefiskleverandørene som rammes år etter år. Dette ble bekreftet ved analyse av produksjonsdata i AP3, der det tydelig ble observert en sammenheng mellom settefiskleverandør og risikoen for utbrudd av CMS, også på tvers av lokaliteter.
En av flere mulige årsaker til dette kan være at smolten har med seg PMCV fra settefiskfasen. I en studie utført under AP1 ble det tatt prøver av stamfisk, egg og yngel fra to ulike anlegg. Resultatene indikerer at PMCV kan overføres fra stamfisk til yngel, under gjeldende produksjonsforhold. Det samme har blitt rapportert fra Irland. Denne overførselen kan enten skje ved at virus er assosiert til egget og ikke fjernes under desinfeksjon, eller ved re-kontaminasjon etter desinfeksjon. Vi fant at desinfeksjon fjernet det meste virus RNA, men ikke alt, og dette aspektet bør undersøkes nærmere. I studien vår fant vi PMCV ved PCR-analyser, men det vites ikke om det som ble funnet var infektivt virus eller rester av virusgenom. For å konkludere med at denne smitteveien har noe å si for spredningen av CMS, bør det gjennomføres kontrollerte laboratorieforsøk med PMCV-positive egg og/eller yngel. I påvente av mer kunnskap om mulig overførsel vertikalt, bør en søke å få bedre kontroll med smittestatus i settefiskanlegg, samt jobbe med seleksjon av stamfisk med ingen eller en lav mengde PMCV.
Fra feltstudien iAP2 fant vi PMCV i 25 fiskegrupper fordelt på 12 lokaliteter langs hele kysten mellom 0 og 7 måneder etter sjøsetting. Dette tilsier enten at smolten allerede var infisert når den ble satt i sjø, eller at smittepresset av PMCV er langt større enn vi tidligere har antatt. Bare halvparten av de 12 lokalitetene som ble fulgt utviklet klinisk CMS. Det er derfor ikke enkelt å bruke screening for PMCV ved PCR som et verktøy til å forutsi om et utbrudd er på gang. En bør i tillegg bruke histopatologi og kliniske symptomer, men på det tidspunkt da det ses klinisk CMS er det trolig for sent å gjøre noe for å hindre et utbrudd.
PMCV ble detektert helt frem til slakt, noe som tyder på at fisken ikke kvitter seg med infeksjonen, selv når det kliniske utbruddet er over. Dette samsvarer med rapporter om høy forekomst av PMCV i stamfisk, noe som også ble observert i studien i AP1. Dette er et aspekt som en bør tenke over i forbindelse med smittevern; at fisk som tilsynelatende er klinisk frisk godt kan ha høye mengder av PMCV, og dermed kan bidra til å spre smitte ved flytting i forbindelse med for eksempel slakt.
I studien fant vi at tiden fra infeksjon til utvikling av klinisk CMS varierte fra 3 til 13 måneder. Det kan altså gå ganske lang tid fra fisken smittes med PMCV til den blir syk av CMS. Vi vet fortsatt lite om hva som forårsaker utviklingen av klinisk CMS. Det kan være noe med hvordan virus infiserer og påvirker cellene i spesielt hjertet, og dette bør undersøkes mer. Det kan også være, at visse eksterne risikofaktorer som stress eller andre sykdommer kan forårsake overbelastning i et hjerte som allerede er svekket av skader forårsaket av infeksjon med PMCV. I AP3 fant vi at fiskegrupper som hadde hatt PD eller HSMB hadde større risiko for senere å utvikle CMS. Vi fant også at høstutsatte fisk hadde større risiko enn vårutsatte, uansett region. Det kan finnes andre risikofaktorer, og disse bør undersøkes. For eksempel er det mulig at påkjenningen ved mekanisk avlusning øker risikoen for å få CMS i dagene etter, noe som trolig kan undersøkes ved analyse av mer oppdaterte produksjonsdata.

CMS is an increasing problem. Both in Norway, where it affects earlier, more often and harder than before, but also in the other salmon-producing countries around the Atlantic sea. In the fish health report for 2018, CMS is highlighted as the most important infectious disease in Norwegian salmon farming after salmon lice. Solutions for mitigating and controlling the disease are therefore very relevant.
The project "Epidemiological study of cardiomyopathy syndrome (CMS): Spread, risk factors and disease course in Norwegian salmon farming" (CMS-Epi) was initiated to seek to answer questions regarding when the fish are infected with PMCV, how long it is infected, which factors influence development into clinical outbreaks and what can be done to break the chain of infection or limit the damage due to CMS. The project was launched in November 2015, and ended with the end of 2018. The project was organized in five work packages, which are connected to sub-goals. The project group consisted of the National Veterinary Institute, which was responsible for the implementation of all work packages as well as Pharmaq Analytiq, who was responsible for carrying out laboratory analyzes. The steering committee consisted of representatives from four (eventually five) different salmon producing companies, and these have been actively involved in the planning and execution of the studies, as well as discussion of the results.
Through the project, it has been found that several things indicate that there may be a connection between outbreaks of CMS and what happens during the freshwater phase. It has been reported from the field that there are large differences in which fish groups are affected during a CMS outbreak at the same site, and that fish from the same hatchery suppliers are often affected year after year. This was confirmed by analysis of production data in AP3, where there was clearly a connection between the smolt supplier and the risk of outbreaks of CMS, both within and between farms.
One of several possible reasons for this may be that the smolts carry PMCV with them from the freshwater phase. In a study conducted during AP1, samples of broodfish, eggs and fry were taken from two different breeding facilites. The results indicate that PMCV can be transferred from broodstock to fry, under current production conditions. The same has been reported from Ireland. This transfer can be either by the virus being associated with the egg and not removed during disinfection, or by re-contamination after disinfection. We found that disinfection removed most of the virus, but not all, and this aspect should be investigated closer. In our study we found PMCV in PCR assays, but it is not known whether what was found was infectious virus or residues of virus genome. To conclude that this pathway has something to say about the spread of CMS, controlled laboratory trials with PMCV positive eggs and / or fry should be performed. In anticipation of more knowledge about possible vertical transfer, one should seek to gain better control of the infection status in hatcheries, and work on selection of broodstock with no or a small amount of PMCV.
From a field study under AP2, we found PMCV in 25 fishgroups from 12 farms along the entire coast between 0 and 7 months after seatransfer. This either indicates that the smolt was already infected when it was put into the sea, or that the infection pressure of PMCV is far greater than we previously assumed. Only 6 of the 12 sites followed developed clinical CMS. It is therefore not straightforward to use screening for PMCV by PCR as a tool to predict if an outbreak is underway. One should also use histopathology and clinical symptoms, but at the time of clinical CMS, it is probably too late to do anything to prevent an outbreak.
PMCV was detected right up to slaughter, suggesting that the fish do not get rid of the infection, even when the clinical outbreak is over. This is consistent with reports of high incidences of PMCV in broodstock, which was also observed in the study in AP1. This is one aspect that should be considered in the context of communicable disease; that fish that are apparently clinically healthy can have high amounts of PMCV, and thus can help spread infection when being transferred in connection with, for example, slaughter.
In the study, we found that the time from infection to development of clinical CMS varied from 3 to 13 months. So it can take quite a while from the fish gets infected with PMCV until it develops clinical CMS. We still know little about what causes the development of clinical CMS. There may be something about how the virus infects and affects the cells in particular the heart, and this should be investigated more. It may also be risk factors outside the fish that trigger a pre-infected heart to develop disease. In AP3 we found that fishgroups that had had PD or HSMI were more likely to develop CMS later. We also found that fallstocked fish were more at risk than springstocked, regardless of region. There may be other important risk factors besides those we were able to pick up, and this should be investigated. For

example, it is possible that the stress of mechanical delousing increases the risk of getting CMS in the following days. This can probably be investigated by analysis of more updated production data.

2. Innledning

Bakgrunn for prosjektet:

Kardiomyopatisyndrom (CMS), også kalt hjertesprekk, er en alvorlig hjertelidelse som rammer oppdrettslaks i sjø. Fordi det oftest er stor og slaktemoden fisk som rammes, kan de økonomiske tapene bli betydelige.
I noen år så det ut som problemet var avtagende: Fra 80 tilfeller i 2006, ble det bare stilt CMS diagnose på 47 lokaliteter i 2010. Men siden da har antallet av påvisninger økt, og i 2014 ble det rapportert om 104 nye tilfeller i Veterinærinstituttet sin fiskehelserapport. I noen områder er dette den økonomisk mest betydningsfulle sykdommen i lakseoppdrett (1).
Årsaken til CMS har ikke tidligere vært kjent, men i 2010 ble det identifisert et nytt virus, piskint myokardittvirus (PMCV), som ser ut til å forårsake CMS. Det ser ut til å være en klar sammenheng mellom påvist virus og diagnostisert sykdom, og mellom mengde virus og grad av patologiske forandringer i hjertet (2).
Screening for PMCV tilbys kommersielt av Pharmaq Analytiq AS, som bruker realtime RT-PCR for å påvise virus. Virus påvises som regel i forbindelse med utbrudd av CMS eller i laks med CMS-forandringer i hjertet, men det er også påvist PMCV på settefisk i settefiskanlegg med og uten sjøvanstilsetting, uten at det har vært kliniske tegn på CMS. Det er fortsatt ukjent når fisken infiseres med virus. PMCV finnes i en del stamfisk, og siden det nå også er funnet i settefisk, er det oppstått mistanke om at PMCV kan bli vertikalt overført under gjeldende produksjonsforhold. Som understøttelse til dette, er det i de seneste år blitt påvist CMS så tidlig som 3 mnd etter sjøsetting. Det er lite som tyder på at villfisk har noen betydning for spredning av CMS (3).
En studie har vist at det forekommer vannbåren smitte mellom anlegg, og at anlegg med mange fisk og høyt smittepress fra omkringliggende anlegg har høy risiko for å få CMS (4). Det er også vist at risikoen for å få CMS er større i anlegg hvor det har vært påvist CMS i forrige innsett, enn der det ikke er påvist CMS tidligere. Dette studiet baserte seg på landsdekkende data fra offisielle registre, og kunne ikke konkludere noe om hvilke miljø- og driftsfaktorer som har innflytelse på utviklingen av CMS. Det er uvisst når fisken smittes med PMCV, hvor lenge den kan ha virus i seg, vevstropismen til viruset, hvilke faktorer som påvirker utvikling til klinisk utbrudd og hva som kan gjøres for å bryte smittekjeden eller begrense skadene ved CMS. Prosjektet «Epidemiologisk studie av Kardiomyopatisyndrom (CMS): Spredning, risikofaktorer og sykdomsforløp i norsk lakseoppdrett» (CMS-Epi) ble satt i gang for å forsøke å gi svar på disse spørsmålene.

Prosjektets omfang og organisering:

Prosjektet ble satt i gang November 2015, og avsluttet ved utgangen av 2018. Prosjektet hadde et overordnet budsjett på 15.3 mill. kroner, hvorav de 4.6 mill. var egenandel fra prosjektpartnerne, mens resten (10.7 mill.) ble finansiert av FHF.
Prosjektet var organisert i fem arbeidspakker, som er koblet opp mot delmålene. Prosjektgruppen besto av Veterinærinstituttet som var ansvarlig for gjennomførelse av alle arbeidspakker samt Pharmaq Analytiq som var ansvarlig for utførelse av laboratorieanalyser. Styregruppen besto av representanter fra fire (etter hvert fem) ulike oppdrettsselskap, og disse har vært aktivt involvert i planlegging og utførelse av studiene, samt diskusjon av resultatene.

- Styregruppe:
    - Harald Takle, Marine Harvest (Leder av styregruppe fra nov. 2015 til feb. 2017). Erstattet av Farah Manji mai 2017
    - Arne Guttvik, Salmar (Leder av styregruppe fra april 2017)
    - Julia Fossberg Buhaug, Lerøy Midt
    - Henrik Duesund, Cermaq. Erstattet av Harald Takle mars 2018
    - Merete Bjørgan Schrøder, Norwegian Royal Salmon. Fra Mars 2017.

- Prosjektgruppe:
    - Britt Bang Jensen, VI. Prosjektleder
    - Stian Nylund, Pharmaq Analytiq
    - Siri Vike, Pharmaq Analytiq
    - Julie Christine Svendsen, VI. Fra juli 2016 til august 2018. (Involvert i AP1, AP2 & AP4)
    - Arthur Mårtensson, VI. Fra juni 2017 (Involvert i AP3)
    - Anja B. Kristoffersen, VI. (Involvert i AP1, AP2 & AP3)
    - Åse Helen Garseth, VI (AP-leder AP4)
    - Camilla Fritsvold, VI. (Involvert i AP4)
    - Øystein Evensen, NMBU. fra Januar 2017.
    - Aase B. Mikalsen, NMBU. Fra Januar 2017.

I tillegg fantes en referansegruppe med følgende medlemmer:

- Torkjell Bruheim, Aquagen
- Øyvind Haugland, Pharmaq AS
- Aase B. Mikalsen, NMBU. Overgikk til prosjektgruppe januar 2017.
- Sven Martin Jørgensen, Nofima. Fratrådte referansegruppen mars 2017.

Observatør fra FHF:

- Merete Bjørgan Schrøder. Erstattet av Sven Martin Jørgensen mars 2017.

FHF ga i januar 2017 tilsagn til prosjektet «Kardiomyopatisyndrom (CMS) - påvisning av egenskaper hos piscint myokardittvirus som forklarer opptreden av klinisk sykdom i ulike faser av lakseproduksjonen» (prosjektnummer 901179) som eies av Norges miljø- og biovitenskapelige universitet, Veterinærhøgskolen og ledes av Øystein Evensen. Dette prosjektet er i høy grad basert på tilgang til prøver fra CMS-Epi, og ble derfor koordinert sammen med dette. Prosjektet har samme styregruppe som CMS-Epi, og prosjektlederne fra de to prosjektene inngår i begge prosjektgrupper fra oppstarten av det nye prosjektet i januar 2017.

3. Problemstilling og formål

Prosjektets overordnede mål var å øke kunnskapen om spredning av PMCV og faktorer som påvirker utviklingen av klinisk CMS.
Prosjektet var organisert i fem arbeidspakker, og hadde følgende delmål:

- Finne ut om PMCV overføres vertikalt fra stamfisk til matfisk under vanlige produksjonsforhold (AP1)
- Kartlegge forløpet av PMCV infeksjon i sjø (AP2)
- Identifisere risikofaktorer for infeksjon med PMCV (AP3)
- Identifisere risikofaktorer for utvikling av klinisk CMS (AP3)
- Å gi en samlet fremstilling av publisert og upublisert viten om CMS og PMCV, med fokus på epidemiologi og sykdomsutvikling (AP4)
- Gi en samlet vurdering av tilgjengelige muligheter for å begrense spredning av PMCV og kliniske utbrudd av CMS –Både for enkelte anlegg og for næringen som helhet (AP5)

4. Prosjektgjennomføring, resultater, diskusjon og konklusjon

Arbeidspakke 1: Avklaring av om PMCV overføres fra stamfisk til settefisk (vertikal overføring)

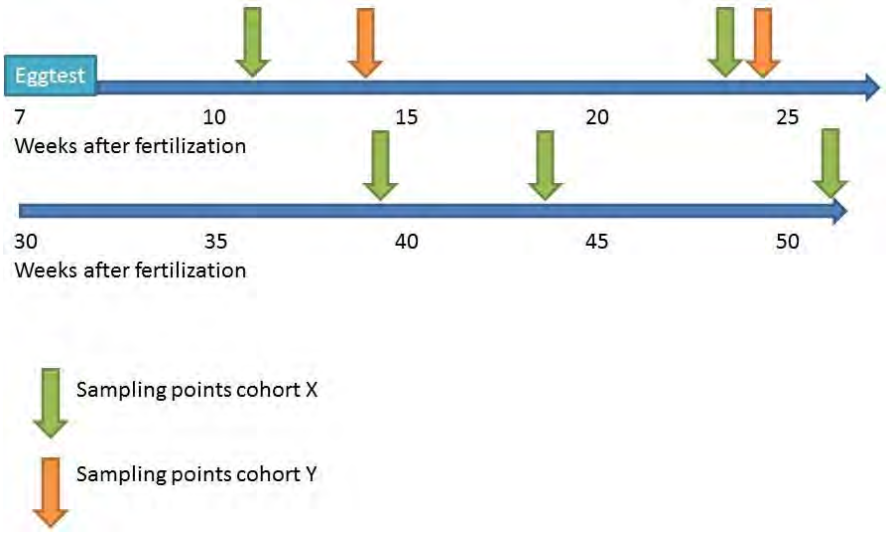
Mål med AP1:

- Finne ut om PMCV overføres vertikalt fra stamfisk til settefisk
- Finne ut om PMCV føres med smolten videre i fra settefiskfasen frem til sjøsetting

Fremgangsmåte
To av næringsaktørene deltok med hvert sitt stamfiskanlegg. Det ble tatt ut prøver av stamfisken og egg og avkom fra stryking og så langt det gikk an å følge de frem mot sjøsetting, som illustrert i figur 1.

I tillegg ble det på det ene anlegget tatt ut prøver av egg før og etter desinfeksjon, og disse ble også testet med RT-PCR for PMCV.
Alle prøver ble analysert med RT-PCR for PMCV hos Pharmaq analytiq. Detaljert beskrivelse er gitt i artikkelen AV Bang Jensen et al (5, vedlegg 2). Alle prøver ble kjørt i triplikat, og hvert kjør besto av 45 cykler. PMCV testen har en repliserbar Ct-verdi på 34,7. Denne verdien er funnet ved å kjøre 10-folds fortyndinger av DNA, og regne gjennomsnittlig Ct-verdi på den høyeste fortynning der alle 10 repplikater er positive. Denne verdien brukes ofte som diagnostisk cut-off verdi, da Ct-verdier over denne grensen ofte ikke er reproduserbare (6). I dette studiet ble alle prøver det det detektert PMCV regnet som positive, uansett Ct-verdi.

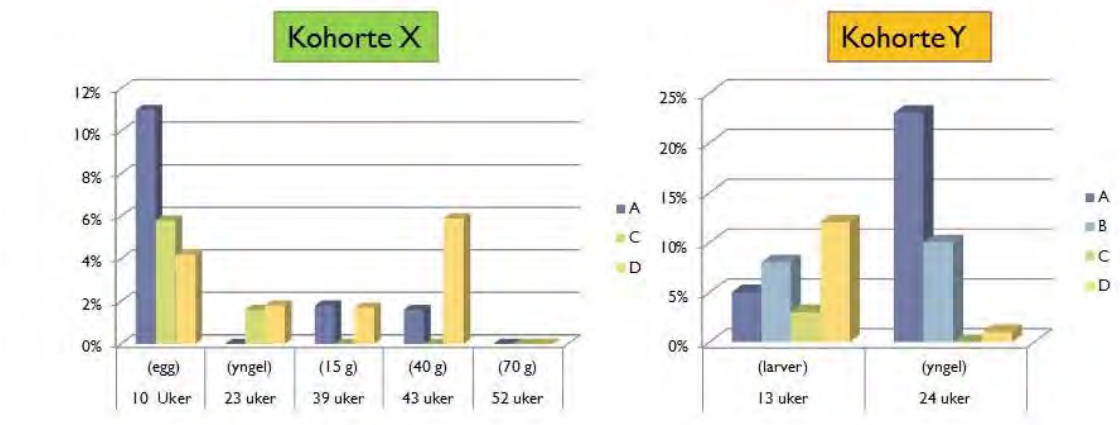
Detaljer rundt dette studiet er beskrevet i vedlagt artikkel (Vedlegg 2), som er publisert i mars 2019.



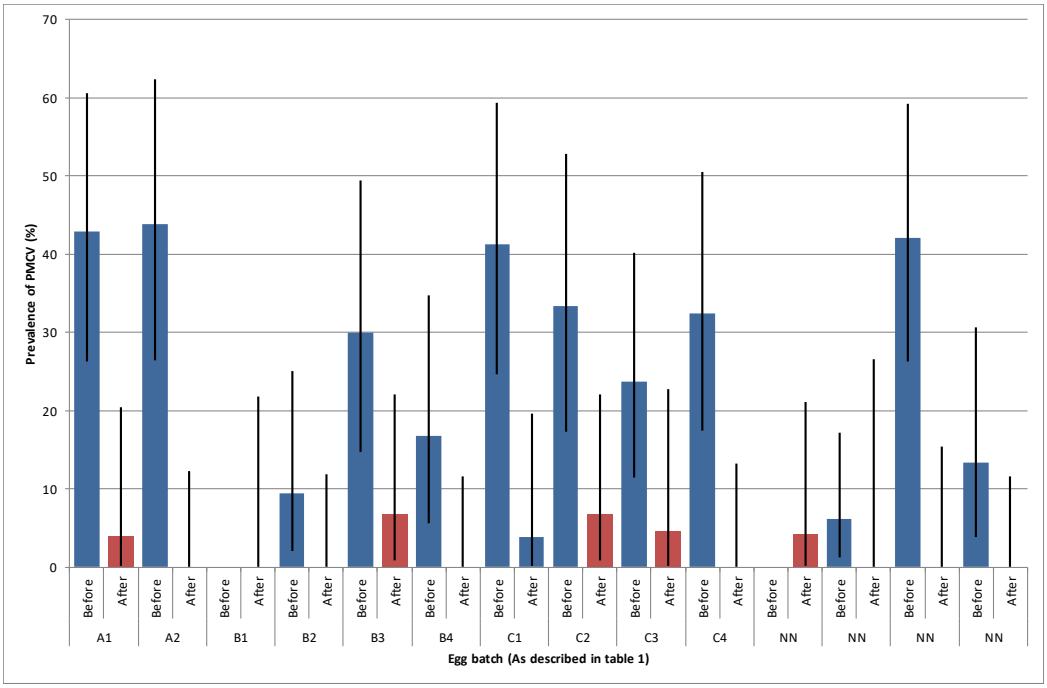
Figur 1. Oversikt over prøvetaking i to kohorter av stamfisk og avkom i AP1.

Resultater
Kort oppsummert var resultatet av studien:

- PMCV ble funnet i hjertet på 128 av 130 testede stamfisk. Prevalensen av PMCV i rognveske var 69%, og 59% i melke.
- PMCV ble funnet i alle stadier fra egg til pre-smolt, men med prevalenser <25% og lave mengder virus (høye Ct-verdier) (Figur 2).
- PMCV ble funnet i alle egg-batcher før desinfeksjon, med en prevalens på 27,5%. Etter desinfeksjon, ble PMCV funnet i 6 av 14 batcher, med prevalenser på 4-6,7%.



Figur 2. Forekomst av PMCV i avkom fra PMCV-infisert stamfisk. For flere detaljer, se vedlegg 2.



Figur 3. Prevalens av PMCV i prøver av egg tatt før og etter desinfeksjon. For flere detaljer, se vedlegg 2

I tillegg til det som er beskrevet over, ble det gjort en liten studie på vevstropisme i stamfisk. Det ble tatt ut prøver fra 13 vev fra 6 hannfisk og 10 hunnfisk på en av stamfisklokalitetene som var med i studien beskrevet over. Det ble tatt ut følgende vev fra alle fisk: Hjerte (atrium), ventrikkel (a), ventrikkel (b), gjeller, tunge, fornyre, lever, milt, muskel (hvit), muskel (rød), baktarm, hud, slim, blod (serum), blod (cellefraksjon), rogn/melke (høyre side) og rogn/melke (venstre side). Hjerne var ikke mulig grunnet fiskens størrelse.

Resultatene av dette vevstropismestudiet var:

- Det sees ikke noen umiddelbar forskjell mellom hann- og hunnfisk.
- Det er funnet virus i alle vev som er testet.
- Følgende vev egner seg til prøvetaking (ct-verdi under cut-off): Hjerte (forkammer og hjertekammer), lever, milt, baktarm, fornyre og evt gjelle.
- Resten av vevene har en del/alle prøver som ligger over cut-off, og er derfor ikke egnet til test.

Øvrige aktiviteter i AP1:

-Det ble gjort forsøk på sekvensering av prøver fra egg og yngel, for å evt kunne sammenligne sekvenser med de fra stamfisk. Dessverre var det så lite materiale og så lav mengde virus at det ikke lyktes å sekvensere fra disse prøvene.

-For å etterprøve resultatene av studien, ble det planlagt å kopiere det ved stryking av stamfisk på et tredje stamfiskanlegg i høsten 2017. Dessverre var alle prøver fra stamfisken negative for PMCV, så forsøket måtte avlyses. Det var i dette studiet også planlagt å teste effekten av ulike desinfeksjonsmetoder på egg, men også dette måtte avlyses siden eggene var negative for PMCV før desinfeksjon.

-PMCV-positive prøver fra stamfisken ble delt med prosjektet «Kardiomyopatisyndrom (CMS) –påvisning av egenskaper hos piscint myokardittvirus som forklarer opptreden av klinisk sykdom i ulike faser av lakseproduksjonen» ved NMBU.

Diskusjon av funn i AP1

Studien som ble gjennomført hadde som formål å finne ut om PMCV kan overføres fra stamfisk til settefisk under vanlige produksjonsforhold. Resultatene indikerer at dette kan skje. Det er noen forhold vedrørende resultatene som vanskeliggjør en entydig konklusjon. Det ble bare funnet små mengder virus, og i en liten andel av avkommet. Dette kan tyde på at viruset ikke replikerer aktivt i avkommet i settefiskfasen. Siden

virus bare ble detektert ved PCR, er det ikke mulig å si om det var infektivt virus som ble funnet, eller eventuelt bare rester av virusgenom. Et smitteforsøk der en bruker materiale fra PMCV-positive egg og yngel som inokulum vil trolig kunne avklare dette.
De fleste av de positive prøvene fra avkom hadde Ct-verdier mellom 35 og 39 (5) Det betyr, at de ville ha blitt ansett som negative ved vanlig diagnostisk undersøkelse, da en da forholder seg til den fastsatt cut-off verdien. Dette er også viktig i forskning, hvor forskere bør kunne etterprøve resultater generert av andre forskere. Ulempen ved å bruke en slik cut-off er på den andre siden, at en mister informasjon om prevalens og tilstedeværelse av virus for eksempel hos bærere av virus. Derfor har vi hatt med relevante kontroller i analysene for å sikre at det er den riktige virus-sekvensen vi finner (mer diskusjon rundt dette i referanse 5).
Andelen av PMCV-positiv stamfisk var høy. Dette kan gjøre det vanskelig å selektere PMCV-fri stamfisk i produksjonen. I studien viste det seg at avkom fra hannfisk der det ikke hadde blitt funnet PMCV i melken, men bare i hjertet, hadde lavest forekomst av PMCV. Dette kan være en tilfeldighet, men det bør underøkes nærmere. Om det viser seg at overførsel fra hannfisk har mer å si for forekomsten av PMCV i avkom, kan dette være en mulig måte å begrense smitte, siden det trengs mye færre hann- enn hunnfisk for produksjon, og det derfor er lettere å velge bort de med PMCV-positiv melke.
Effekten av desinfeksjon av egg mot PMCV bør også undersøkes nærmere. I studien vår ble det observert en reduksjon av mengden og forekomsten av PMCV-RNA fra egg ved den mest anvendte metoden for desinfeksjon i Norge. Men vi vet ikke om det betyr at metoden ikke effektiv til å fjerne infektivt PMCV, siden det igjen bare er virus-RNA vi detekterer ved PCR-metoden.
Resultatet av vevstropisme-studien tilsier at blod, slim, gjelle, melke og rognveske ikke er egnet som materiale for screening av stamfisk for PMCV. Gjelle kan muligens være egnet, da de fleste av gjelleprøvene var positive. Dermed finnes fortsatt ingen ikke-letale metoder for screening, noe som ellers kunne ha lettet arbeidet med seleksjon av PMCV-fri stamfisk for produksjon.

Leveranser fra AP1
- Populærvitenskapelig fremstilling av resultatene av studien om vertikal overføring av PMCV. Publisert i Norsk Fiskeoppdrett desember 2017 (Vedlegg 1).
- Muntlige presentasjoner på følgende konferanser:
  - -Workshop om vertikal overføring av PMCV på Frisk Fisk, Februar 2017
  - -18th International conference on Fish and Shellfish Diseases, Belfast, september 2017
  - -TriNation møte og CMS-workshop, Bergen, Mars 2018
  - -Havbrukskonferansen, Oslo, April 2018
- Posterpresentasjon på 15th International symposium on Veterinary epidemiology and Economics, Chiang Mai, November 2018
- Vitenskapelig artikkel "Indications for a vertical transmission pathway of Piscine Myocarditis virus (PMCV) in Atlantic salmon (Salmo salar)". Bang Jensen B, Nylund S, Svendsen JC, Ski P-MR, Takle H. (2019). Journal of Fish Diseases, vol 00, p 1–9. (Vedlegg 2)

Arbeidspakke 2: Kartlegging av forløpet av PMCV-infeksjon fra settefisk til slakt

Mål med AP2:
- Kartlegge forløpet av PMCV infeksjon hos oppdrettslaks i sjø
- Finne ut hva som er det beste vevet å screene for PMCV fra fisk i settefiskfasen

Fremgangsmåte
Fire oppdrettsselskap deltok i studien. Matfisklokaliteter fra selskapene ble selektert basert på høy risiko for å få CMS, og supplert med anlegg som hadde lav risiko. Det var selskapene selv som stod for utvelgelsen, basert på egne historiske erfaringer. Siden CMS hos noen aktører oftest er et problem hos høstsmolt, var det ønskelig å følge settefisken som ble satt ut høsten 2015. Totalt ble det fulgt fisk på tre H15 lokaliteter, samt fra fire V16 og fem H16 lokaliteter. Lokalitetene var plassert langs hele kysten, med 2 i Finnmark, 1 i Troms, 2 nord i Nordland, 1 i Sør-trøndelag, 3 i Møre- og Romsdal, 1 i Hordaland og 2 i Vest-Agder (Se evt kart i vedlegg 4)

På hver lokalitet ble det valgt ut to fiskegrupper som ble fulgt fra utsett til slakt. Dette med unntak av èn lokalitet, hvor en fulgte tre grupper. Fisken ble prøvetatt for PMCV med regelmessige tidsintervaller fra etter sjøsetting og frem til slakt. Høyrisikolokalitetene ble prøvetatt etter utsett og deretter ca. hver måned frem til slakt, mens det ble tatt prøver ca. annenhver måned frem til slakt på lavrisikolokalitetene. Detaljer rundt dette studiet er beskrevet i vedlagt artikkel (Vedlegg 4), som ble publisert i februar 2019.

Resultater

Resultatene er oppsummert her, men for ytterligere detaljer, se vedlegg 3.

PMCV ble påvist gjennom RT-qPCR på alle lokalitetene, i alle prøvetatte merder. Klinisk CMS utviklet seg på seks av de 12 lokalitetene som ble fulgt, mens fire av lokalitetene forble CMS negative. På to lokaliteter var det en økning i virusforekomst samt et fall i CT verdiene, men en sikker CMS diagnose kunne ikke stilles basert på histopatologiske funn. Disse lokalitetene ble definert som med mistanke om CMS. Åtte av lokalitetene hadde høstsmolt, mens fire hadde vårsmolt. Klinisk sykdom utviklet seg på en høyere andel av høstlokalitetene (5/8), sammenlignet med vårlokalitetene (1/4). Begge lokalitetene med mistanke om klinisk CMS hadde høstsmolt.

Tid for første PMCV påvisning varierte fra en til 13 måneder etter sjøutsett, mens tiden fra sjøutsett til klinisk sykdom varierte fra seks til 17 måneder (tabell 1). Mediantiden fra PMCV infeksjon til klinisk utbrudd var fire måneder, med et spenn fra tre til 10 måneder.

Tabell 1. Tid fra sjøsetting til påvisning av PMCV ved RT-PCR, tid fra påvisning av PMCV til utvikling av klinisk CMS, og tid fra sjøsetting til klinisk CMS for 12 lokaliteter som ble fulgt i AP2.

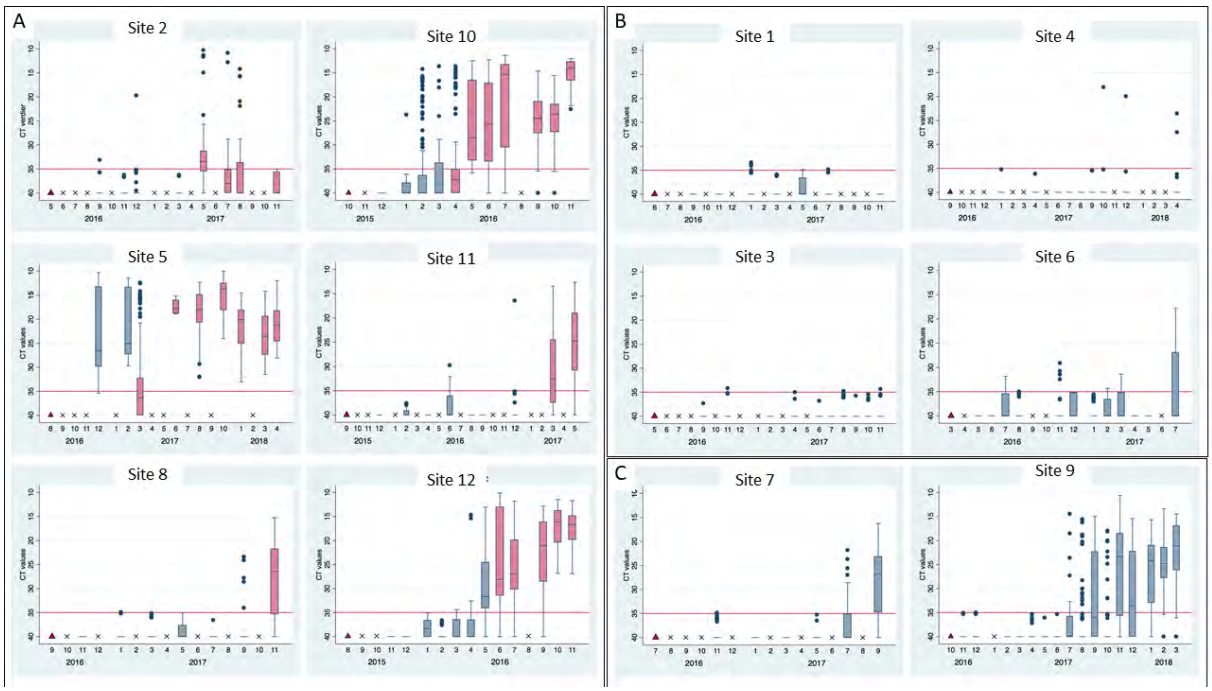
Disease status	**Months from sea transfer → PMCV (+) (median (range))**	**Months from PMCV (+) → CMS (+) (median (range))**	**Months from sea transfer → CMS (+) (median (range))**
CMS positive (n=6)	4 (3 – 5)	6,5(3 - 13)	11 (6 - 18)
CMS negative (n=4)	4 (4 –7)	n/a	n/a
CMS suspect (n=2)	2,5 (1 – 4)	n/a	n/a

Det ble utført utvidet prøvetaking på sju lokaliteter, på ulike tidspunkt i sjøfasen. Ut av disse ble fire CMS positive, to forble CMS negative og en hadde CMS mistanke. PMCV ble påvist i fisk fra alle merdene som ble prøvetatt ifm. de utvidete uttakene, også de som forble CMS negative. I tillegg ble CMS påvist ved to ytterligere lokaliteter, hvor prøver til RT-qPCR og histologisk undersøkelse kun ble tatt fra de to merdene som ble fulgt gjennom studiet.

PMCV ble påvist etter tre til åtte måneder i sjøen på de seks lokalitetene som ble CMS positive. På fire av disse var det noen måneder med høye CT verdier, før en heller markert økning i virusmengden (figur 4A). CMS diagnosen ble stilt enten samtidig som, eller kort tid etter denne økningen, og virusmengden forble høy resten av produksjonssyklusen.

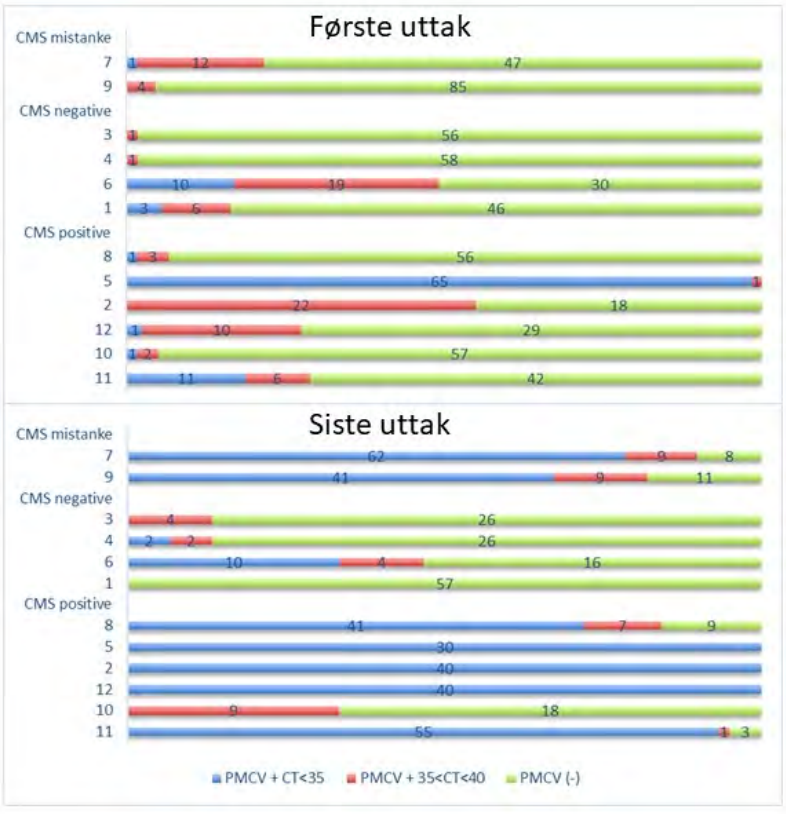
På de fire lokalitetene uten klinisk CMS var bildet annerledes: PMCV ble påvist, men i generelt lave mengder. PMCV ble påvist på tre av lokalitetene helt fram til fisken ble slaktet ut. På den siste lokaliteten ble det ikke påvist PMCV i prøvetakingen før slakt, men forekomsten på tidligere uttak var så lav at et negativt resultat ikke utelukker tilstedeværelse av virus (figur 4B).

På de to lokalitetene med mistanke om CMS, fulgte infeksjonsforløpet et veldig lignende mønster som på de CMS positive lokalitetene (figur 4C).



Figur 4. Utvikling av PMCV i 12 lokaliteter som ble fulgt i sjø. For flere detaljer, se vedlegg 4

Andelen av PMCV-positive fisk ved første påvisning (figur 5) var generelt lav på de fleste lokalitetene (2% - 55%), og hovedandelen av påvisninger hadde CT verdier mellom 35 – 40. Ved det siste prøveuttaket før slakt var andelen av PMCV-positive fisk høyest hos de CMS positive (33,3% - 100%) og CMS mistanke (82% og 90%) lokalitetene. På de CMS negative lokalitetene var forekomsten mellom 0% - 46,7%. På dette siste uttaket lå hovedvekten av CT verdiene under 35.



Figur 5. Forekomst av PMCV ved første og siste prøveuttak i hvert av de 12 lokalitetene.

CT verdiene fra all prøvetatt fisk ble inndelt i kategorier etter helsestatus og sammenlignet. Kategoriene var "ukjent", "tilsynelatende normal", "død/svimer" og "tapere". Fisk i kategorien "død/svimer" hadde generelt lavere CT verdier.

Øvrige aktiviteter i AP2:

- Det ble gjort en liten studie på hvilket vev som er best å bruke når en ønsker å evaluere forekomsten av PMCV i klinisk frisk fisk i settefiskfasen. Dette ble undersøkt ved å ta en større organpakke fra et utvalg av fisk, som inkluderte hjerte, nyre, gjelle, hjerne, milt og lever.
  Det ble gjort to uttak til vevstropisme, på to ulike settefiskanlegg. Ved begge uttakene var det smolt som ble prøvetatt rett før sjøutsett.
  På det ene anlegget ble det tatt prøver av totalt 20 individ. Av samtlige prøver var det kun èn miltprøve som var positiv, da med høy CT-verdi (36,2).
  På det andre anlegget ble det tatt prøver av ni fisk, av de samme organene som nevnt over. Viralt RNA ble påvist i seks av ni individ, fra organ som vist i tabell under:

Tabell 2. Funn av PMCV i ulike vev fra 9 settefisk.

Individ	PMCV positiv	Vev	CT verdi
1	Nei		
2	Ja	Lever	35,6
3	Nei		
4	Nei		
5	Ja	Lever	35,8
6	Ja	Hjerte	34,6
7	Ja	Milt	36,9
8	Ja	Hjerte	35,2
9	Ja	Lever	36,0

- Fra et anlegg med klinisk utbrudd av CMS, ble det i mai 2016 tatt ut prøver fra 60 blåskjell og 89 berggylt som oppholdt seg rundt anlegget. Gjeller fra blåskjell og hjerte, nyre og gjeller fra berggylt ble testet for PMCV ved PCR. Alle prøver var negative.

Diskusjon av funn i AP2:

Når en ser på de CMS positive lokalitetene i figur 4 ser en noe variasjon i bildet, men flere lokaliteter følger det samme mønsteret. På disse ser en at de første påvisningene av PMCV er med høye CT-verdier, som så fortsetter noen få måneder, før en får en kraftig økning i både forekomst av virus og virusnivå. Denne økningen fant sted kort tid før, eller parallelt med, påvisning av klinisk CMS på lokaliteten. Men, på en av lokalitetene (2) endret ikke CT nivåene seg på samme vis over tid. På denne lokaliteten ble klinisk CMS diagnostisert i mai 2017, og fisken forble i sjøen i ytterligere ett halvår. Det ble rapportert at sykdommen tok en klinisk mild form på lokaliteten. Dette setter lys mot spørsmålet om hvorfor noen lokaliteter opplever alvorlige sykdomsutbrudd med store tap, mens andre har et mye mildere forløp.
Ved den første påvisningen av PMCV varierte forekomsten noe mellom lokalitetene, men den er relativt lav på flere. Dette bildet blir også forsterket av at en stor andel av påvisningene er med CT verdier over den vanlig brukte cut off verdien (Ct = 35). Dette medfører at disse resultatene ville blitt vist som negative i en vanlig screening-rapport.
På den siste prøvetakingen før slakt hadde dette endret seg betydelig. På CMS (+) lokalitetene var forekomsten svært høy på alle lokalitene, med unntak av den ene hvor det kun var et mildt klinisk utbrudd. Prøvene fra disse lokalitetene var også dominert av lave CT verdier. Dette var også tilfellet for de to lokalitetene med mistanke om klinisk sykdom. De CMS negative lokalitetene derimot, hadde generelt lavere forekomst av virus og lavere virusnivå.
Disse resultatene underbygger tidligere studier som har vist tydelig korrelasjon mellom virusnivå og klinisk sykdom.
Basert på funnene i dette studiet virker viruset å være vidt utbredt i norsk lakseoppdrett. Det ble også funnet på fire lokaliteter hvor infeksjonen ikke resulterte i klinisk CMS. Dette indikerer at viruset kan eksistere i populasjoner uten påfølgende sykdomsutbrudd. Å påvise PMCV betyr dermed ikke at

sykdomsutbrudd nødvendigvis er forestående. Den lave forekomsten samt virusnivåene som karakteriserer den første fasen av en infeksjon svekker også påliteligheten til screening som et verktøy for tidlig påvisning av sykdomsutbrudd. Men, screening kan være nyttig for å følge helhetsbildet på en lokalitet. En utvikling med økt forekomst, samt økte virusnivå, kan være et varselssignal. Brukt sammen med vurderinger av fiskehelsen på lokaliteten, kan dette danne basis for avgjørelser om å iverksette forebyggende tiltak. CT verdiene var lavest i "død/svimer" gruppen, noe som indikerer at dette kan være den mest hensiktsmessige fisken å prøveta ifm. evt. screening.
Det ble ikke påvist PMCV i berggylt eller blåskjell som oppholdt seg nær et oppdrettsanlegg med klinisk CMS. Dette tyder på at disse artene ikke kan smittes med sykdommen eller fungere som reservoir.

Leveranser fra AP2
- Populærvitenskapelig fremstilling av resultatene av studien om overvåking av PMCV. Publisert i Norsk Fiskeoppdrett januar 2018 (Vedlegg 3).
- Muntlige presentasjoner på følgende konferanser:
  - -Frisk Fisk, Bergen, Februar 2017
  - -18th International conference on Fish and Shellfish Diseases, Belfast, september 2017
  - -TriNation møte og CMS-workshop, Bergen, Mars 2018
  - -Havbrukskonferansen, Oslo, April 2018
- Vitenskapelig artikkel "Monitoring infection with Piscine myocarditis virus and development of cardiomyopathy syndrome in farmed Atlantic salmon (Salmo salar L.) in Norway". Svendsen JC, Nylund S, Kristoffersen AB, Takle H, Fossberg Buhaug J, Bang Jensen B. (2019). Journal of Fish Diseases, vol 42, p 511–518. (Vedlegg 4)

Arbeidspakke 3: Risikofaktorer for klinisk CMS i felt

Mål med AP3:
- Identifisere risikofaktorer for infeksjon med PMCV
- Identifisere risikofaktorer for utvikling av klinisk CMS
- Finne ut hvorfor noen lokaliteter oftere får CMS enn andre

Fremgangsmåte
Denne arbeidspakken ble utviklet løpende underveis i prosjektet gjennom dialog med styregruppen. Flere forskjellige tilnærminger ble diskutert, deriblant:
- Kasus-kontroll studie der det plukkes ut lokaliteter som ofte har hatt CMS og tilsvarende lokaliteter som aldri/sjeldent har hatt det. Data om produksjonsforhold innhentes fra alle lokalitetene. Ulempen ved denne tilnærmingen er at en trenger å ta ut prøver fra mange lokaliteter for å avgjøre CMS-status. Kan dessuten være tungvint å hente inn data fra ulike lokaliteter og næringsaktører.
- Innsamling av retrospektive data fra alle aktører på utsett fra vår 2012 til og med høst 2014. Det var enighet om å gjennomføre dette, men pga. vanskeligheter med å få ta ut data fra alle aktører, endte vi med å bare bruke data fra en aktør. Dette er beskrevet mer i detalj under.
- Innsamling av data fremadrettet fra fiskegrupper satt ut fra 2016. Vi forsøkte å få til dette, men det viste seg vanskelig å få oversikt over alle utsett. Planen var at det skulle tas ut prøver for PMCV rett før eller rett etter utsett. Underveis ble det besluttet at det var for stor usikkerhet forbunnet med nytteeffekten av denne data- og prøveinnsamlingen, og det ble besluttet å heller fokusere på grundig gjennomgang av de retrospektive dataene.

Det ble i april 2016 avholdt en workshop med formål å diskutere AP3. I denne workshopen inngikk en diskusjon av casedefinisjon på klinisk CMS. Resultatet av denne diskusjonen var at det i prosjektet ble brukt tre ulike nivåer:
1. Tilstedeværelse av virus
2. Tilstedeværelse av histologiske funn forenelig med CMS
3. Tilstedeværelse av økt dødelighet samt enten 1) og/ eller 2)

Resultater

Fremgangsmåte for analyse og tilhørende resultater fra det retrospektive datasettet er presentert i vitenskapelig artikkel som er sendt inn til vitenskapelig tidsskrift i april 2019. Kort oppsummert var resultatet av studien:

- Klinisk CMS (akkumulert CMS-relatert dødelighet over 0,1%) forekom i 470 av de 1395 fiskegruppene som var inkludert i studien Opprinnelig antall fiskegrupper var 1536, men vi måtte ta bort de som det ikke var mulig å følge gjennom hele produksjonssyklus -Se tabell 3.
- Generelt sett var andelen av fiskegrupper som fikk CMS høyere i høstutsatte fisk enn i vårutsatte (5-29% vs 30-52%), men det var variasjoner mellom områder.
- Det var ikke noen sammenheng mellom CMS-utbrudd og sesong eller temperatur

Tabell 3. Oversikt over fiskegrupper som ble analysert i AP3

Grupper per generasjon	2012	2013	2014	Totalt
Vår	236	213	197	646
Høst	251	210	288	749
Grupper med CMS				
Vår	21	57	36	114
Høst	117	100	139	356

Datasettet inneholdt fiskegrupper fra fire produksjonsområder, men det var bare en fiskegruppe i produksjonsområde nord som hadde CMS (Se figur 6), så dette området ble tatt ut av analysen. Det ble laget en modell som ble kjørt for hvert område separat, for å ta høyde for geografiske- og administrative forskjeller.

Fra start ble det i modellen forsøkt inkludert mange forskjellige faktorer, blant annet:

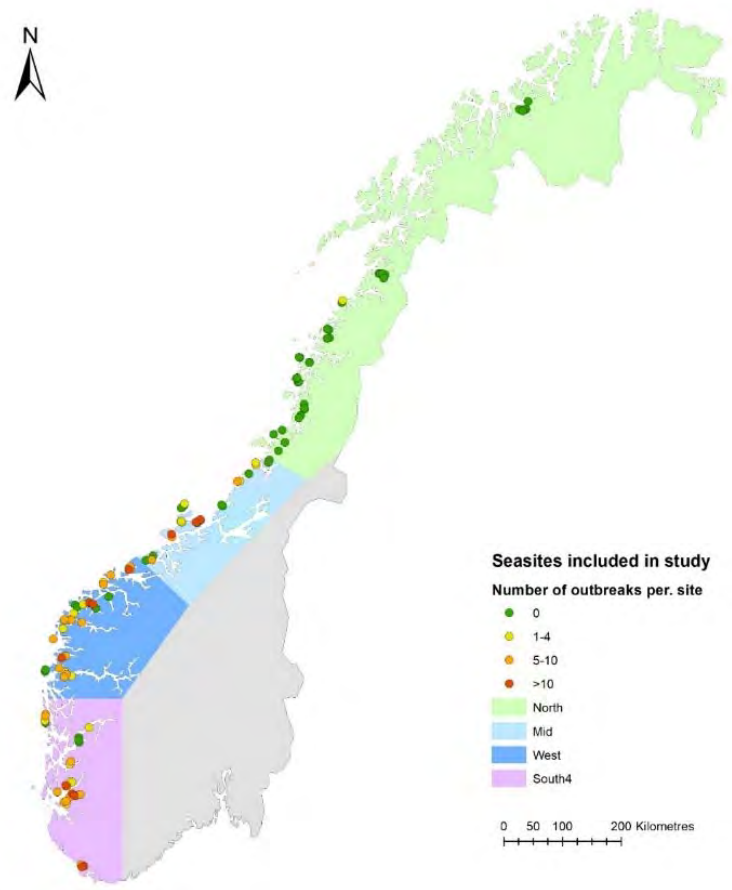
- Temperatur
- Fôring
- Lusebehandling
- Smittepress
- Størrelse på anlegg
- Forskjellige sykdommer
- Miljøfaktorer

Tabell 4. Oversikt over fordeling av lokaliteter som inngikk i studien i AP3

Region	Total	CMS	Andel CMS
Sør	319	196	61,4%
Vest	361	160	44,3%
Midt	402	111	27,6%
Nord	308	1	0,3%

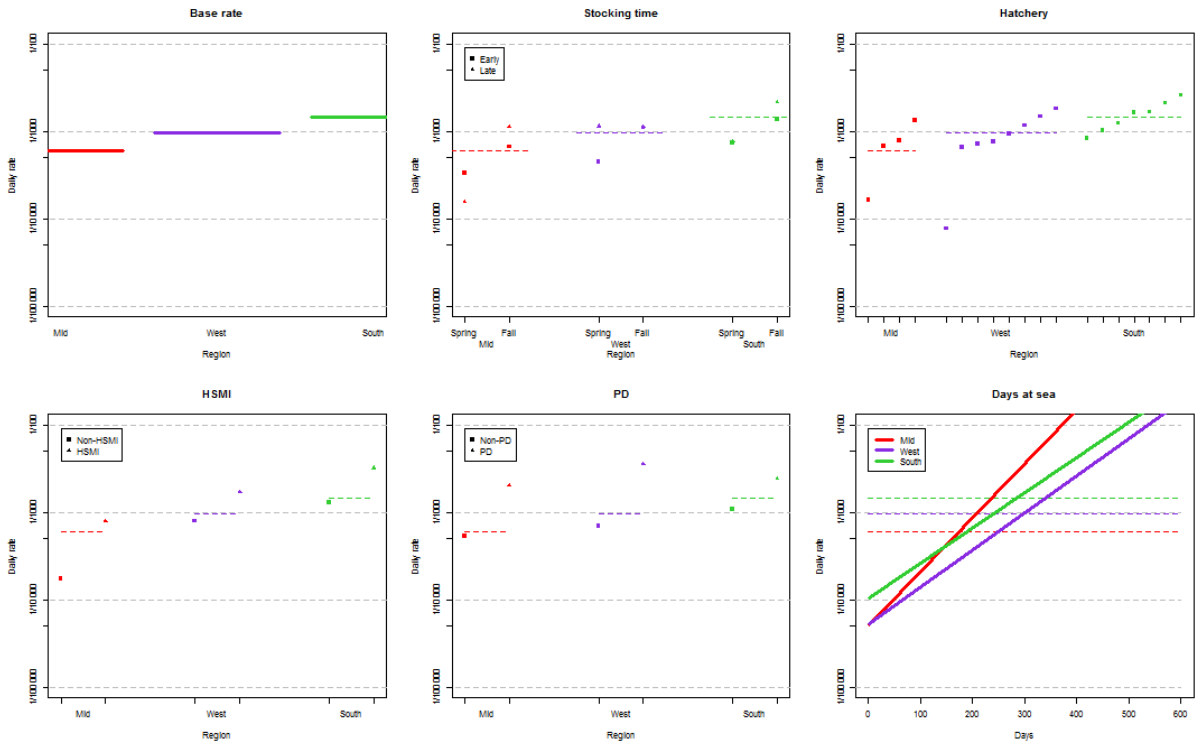
Modellen er satt opp slik at den beregnet den totale sannsynligheten for observerte utbrudd (og ikke-utbrudd). I modellen ble parametere som maksimerte denne sannsynligheten etterfølgende avprøvd. Det ble funnet at følgende parametere hadde innflytelse på risikoen for å få CMS (Figur 7):

- Utsettstid: Vårutsett hadde betydelig mindre risiko for å få CMS enn høstutsett. I område midt hadde især de som var satt ut sent på våren lav risiko, mens det i område vest var de som var satt ut tidlig på våren som hadde lav risiko. I sør var risikoen like lav for tidlig- og sent vårutsett.
- Tidligere utbrudd av HSMB i fiskegruppen: I alle områder hadde fiskegrupper som hadde hatt utbrudd av HSMB større risiko for også å få CMS. Forskjellen var mest synlig i region midt og sør.
- Tidligere utbrudd av PD i fiskegruppen: I alle områder hadde fiskegrupper som hadde hatt utbrudd av PD større risiko for også å få CMS. Forskjellen var mest synlig i region midt og vest.
- Tid i sjø: Utbrudd av CMS var mer vanlige jo lenger fisken hadde stått i sjø i alle regioner



Figur 6. Oversikt over fordeling av lokaliteter som inngikk i studien i AP3

I tillegg fant vi ved univariat analyse, at hvilket settefiskanlegg som fiskegruppen kom fra hadde stor innflytelse på risikoen for å få CMS. Dette kunne ikke inkluderes i den fulle modellen pga antallet av settefiskanlegg, men effekten ses i figur 7.



Figur 7. Risiko for utvikling av CMS i fiskegrupper avhengig av ulike faktorer. Øverst til venstre ses den daglige basisrate for utvikling av CMS i de tre regionene. Denne raten er satt inn som stiplet linje i de andre figurene. Faktorer som er plassert over linjen øker den daglige risikoen for CMS, mens faktorer under er beskyttende.

Diskusjon av funn i AP3

Da prosjektet begynte, var det en utbredt oppfattelse at det var en klar sammenheng mellom infeksjon med PMCV og klinisk utbrudd av CMS, slik at de anlegg som hadde fått påvist PMCV også utviklet klinisk CMS. Fra AP2 fant vi at det ikke alltid er slik. Alle lokaliteter i AP2 fikk påvist PMCV, men bare halvparten utviklet CMS. Derfor er det ikke mulig å anta at det bare er fiskegrupper som har fått CMS som er infisert med PMCV. Fra AP2 viste det seg også at mange anlegg var infiserte med PMCV tidlig etter sjøsetting. Derfor må en tenke nytt om hvordan og når fisken infiseres med PMCV, før en kan gjennomføre studier om årsakene til dette.

I studien viste det seg at det var en viktig sammenheng mellom hvilket settefiskanlegg som hadde levert smolten og utviklingen av CMS. Dette kan være en av årsakene til at det ses forskjeller mellom fiskegrupper i samme anlegg. Det underbygger også resultater fra AP1 og AP2, som tyder på at noen smolt kan være infisert med PMCV fra settefiskfasen.

Vi forsøkte å se på mange ulike risikofaktorer utifra datasettet. For eksempel så vi på effekten av avlusning, miljøpåvirkninger og andre sykdommer. Men det var ikke mulig med det gjeldende datasettet å se noen andre sammenhenger enn de som er presentert over. For å undersøke for eksempel effekten av mekanisk avlusning, bør en inkludere data fra senere utsett, siden disse metodene først for alvor ble tatt i bruk etter den perioden vi studerte. Siden modellen er på plass, forventes det at et slikt studie vil kunne gjennomføres relativt raskt, om ønskelig, og dersom data blir tilgjengelig fra næringen.

Fra dette datasettet fant vi ikke noen tydelige sammenhenger mellom utbrudd og sesong eller år. Tidligere har CMS blitt oppfattet som en vintersykdom. Vi brukte datoen for når de første CMS-døde fisk ble registrert som utbruddsdato, noe som trolig gir et mer nøyaktig tidspunkt enn tidligere brukt.

Det var store forskjeller mellom regionene i forhold til andelen av vår- og høstsmolt som fikk CMS. Generelt sett var andelen størst i høstsmolt, og spesielt de som ble satt ut sent på høsten i sør og midt,

mens det i region vest var en høyere andel CMS blant fiskegrupper som ble satt ut tidlig på høsten de to siste årene.

Leveranser fra AP3:
- Muntlige presentasjoner er gitt på følgende konferanser/møter:
  - TriNation møte og CMS-workshop, Bergen, Mars 2018
  -Frisk Fisk, Tromsø, Februar 2019
- Posterpresentasjon på 15th International symposium on Veterinary epidemiology and Economics, Chiang Mai, November 2018
- Vitenskapelig artikkel "Estimating risk factors for development of clinical Cardiomyopathy syndrome on a fishgroup level)" forventes insendt til Preventive Veterinary Medicine april 2019.

Arbeidspakke 4: Kunnskapssammenstilling

Mål med AP4:

- Lage en samlet oversikt (review) over publisert og upublisert viten om CMS og PMCV, med fokus på epidemiologi og sykdomsutvikling.

Resultat

Arbeidspakkens hovedleveranse er en norsk populærvitenskapelig rapport og et faktaark. I tillegg ble publisering av en review i tidsskrift skissert i prosjektbeskrivelsen. Det ble tidlig avgjort at reviewen skulle gjennomføres.

Ut over disse leveransene ble det, etter forslag fra styregruppeleder Harald Takle (MH), utarbeidet en kortfattet PowerPoint presentasjon på engelsk som kan benyttes i opplæring og informasjonsarbeid internt i selskap.

Diskusjon av AP4

Norsk rapport og den vitenskapelige reviewen har fått stor oppmerksomhet. Reviewen er blant de 20 mest nedlastede artiklene fra Journal of Fish Diseases i de to siste årene. Reviewen brukes også som pensum for fiskehelsebiolog-, og veterinærstudenter. I følge statistikk på Researchgate har reviewen blitt lastet ned 129 ganger fra dette nettstedet.

Leveranser fra AP4
- Populærvitenskapelig sammenstilling på norsk «Kardiomyopatisyndrom (CMS) hos laks Sykdomsutvikling - Agens – Epidemiologi». Tilgjengelig på https://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2017/kardiomyopatisyndrom-cms-hos-laks. Publisert januar 2017.
- Faktaark om CMS «Kardiomyopatisyndrom (CMS) hos laks. Sykdomsutvikling - Agens – Epidemiologi». Vedlegg 5
- Powerpointpresentasjon om CMS. Distribuert til styregruppen Juni 2017.
- Munntlige presentasjoner er gitt på følgende konferanser/møter:
  - TriNation møte og CMS-workshop, Bergen, Mars 2018
- Vitenskapelig artikkel "Cardiomyopathy syndrome in Atlantic salmon Salmo salar L.: A review of the current state of knowledge". Å H Garseth, C Fritsvold J C Svendsen B Bang Jensen A B Mikalsen. Journal of Fish Diseases, 41-1, pp. 11-26. Publisert oktober 2017. Vedlegg 6

Arbeidspakke 5: Muligheter for begrensning av CMS

Mål med AP5:

- Gi en samlet vurdering av tilgjengelige muligheter for å begrense spredning av PMCV og kliniske utbrudd av CMS –Både for enkelte anlegg og for næringen som helhet

Fremgangsmåte

Gjennom denne arbeidspakken har det blitt avholdt to workshops:

- «Workshop om vertikal overføring av virus i lakseoppdrett». Avholdt i forbindelse med Frisk Fisk i Bergen, februar 2017.
- «CMS-workshop» Avholdt i forbindelse med TriNation møte i Bergen, mars 2018.

Målet med den første workshopen var å diskutere mulighetene for vertikal overføring av CMS. Det var invitert foredragsholdere som har forsket på vertikal overføring av ulike virussykdommer. I tillegg ble foreløpige resultater fra AP1 i dette prosjektet lagt frem og diskutert.
Målet med den andre workshopen var å utveksle erfaringer fra felt og forskning på PMCV og CMS og diskutere muligheter for kontroll. I tillegg å utarbeide en liste med kunnskapshull.
Resultater fra AP1-4 har blitt inkludert i en vurdering av mulighetene for å hindre introduksjon av PMCV i sette- og matfiskanlegg, og for å hindre utviklingen av klinisk CMS.
Muligheter for å begrense spredning av PMCV i næringen samt for utryddelse av klinisk CMS i norsk lakseoppdrett har også blitt diskutert.

Resultater
Til workshopen om vertikal overføring av virus i lakseoppdrett var det 27 påmeldte deltakere. Program, deltakerliste og referat av workshopen finnes i vedlegg 7. Kort oppsummert ble følgende emner behandlet:
- Hvordan skjer vertikal overførsel rent biologisk, og hva skal til for å si at det er snakk om «ekte» vertikal overføring. Om virus skal kunne overføres vertikalt, må det tåle overflatedesinfeksjon av egget, være svært motstandsdyktig i miljøet, forbli assosiert med rognkorn helt til klekking, smittes fra rognkorn til yngel etter klekking og forårsake sykdom hos yngel. Det kan også hende, at det heller er snak om mekanisk overføring av virus etter desinfeksjon.
- Når en påviser virus ved PCR-metoder, må en huske på at slike metoder, som ikke innebærer dyrkning av agens, ikke nødvendigvis påviser et smittsomt agens. Derfor er det en vurderingssak hva betydningen er ved PCR-funn av virus i for eksempel kjønnsprodukter og befruktede egg.
- Status vedr kunnskap om vertikal overføring for ulike virus. Det er fortsatt en del som er uavklart angående dette, og forskere er ikke alltid enige om tolkingen av forsøksresultater. Det kan være fornuftig å anta en føre-var holdning der en tenker som om de fleste virus er vertikal overførbare, inntil det motsatte er bevist.
- I genbank for villlaks bruker en nettopp føre-var prinsippet når en jobber med bevaring av den ville laksebestanden. I genbanken er det fokus på å hindre introduksjon, oppformering og spredning av vertikal overført sykdom. Stamfisken screenes for IPNV, BKD og PMCV, og egg fra positive stamfisk kasseres.
- Det trengs mer forskning på muligheten for vertikal overføring. Blant annet bør en utføre mer kontrollerte forsøk med PMCV-infiserte egg som smittestoff.

Til CMS-workshopen som ble avholdt i forlengelse av TriNation møtet var det 123 deltagere. Det var 14 foredrag, og de fleste er publisert på http://trination.org/past-meetings/bergen-2018/
Et sammendrag av workshopen er presentert i vedlegg 8.
Noen av de viktigste budskapene fra presentasjonene var:
- CMS er blitt et stort problem også i Irland og Skottland i de senere år. På Færøyene har det også nylig blitt introdusert. Det virker som om det i alle tre land er skjedd en ny introduksjon, siden screening av gamle prøver har vært negative.
- I Norge har CMS også økt som problem; det er mer utbredt enn før, og det rammer hardere.
- I Irland har det blitt gjennomført et studie som var veldig likt det som ble utført i AP1, og med samme resultater.
- I felt ses det ofte store forskjeller i CMS-dødelighet mellom fiskegrupper fra ulike settefisk leverandører, i samme anlegg. Og det kan virke som om det er de samme leverandører som har problemet år etter år.
- Sekvensering av isolater fra flere fisk fra samme utbrudd av CMS har vist at det finnes mange quasi-species av PMCV under en infeksjon. Men den samme «grunn-sekvensen» finnes alltid i alle utbrudd. Så spørsmålet er om det bare er denne som kan infisere, og det så skjer mutasjoner innad i utbrudd etterpå.
- I tillegg ble laget en liste over kunnskapshull, som ses i vedlegg 8.

Diskusjon av funn i AP5

CMS er et økende problem. Både i Norge, der det rammer tidligere, oftere og hardere enn før, men også i de andre lakseproduserende land rundt Atlanterhavet. I Fiskehelserapporten for 2018, fremheves CMS som den viktigste infeksiøse sykdommen i norsk lakseoppdrett etter lakselus. Det er derfor enda mer aktuelt med løsninger på begrensing og kontroll av sykdommen.

Gjennom prosjektet er det funnet at flere ting peker på at det kan være en sammenheng mellom utbrudd av CMS og hva som skjer i settefiskfasen. Fra felt har det blitt rapportert, at det ses store forskjeller i hvilke fiskegrupper som blir berørt under et CMS-utbrudd på samme lokalitet, og at det ofte er fisk fra de samme settefiskleverandørene som rammes år etter år. Dette ble bekreftet ved analyse av produksjonsdata i AP3, der det tydelig sås en sammenheng mellom settefiskleverandør og risikoen for utbrudd av CMS, også på tvers av lokaliteter.

En av flere mulige årsaker til dette kan være, at smolten har med seg PMCV fra settefiskfasen. I studiet utført i AP1 fant vi indikasjoner på at PMCV kan overføres fra stamfisk til yngel, under gjeldende produksjonsforhold. Det samme har blitt rapportert fra Irland. Denne overførselen kan enten skje ved at virus er assosiert til egget og ikke fjernes under desinfeksjon, eller ved re-kontaminasjon etter desinfeksjon. Vi fant, at desinfeksjon fjernet det meste virus, men ikke alt, og dette aspektet bør undersøkes nærmere. I studien vår fant vi PMCV ved PCR-analyser, men det vites ikke om det som ble funnet var infektivt virus eller rester av virus genom. For å konkludere med at denne smittevei har noe å si for spredningen av CMS, bør det gjennomføres kontrollerte laboratorieforsøk med PMCV-positive egg og/eller yngel. I påvente av mer kunnskap om mulig overførsel vertikalt, bør en søke å få bedre kontroll med smittestatus i settefiskanlegg, samt jobbe med seleksjon av stamfisk med ingen eller en lav mengde PMCV.

Fra feltstudiet i AP2 fant vi PMCV i alle fiskegrupper på alle lokaliteter langs hele kysten mellom 0 og 7 måneder etter sjøsetting. Dette tilsier enten at smolten allerede var infisert når den ble satt i sjø, eller at smittepresset av PMCV er langt større enn vi tidligere har antatt. Bare halvparten av de 12 lokalitetene som ble fulgt utviklet klinisk CMS. Det er derfor ikke enkelt å bruke screening for PMCV ved PCR som et verktøy til å forutsi om et utbrudd er på gang. En bør i tillegg bruke histopatologi og kliniske symptomer, men på det tidspunkt da det ses klinisk CMS er det trolig for sent å gjøre noe for å hindre et utbrudd. PMCV ble detektert helt frem til slakt, noe som tyder på at fisken ikke kvitter seg med infeksjonen, selv når det kliniske utbruddet er over. Dette samsvarer med rapporter om høy forekomst av PMCV i stamfisk, noe som også ble observert i studien i AP1. Dette er et aspekt som en bør tenke over i forbindelse med smittevern; at fisk som tilsynelatende er klinisk frisk godt kan ha høye mengder av PMCV, og dermed kan bidra til å spre smitte ved flytting i forbindelse med for eksempel slakt.

I studien fant vi at tiden fra infeksjon til utvikling av klinisk CMS varierte fra 3 til 13 måneder. Det kan altså gå ganske lang tid fra fisken smittes med PMCV til den blir syk av CMS. Vi vet fortsatt lite om hva som forårsaker utviklingen av klinisk CMS. Det kan være noe med hvordan virus infiserer og påvirker cellene i spesielt hjertet, og dette bør undersøkes mer. Det kan også være utefra kommende risikofaktorer som trigger et i forveien infisert hjerte. I AP3 fant vi at fiskegrupper som hadde hatt PD eller HSMB hadde større risiko for senere å utvikle CMS. Vi fant også at høstutsatte fisk hadde større risiko enn vårutsatte, uansett region. Det kan finnes andre risikofaktorer, og disse bør undersøkes. For eksempel er det mulig at påkjenningen ved mekanisk avlusning øker risikoen for å få CMS i dagene etter, noe som trolig kan undersøkes ved analyse av mer oppdaterte produksjonsdata.

Leveranser fra AP5

- Program, deltakerliste og referat fra workshop om vertikal overføring av virus i lakseoppdrett. Vedlegg 7
- Synopsis fra «CMS-workshop». Vedlegg 8.
- Faktaark med muligheter for kontroll av CMS. Vedlegg 9.
- Muntlige presentasjoner er gitt på følgende konferanser/møter:
    - -Annual meeting for the European Reference Laboratories for fish diseases, Lyngby, Mai 2018
    - -Keynote på Frisk Fisk, Tromsø, Februar 2019.

5. Hovedfunn

- PMCV er mer utbredt enn tidligere antatt, og i vår studie påviste vi PMCV første gang mellom 0-7 måneder etter sjøsetting. Produsenter bør dermed forvente at laks i matfiskanlegg har stor sannsynlighet for å bli infisert i løpet av en produksjon, og denne sannsynligheten øker jo lengre fisken står i sjø.
- Ikke alle fiskegrupper som er smittet med virus utvikler CMS, og i vår studie gikk det mellom 3-13 måneder fra påvisning av virus RNA (ved PCR) til klinisk utbrudd hvis det ble utbrudd i fiskegruppen. Selv tidlig PCR-påvisning av PMCV er altså ikke ensbetydende med at fiskegruppen vil gjennomgå et klinisk CMS-utbrudd i løpet av produksjonsperioden.
- Resultatene fra dette prosjektet viser at det ikke kan utelukkes at PMCV kan overføres fra stamfisk til egg og yngel under vanlige produksjonsforhold. Hvordan dette skjer og betydningen av denne smitteveien er foreløpig uvisst.
- Smolt fra enkelte settefiskleverandører har større risiko for å utvikle CMS enn andre, både innad i anlegg og på tvers av anlegg.
- Fiskegrupper som har hatt PD eller HSMB har større risiko for å utvikle CMS enn de som ikke har. Høstutsett er mer utsatt for å utvikle CMS enn vårutsett.
- Fiskegrupper som har blitt smittet med PMCV, forblir smittet helt frem til slakt. Dette bør tas i betraktning ved flytting av ellers klinisk frisk fisk.

6. Leveranser

30.12.2015: Referat fra oppstartsmøte, inkl. endelig prosjektplan
30.06.2016: Statusrapport til FHF samt referat fra møte med styringsgruppe
31.10.2016: Presentasjon på TriNation møte
31.12.2016: Manus kunnskapssammenstilling og faktaark
31.12.2016: Statusrapport til FHF
31.03.2017: Program, deltakerliste og referat fra workshop om vertikal overføring av PMCV
30.06.2017: Presentasjon fra region vise møter med næringsaktørene om beste praksis angående vertikal overføring av PMCV –Erstattet av power point presentasjon.
30.06.2017: Statusrapport til FHF samt referat fra møte med styringsgruppe
30.06.2017: Presentasjon på EAFP konferansen
31.12.2017: Manus populærvitenskapelig artikkel AP2
31.12.2017: Manus vitenskapelig artikkel AP1
31.12.2017: Statusrapport til FHF
30.06.2018: Statusrapport til FHF samt referat fra møte med styringsgruppe
31.12.2018: Faktaark: «Veiledning om muligheter for kontroll av CMS» (AP5)
31.12.2018: Manus vitenskapelig artikkel AP3
31.12.2018: Presentasjon fra region vise møter med næringsaktørene om beste praksis for kontroll av CMS
31.12.2018: Sluttrapport til FHF samt program deltakerliste og referat fra workshop om muligheter for kontroll av CMS.

7. Referanser

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6. Svendsen JC, Nylund S, Kristoffersen AB, Takle H, Fossberg Buhaug J, Bang Jensen B. (2019) Monitoring infection with Piscinemyocarditis virus and development of cardiomyopathy syndrome in farmed Atlantic salmon (Salmo salar L.) in Norway. Journal of Fish Diseases, vol 42, p 511–518

8. Vedlegg

Vedlegg 1. AP1: Populærvitenskapelig fremstilling av resultatene av studien om vertikal overføring av PMCV. Publisert i Norsk Fiskeoppdrett desember 2017
Vedlegg 2. AP1: Vitenskapelig artikkel "Indications for a vertical transmission pathway of Piscine Myocarditis virus (PMCV) in Atlantic salmon (Salmo salar)". Publisert i Journal of Fish Diseases.
Vedlegg 3. AP2: Populærvitenskapelig fremstilling av resultatene av studien om overvåking av PMCV. Publisert i Norsk Fiskeoppdrett januar 2018
Vedlegg 4. AP2: Vitenskapelig artikkel "Monitoring infection with Piscine myocarditis virus and development of cardiomyopathy syndrome in farmed Atlantic salmon (Salmo salar L.) in Norway". Publisert i Journal of Fish Diseases Februar 2019.
Vedlegg 5. AP4: Faktaark om CMS «Kardiomyopatisyndrom (CMS) hos laks. Sykdomsutvikling – Agens – Epidemiologi».
Vedlegg 6. AP4: Vitenskapelig artikkel "Cardiomyopathy syndrome in Atlantic salmon Salmo salar L.: A review of the current state of knowledge". Journal of Fish Diseases, 41-1, pp. 11-26. Publisert oktober 2017.
Vedlegg 7. AP5: Program, deltakerliste og referat fra workshop om vertikal overføring av virus i lakseoppdrett. Sendt til styregruppe mars 2017
Vedlegg 8. AP5: Synopsis fra «CMS-workshop». Sendt styregruppe mai 2018.
Vedlegg 9. AP5: Faktaark med muligheter for kontroll av CMS. Sendt til styregruppe mars 019

Vedlegg 1

KUNNSKAP OM FISKEHELSE
I denne spalten vil Veterinærinstituttet i hvert nummer bidra med oppdatert kunnskap om fiskehelse. Ansvarlig for spalten er fiskehelseansvarlig Anne-Gerd Gjevre
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Av plasshensyn har vi valgt å utelate kildehenvisninger. Ta kontakt med spalteansvarlig dersom du ønsker opplysninger om dette.

Veterinærinstituttet
Norwegian Veterinary Institute

Virusinfeksjon hos stamfisk

kan gi hjerte-smerte i smolten

Julen er hjertenes fest! Derfor setter vi i denne utgaven fokus på en av lakseoppdrettets viktigste hjertesykdommer: Kardiomyopatisyndrom eller «Hjertesprekk». Gjennom et omfattende prosjekt prøver vi å finne ut mer om hvordan denne sykdommen sprer seg, og hva en kan gjøre for å kontrollere den. Vi har blant annet funnet tegn på at viruset som gir CMS kan overføres fra stamfisk til smolt gjennom settefiskfasen.

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CMS er et alvorlig problem i mange oppdrettsanlegg der man produserer laks. I fjor ble 90 tilfeller av CMS diagnostisert ved Veterinærinstituttet, og 108 tilfeller ved private laboratorier, men det er trolig en del overlapp mellom disse påvisningene. CMS er ikke meldepliktig, så det er vanskelig å få et presist overblikk over utbredelsen. Sykdommen er bare kjent hos laks, og fisken må først bli smittet med Piscint myokarditt virus (PMCV) for å få CMS. Det begynner å bli mer og mer tydelig at infeksjon med PMCV alene ikke er nok til å gi sykdom. Dette understøttes av at PMCV kan finnes i både smolt, matfisk og stamfisk, uten at fisken viser tegn på sykdom. Fisk med CMS kan få blødningsforstyrrelser og i alvorlige tilfeller kan hjertesekken sprekke og medføre rask død. Det typiske CMS-tilfellet har tidligere vært plutselig død blant slaktemoden fisk i god kondisjon. I de senere år har det vært flere rapporter om CMS kort tid etter sjøsetting, og det har vist seg at PMCV er utbredt blant stamfisken. Dette har naturligvis fått oppdrettere og forskere til å spekulere på om PMCV faktisk kan overføres fra stamfisken via egg til smolten, der det ultimate utfallet kan bli klinisk utbrudd av CMS hos matfisken.

CMS i stamfisk

Laboratorier som utfører screening av stamfisk for PMCV melder om høye forekomster av virus; så mye som 70 % av den undersøkte stamfisken kan være infisert. I vår undersøkelse, fant vi PMCV i hjertet hos 98 % av stamfisken vi testet. Vi testet da 65 stamfisk fra hvert av to forskjellige anlegg. Denne fisken fremsto som klinisk frisk og i god kondisjon, og det var derfor noe overraskende at noen av

CMS-Epi

«Epidemiologisk studie av Kardiomyopatisyndrom (CMS): Spredning, risikofaktorer og sykdomsforløp i norsk lakseoppdrett» (CMS-Epi) er et tre-årig forskningsprosjekt ledet av Veterinærinstituttet. Prosjektet er finansiert av FHF og private aktører som også deltar som samarbeidspartnere i prosjektet. Prosjektets overordnede mål er å øke kunnskapen om spredning av PMCV og faktorer som påvirker utviklingen av klinisk CMS.

Prosjektet har som mål å gi en samlet vurdering av tilgjengelige muligheter for å begrense spredning av PMCV og kliniske utbrudd av CMS – både for enkeltanlegg og for næringen som helhet.

Du kan lese mer om CMS-Epi og de foreløpige resultatene her:

http://www.fhf.no/prosjektdetaljer/?projectNumber=901118

PMCV

Piscint myokarditvirus (PMCV) er et nakent RNA-virus, som er nært beslektet med medlemmer i *Totiviridae* – familien. Viruset ble først oppdaget og knyttet til CMS hos laks i 2010, og laks er det mest viktige reservoaret en kjenner til så langt. Horisontal smitte er påvist, og det er også indikasjoner på en mulig vertikal smittevei. Resultater fra en pågående epidemiologisk studie peker mot at tilstedeværelsen av virus på norske matfiskanlegg er svært utbredt.

fiskene hadde virusnivå i hjertet som man vanligvis assosierer med klinisk CMS. Fra den samme fisken testet vi også melke og rognvæske, for å se om viruset er tilstede i kjønnsproduktene. Her fant vi PMCV i melkeprøver fra 59 % av hannfisken, og i prøver av rognvæske fra 69 % av hunnfisken. Men der hvor virusmengden i hjertene var høy, var virusmengden i kjønnsproduktene såpass lav at det var på grensen av hva som er mulig å detektere med den PCR-metoden som brukes i dag. Disse funnene antyder to ting: at forekomsten av PMCV i stamfisk er meget høy, og at virus som finnes i rogn og melke er tilstede i så små mengder at det ikke nødvendigvis oppdages ved screening av kjønnsprodukter under stryking.

PMCV i egg og yngel

I denne undersøkelsen plukket vi ut familiegrupper som vi ønsket å følge frem mot sjøsetting. Basert på resultatene fra prøvene av stamfisken, valgte vi ut familier der enten hann- eller hunnfisken eller begge to hadde hatt PMCV. Befruktet egg fra hver gruppe ble singelinkubert, og deretter prøvetatt på ulike tidspunkt og testet for PMCV. På øyerognstadiet, ble det testet mellom 30 og 60 rognkorn fra hver familiegruppe. På dette stadiet fant vi PMCV i 13 av 13 familiegrupper på det ene anlegget, og i 6 av 14 grupper på det andre. Prevalensen varierte fra 1,5 til 23 %, og i alle prøvene var mengden av virus på deteksjonsgrensen for metoden.

I det ene anlegget ble det tatt ut prøver fra fire av familiegruppene da de var på plommesekkstadiet, og igjen da yngelen var omkring 5 gram. PCMV ble funnet på begge prøvetidspunkt, i alle gruppene på plommesekkstadiet og i tre av fire grupper på yngelstadiet. Forekomsten av virus i gruppene varierte fra 1 til 23 %, og det var ingen sammenheng mellom virusforekomst og prøvetidspunkt. Det hadde heller ikke noe å si for viruspåvisning i avkommet om en eller begge foreldre var positive for PMCV.

I det andre anlegget ble det tatt ut prøver fra tre familiegrupper på fire forskjellige

tidspunkt; yngel rett før startfôring, yngel ved 15g og parr ved 40g og 70g. I dette anlegget fant vi PMCV i to av fire grupper frem til 40g, med påvisning av virus i mellom 1,6 og 11% av avkommet. I den siste prøvetakingen fant vi ingen PMCV-positive yngel. Men fra en av gruppene tok vi ut et ekstra prøveuttak for å undersøke hvilke vev virus fordeler seg i, og her fant vi PMCV i seks av ni prøvetatte fisk.

Disse funnene antyder at PMCV kan følge med fra stamfisken til yngelen via egg, men at forekomsten i avkommet er så lav og virusmengden i hvert individ så liten at det er stor sannsynlighet for at det ikke oppdages om det er der.

Fra virus i egg til CMS i matfisk

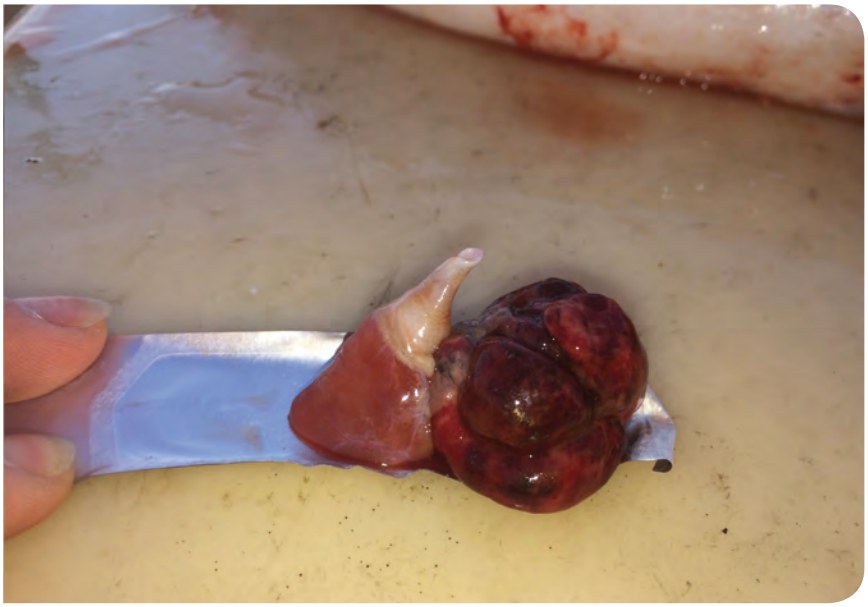
Vi er ikke de eneste som har funnet PMCV i egg og yngel som stammer fra PMCV-infisert stamfisk. Lignende studier er utført i Irland med resultater som støtter opp under våre. Det er også flere rapporter om funn av PMCV i settefisk, så vi vet det kan finnes der. Vi har også selv testet settefisk fra ferskvannsfasen og etter sjøsetting, og funnet PMCV i samme gruppe fisk både før og etter sjøsetting. Dessverre er det ingen som har fulgt en fiskegruppe helt fra egg via smolt til slaktet matfisk, og påvist PMCV hele veien. Derfor er vi fortsatt litt usikre på betydningen av PMCV i stamfisken for forekomst av CMS i matfisk. Vi tenker det vil være lurt å anta en føre-var tilnærming, der en gjør hva en kan for å bryte en eventuell smittevei fra stamfisken til smolten. Siden såpass mange av stamfiskene har virus, er det ikke enkelt å basere seg på en virusfri stamfisk-stamme. I prosjektet vårt gjorde vi derfor et lite forsøk, som visete at nøye desinfeksjon av egg kan minimere smitten. På det ene av de to anleggene som var med prosjektet, ble det tatt ut egg til PCR-undersøkelse både før og etter desinfeksjon, Her ble det tatt ut 30-40 egg fra hver av 12 familier før desinfeksjon og 15-30 egg etter desinfeksjon. Resultatene tilsier at det er en god effekt av desinfeksjon: I alle 12 familier ble det funnet PMCV i mellom 6

og 44 % av eggene før desinfeksjon, men etter desinfeksjon ble det bare funnet virus i 5 av de samme 12 familiene, og da bare i 4-7% av eggene.

Hvordan unngå hjerte-smerte?

Det er fortsatt et stykke å gå før vi kan si noe konkret om hvordan vi kan unngå hjertesprekk. De resultatene som er presentert her har en del usikkerheter, og genererer også nye spørsmål. Et sentralt spørsmål er om viruset vi finner i yngelen er infektivt og kan gi sykdom, eller om det er rester av virusgener som har blitt båret med gjennom produksjonen. Det er også viktig å huske på at dette bare er èn mulig smittevei. Vi har ganske gode bevis på at virus overføres fra fisk til fisk gjennom vann, både under eksperimentelle forhold og i felt. I neste utgave av Norsk Fiskeoppdrett skal vi fortelle om en undersøkelse vi har gjennomført for å finne ut når matfisk blir smittet med PMCV i sjøen.

Men uansett hvor viruset kommer fra, vil en trolig oppnå en fordel av å bryte èn smittevei, slik at det generelle smittepresset blir lavere. Dette kan igjen føre til lavere forekomst i stamfisk, og så er vi på tur inn i en god sirkel. Og da kan faktisk julen bli hjertenes fest også for oppdrettslaksen!



Bildet viser hjerte med svært forstørret forkammer fra CMS-syk fisk. Foto: Julie Svendsen, Veterinærinstituttet

Vedlegg 2

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ORIGINAL ARTICLE

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Indications for a vertical transmission pathway of piscine myocarditis virus in Atlantic salmon (*Salmo salar* L.)

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Abstract

Losses due to cardiomyopathy syndrome (CMS) keep increasing in salmon-producing countries in the North-Atlantic. Recently, Piscine myocarditis virus (PMCV) has been detected in post-smolts shortly after sea-transfer, indicating a possible carry-over from the hatcheries. In addition, there are reports of prevalences of PMCV as high as 70%–90% in certain groups of broodfish, and a recent outbreak of CMS in the Faroe Islands has been linked to the importation of eggs from a CMS-endemic area. Thus, there is a need to investigate whether PMCV can be transmitted vertically from infected broodstock to their progeny. In the present study, samples from eggs, larvae, fingerlings and presmolt originating from PMCV-positive broodstock from two commercial Atlantic salmon producers were tested for PMCV. The prevalence of PMCV in the broodstock was 98% in the hearts, 69% in the roe and 59% in the milt. Piscine myocarditis virus was detected in all stages of the progeny until and including the 40 g stage. Piscine myocarditis virus was also detected in presmolt sampled for tissue tropism. This provides farmers with several options for minimizing the risk of transfer of PMCV from broodstock to progeny, including screening of broodstock and aiming to use only those that are negative for PMCV or have low levels of virus.

KEYWORDS
cardiomyopathy syndrome, epidemiology, piscine myocarditis virus, vertical transmission

1 | INTRODUCTION

Piscine myocarditis virus (PMCV) was identified as the causative agent for cardiomyopathy syndrome (CMS) in 2010 (Haugland et al., 2011; Løvoll et al., 2010). Cardiomyopathy syndrome is an economically important disease that affects Atlantic salmon (*Salmo salar L.*; AS) in marine aquaculture. It was first reported in Norway in 1988 and has been reported from Norway, Scotland, Ireland and the Faroe Islands (Garseth, Fritsvold, Svendsen, Bang Jensen & Mikalsen, 2018).

The disease mainly affects the spongious myocardium, with lesions usually appearing first in the atrium and progressing to the ventricle, and can lead to heart failure and even rupture of the atrial wall (Garseth et al., 2018). Clinical outbreaks of CMS with associated mortalities have primarily been reported from fish in their second year at sea, and the median time reported from sea-transfer to initial diagnosis of CMS in Norwegian aquaculture is 16 months (Bang Jensen, Brun, Fineid, Larssen & Kristoffersen, 2013). Outbreaks of CMS have, however, been reported in post-smolt at 2–300 g size, and recent studies have shown that PMCV can be found within

the first month after sea-transfer, at levels below commonly used cut-off diagnostic values (Hjeltnes et al., 2016; Svendsen, Nylund, Kristoffersen, Takle, Fossberg Buhaug & Bang Jensen, 2019)

To date, clinical outbreaks of CMS have not been reported in fry, smolt or broodfish in the freshwater phase. Piscine myocarditis virus has, however, been detected in broodfish after transfer from sea to freshwater. In a study by Wiik-Nielsen, Ski, Aunsmo, & Løvoll (2012), PMCV was detected in 16 out of 20 tested broodfish before stripping, and Pharmaq Analytiq reports that certain broodfish groups submitted for analysis can have a prevalence as high as 70%–90% (S. Nylund, *personal observation*). Low levels of viral RNA from PMCV have also been detected in fry (Wiik-Nielsen et al., 2012).

There is consensus that the primary route of transmission of PMCV is horizontal, and several studies have shown that PMCV and CMS are transmitted between fish in tanks and between farms in the field (Bang Jensen et al., 2013; Fritsvold et al., 2009; Haugland et al., 2011). However, a recent outbreak of CMS in the Faroe Islands has been linked to eggs imported from Norway (Garseth et al., 2018), and in light of the high prevalence of PMCV among broodfish, and the findings of PMCV early after sea-transfer, a discussion on whether PMCV can be transmitted from broodstock to progeny has been rekindled. Previously, one small-scale study exploring this possibility was performed, in which PMCV was detected at low levels from fertilized eggs and fry, but not in hatchlings after first feeding (Wiik-Nielsen et al., 2012). Thus, the results were not sufficient to form a conclusion on whether vertical transmission of PMCV can or does occur. The consequences of a vertical transmission pathway of PMCV for trade and control could be large, and thus, it is important to ascertain whether this is possible or not.

The aim of the present study was to ascertain whether PMCV can be transmitted from broodstock to their progeny, under normal production systems by sampling from commercial producers in the field.

2 | MATERIALS AND METHODS

2.1 | Fish cohorts

Fish cohorts from two commercial AS producers in Norway were included in this study. Cohort X includes broodfish and eggs and progeny from these fish. The broodstock for this cohort originated from a seafarm which had experienced some mortality due to CMS in the last half year before stripping. Similarly, cohort Y includes broodfish and eggs and progeny from these fish.

Samples were taken at different timepoints as depicted in Figure 1 and described below.

2.2 | Broodstock sampling

Broodstock from both cohorts were stripped in November 2015. At this time, heart (ventricle) and ovarian fluid were sampled from female broodstock. At the same time, milt was sampled from the males used for fertilization from cohort X, and both milt and heart from males from cohort Y. A minimum of 0.2 ml and maximum 1 ml of ovarian fluid or milt were sampled from each fish and stored individually in test tubes without any added media. These samples were refrigerated and kept cold during overnight delivery to the laboratory. All samples from broodfish were analysed by RT-PCR (see description below), and based on the results, broodfish and their corresponding progeny were sorted into groups A to G as presented in Table 1. In group A and D, both parents were positive for PMCV in both hearts and sexual products. In group B and F, the female was positive for PMCV in heart and ovarian fluid and the male negative for PMCV in milt (Group B) and only weak positive in heart (Group F). In group G, the female was negative in both samples and the male positive in both. In cohort X, there were no females that where negative for PMCV in the heart; therefore, the equivalent to group G consisted of a female which was negative for PMCV in the ovarian fluid and a male with PMCV-positive milt (group C). Finally, group E was similar to group C, except that the male was also positive for PMCV in the heart.

2.3 | Egg sampling and disinfection

After fertilization, each batch of eggs (a batch meaning the progeny of one male × one female) was kept separate. Eggs from cohort X were sampled before and after disinfection. These eggs were sent in batches together with the broodfish samples to the laboratory. At the laboratory, 50 eggs from each batch were frozen individually before testing. Eggs from both cohorts were disinfected with Buffodine according to manufacturer's protocol: 100 ml Buffodine

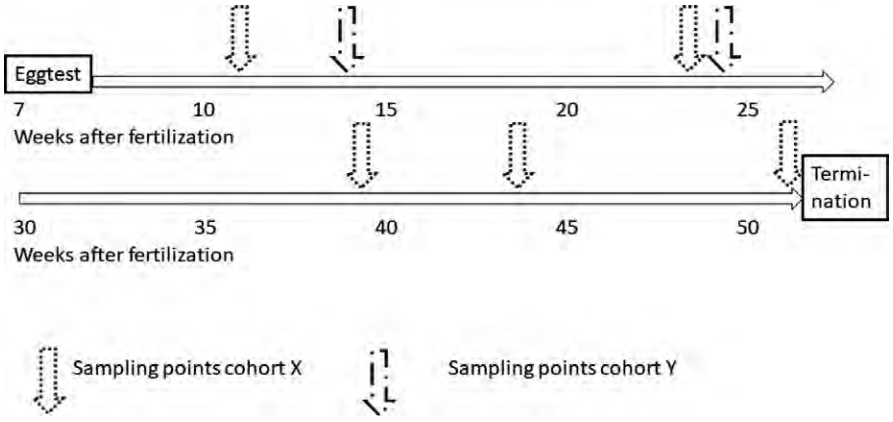

FIGURE 1 Sample points for the samples taken from cohort X and Y in the study

TABLE 1 Overview of the egg batches in each of cohort X and Y

	PMCV in female broodfish		PMCV in male broodfish		
Group	Heart	Ovarian fluid	Heart	Milt	Egg batches
Cohort X					
A	PMCV +	PMCV ++	Nd	PMCV +	A1, A2
B	PMCV +	PMCV +	Nd	PMCV –	B1, B2, B3, B4
C	PMCV +	PMCV –	Nd	PMCV +	C1, C2, C3, C4
Cohort Y					
D	PMCV +	PMCV +	PMCV +	PMCV (+)	D1, D2, D3, D4
E	PMCV +	PMCV –	PMCV +	PMCV +	E1, E2, E3,
F	PMCV +	PMCV +	PMCV (+)	PMCV–	F1, F2, F3
G	PMCV –	PMCV –	PMCV +	PMCV +	G1[a], G2, G3

[a]For this batch, the heart of the female was PMCV +, and the milt from the male was PMCV –

per 10 L of water for 10 min, followed by five times of rinsing in a dilution of 90 g salt in 10 L freshwater. The eggs were then incubated in single incubators containing one batch of eggs each. From each of the groups A to G as described above, two to four egg batches were selected for further sampling. An overview of the batches included is presented in Table 1.

From two egg batches from group A and four egg batches from each of group B and C, approximately 30 of the eggs that were sampled before disinfection and 30 of the eggs sampled after disinfection were tested with RT-PCR for PMCV. Each egg was processed and tested individually.

2.4 | Sampling of progeny

The following sampling and testing were performed from cohort X: From group A, B and C, the egg batches with the highest prevalence of PMCV after disinfection were included in the subsequent study. After approximately 330degree days (in January 2016), the eggs were shocked. At the same time, approximately 120 eggs from each of the three egg batches were sampled and analysed individually. The eggs hatched in February 2016, and samples were taken from each of the three groups four times from hatching until termination right before sea-transfer, as illustrated in Figure 1. At the last sample point, additionally nine fish from group B were sampled for a study on tissue tropism.

The following sampling and testing were performed from cohort Y: In January, when the eggs had become eyed eggs, 30 eggs were sampled and tested from each of four batches of group D and three batches of group E, and 60 eggs from three bathes from each of groups F and G (see Table 1).

In February, after approximately 110 degree days, 60 yolksac fry were sampled from the egg batch with highest prevalence of PMCV from the previous test from each of group D, E, F and G. All sampled fry were tested individually for PMCV by RT-PCR. After first feeding, the hatchlings were transferred to another hatchery, where all fry from each of the groups D to G were merged in each of four tanks.

A final sampling was performed in May, when the fry were approximately 6 g. At this time, 90 fry were sampled from each of the four groups. An overview of all sampling points is presented in Figure 1.

2.4.1 | Sampling

All samples were kept on RNA-later until analysis, except milt and roe which were not kept on RNA-later. From broodstock, pieces of hearts (ventricles) were sampled together with roe and milt during stripping. Eggs were placed in individual containers and tested separately. Yolksac fry were placed whole in containers, whereas the head region (including gills, heart and kidney) was sampled from larger hatchlings/fry. At the last sampling point for cohort X, six tissues were sampled from each of nine fish, in addition to the regular sampling. From these fish, heart, liver, spleen, kidney, brain and gill were sampled separately, in order to study tissue tropism.

2.4.2 | Real-time RT-PCR

Tissue samples were processed using a Qiagen's Universal Biorobot, with the compatible RNA purification kit (RNeasy 96 Universal Tissue Kit), according to the manufacturers' recommendation. Extracted total RNA was eluted in a final volume of 100 µl of the supplied kit elution buffer.

Extracted RNA from tissues of salmon was tested by Taqman real-time RT-PCR (qScript XLT 1- Step RT-qPCR ToughMix, Quantabio). During the real-time RT-PCR screening a house-keeping gene, elongation factor 1 alpha (EF1A) was used as an internal control (Olsvik, Lie, Jordal, Nilsen, & Hordvik, 2005), and a specific assay was used for detection of PMCV (Nylund et al., 2018). The primer and probe concentrations had been optimized and found to be 900 nM for all primers used, and 225 nM for the corresponding probes. The samples were run in simplex for the internal control, and triplicates for detection of PMCV in standard 384-well plates. All assays were run in a total volume of 10 µl in each well, with 2.5 µl of isolated total RNA as the template. Plates were analysed in an Applied Biosystems

TABLE 2 Results from PCR-tests of samples from broodstock of Atlantic salmon (*Salmo salar*) from cohort X and Y for piscine myocarditis virus (PMCV)

	No. of samples	No. of positive samples	Ct-value range (median)
Cohort X			
Females			
Heart	65	65	14.2–34.4 (25.7)
Roe	67	43	34.6–40.55 (36.9)
Males			
Milt	46	28	35.4–37.6 (36.6)
Cohort Y			
Females			
Heart	60	58	15.4–36.5 (27.0)
Roe	60	45	33.0–39.1 (36.3)
Males			
Heart	5	5	25.6–34.8 (31.05)
Milt	5	2	36.7–36.9 (na)

7900 HT real-time machine under standard conditions. Each run consisted of 45 cycles, and the samples were considered positive when the fluorescence signal increased above a set threshold of 0.09. The PMCV assay has a repeatable cycle threshold value (Ct) of 34.7 (Svendsen et al., 2019), and average Ct-values were calculated based on the results from all values found in the triplicates for each sample. Criteria for determination of this average Ct-value are normally based on the requirement that a minimum of two out of three in a triplicate are below the repeatable cycle threshold value. For the purpose of this study, an average of all Ct-values was included in the calculation, regardless of Ct-value.

2.5 | Statistical analysis

For each batch of samples, the prevalence of PMCV with confidence intervals was calculated using the binom.test in R (R Core Team, 2018).

3 | RESULTS

3.1 | Broodfish

The results of the PCR-tests of broodstock are shown in Table 2. The prevalence of PMCV in heart tissue was 98.4%, with only two samples testing negative. In roe and milt, the prevalence was 69.3% and 58.8%, respectively. The viral levels in heart tissue were generally medium to low (median Ct-values of 25.7–31.5), whereas they were low in roe and milt (Ct-value medians of 36.3–36.9).

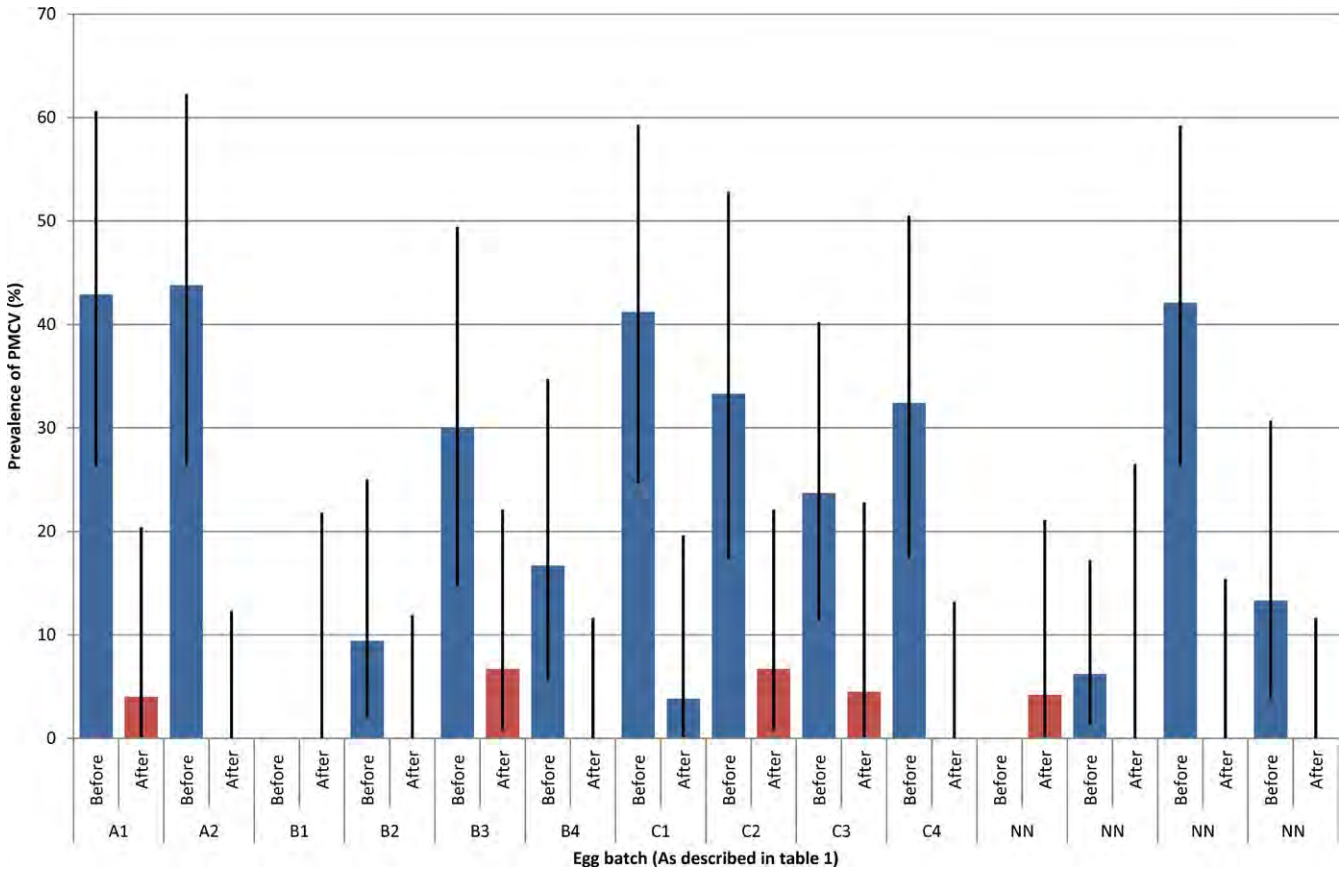

FIGURE 2 Prevalence of Piscine myocarditis virus (PMCV) in egg batches tested before (blue) and after (red) disinfection. Black lines indicate 95% confidence intervals based on sample size

TABLE 3 Results from testing of egg batches from cohort X at different timepoints. CI = 95% Confidence interval based on sample size

Cohort X	Shocked eggs			Larvae			Fry (15 g)			Parr(40 g)			Presmolt (70 g)		
Egg batch	No of samples	Prevalence (%)	CI	No of samples	Prevalence (%)	CI	No of samples	Prevalence (%)	CI	No of samples	Prevalence (%)	CI	No of samples	Prevalence (%)	CI
A1	120	10.8	5.9–17.8	68	0	0–5.3	63	1.6	0–8.5	55	1.8	0–9.7	60	0	0–6.0
B3	120	5.8	2.4–11.6	62	1.6	0–8.7	67	0	0–5.4	54	0	0–6.6	60	0	0–6.0
C2	120	4.2	1.4–9.5	56	1.8	0–9.6	60	1.7	0–8.9	68	5.9	1.6–14.4	60	0	0–6.0

3.2 | Disinfection

PMCV was detected in all egg batches from cohort X before disinfection, with an overall prevalence of 27.5%. Prevalence in individual batches ranged from 6.2% to 43.8% (Figure 2). Ct-values from these samples were between 36.0 and 42.0. After disinfection, PMCV was detected in six of the 14 batches tested, with prevalences from 4%–6.7%. Ct-values ranged from 37.0 to 41.3 in these samples.

3.3 | Progeny

The results from testing of cohort X for PMCV at different time points are presented in Table 3 and Figure 3. At the first sample point, the prevalence in each of the egg batches tested varied from 4.2% to 10.8%, with confidence intervals from 1.4% to 17.8%. At the second and third sample point (larvae and fry stage), the prevalence was between zero and 1.8%, with confidence intervals from 0% to 9.6%. At the 40 g stage, the prevalence varied from zero to 5.9% (CI 0%–14.4%). At 70 g, we were not able to detect any PMCV in any of the groups from cohort X. The Ct-values of all positive samples were between 35.4 and 37.8, except from one sample from 15 g fry which had a Ct-value of 33.9. In 6 of the 9 fish that were sampled for tissue tropism at 70 g, PMCV was detected in either heart, liver or spleen with Ct-values between 34.6 and 36.9. The other five organs tested in each fish were negative, as was the remaining three fish.

The prevalence of PMCV in the eyed eggs from the 13 egg batches tested from cohort Y varied from 1.7% to 23.3%, with confidence intervals from 0 to 42.3 (Table 4). In the four batches tested at the yolksac fry stage, the prevalence was between 3.3% and 11.7% (CI from 0.4 to 22.6). At the last sampling point at 6 g, the prevalence varied from 0% to 23.3%, with confidence intervals from 0 to 33.4. From Figure 3, it can be seen that the prevalence was low in group F2 at all sample times, and this group was the only one found negative at the last sample time. The Ct-values of the positive tests were within the range of 35.8–39.3, except for one sample with Ct of 31.4 in group E at the last sample point, and 4 samples with Ct-values from 27.8 to 34.1 in group F at the same sample point.

In Figure 3, results from the seven batches from both cohort X and Y that were sampled over time are shown. The two batches with persistently low prevalence of PMCV both originate from eggs where the milt was negative and the roe positive for PMCV. In all other batches, the milt was positive for PMCV, and from the figure it does not seem as if there was a difference between those batches were the roe was also positive for PMCV and those in which it was not.

4 | DISCUSSION

In this study, we have found PMCV in both hearts and sexual products of broodfish, and in their progeny at several stages of development. This indicates a possible pathway for transmission from broodstock to smolt.

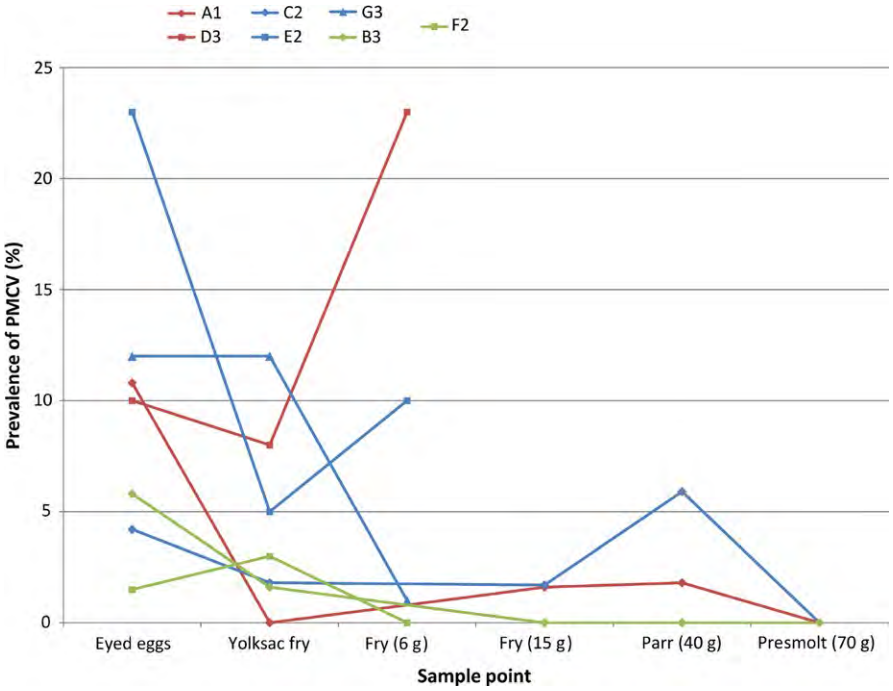

FIGURE 3 Prevalence of Piscine myocarditis virus (PMCV) in seven egg batches from cohort X and Y, sampled at different timepoints (see also Tables 3 and 4). Red lines indicate batches where both milt and roe from the parents were PMCV positive, blue lines indicate batches where milt was positive and roe negative, and green lines indicate batches where milt was negative and roe positive (see also Table 1)

The prevalence of PMCV in the broodfish was very high, with positive real-time RT-PCR results from 128 hearts of the 130 tested. There were no clinical signs of CMS at the time of sampling, and the Ct-values in the hearts were also higher than what is normally seen at clinical outbreaks of CMS. A recent study has shown that fish groups that have gone through a clinical outbreak of CMS remain positive for PCR-testing until the time of slaughter (Svendsen et al., 2019). The broodfish that were used in the present study had gone through a clinical outbreak earlier, so the findings most likely indicate a carrier-state. The prevalence of PMCV in the tested roe (69.3%) and milt (58.8%) was high, but the Ct-values in the positive samples were all just below or just above the diagnostic cut-off value of 34.7. This diagnostic cut-off value is calculated by determining the lowest amount of template in a sample that can be consistently detected and reproduced, using the same reaction conditions (Sloan, 2007). For the purpose of using real-time RT-PCR as a diagnostic tool for detection of viruses, the cut-off value that is normally employed is important to ensure that any positive detections of a virus is consistent when comparing both intra laboratory runs, and analyses performed on the same sample in different laboratories. This is also a hallmark of research, where scientists should be able to reproduce results performed by others to confirm the findings. The disadvantage of this strict approach is that relevant information regarding prevalence and presence of a virus in carrier status individuals can be lost. To compensate for this, studies that include values above the cut-off values should always include relevant controls to ensure that positive detection occurs as a result of amplification of the intended target and also include enough samples in the study to ensure that the overall trends in the results are shown to be consistent. In this study, to ensure that any positive samples appeared as a consequence of detection of PMCV gene sequences, and not as an artefact of the real-time RT-PCR reaction parameters, all runs were performed with negative RNA extraction controls and with the use of positive controls. The negative controls were subjected to the same extraction protocol and reaction parameters as the tissue samples, and were consistently negative in all analyses. This indicated that positive signals above the repeatable threshold for PMCV in tissue occurred as a result of amplification of viral sequence, and not as consequence of either unspecific amplification or degeneration of the fluorescent dye from the PMCV probe.

In addition, Ct-values above the cut-off, while not reproducible, were still within range of the limit of detection for the analysis. We were not able to confirm the presence of PMCV in weakly positive samples, probably due to limitations in sensitivity of the standard RT-PCR analyses that were employed. Although the overall real-time RT-PCR and PCR results put together cannot be considered conclusive, they do suggest that care should be taken when assessing negative test results from sexual products of broodfish.

In six of the 14 egg batches tested, PMCV could be detected by PCR after disinfection, albeit at a lower prevalence than before disinfection. The biophysical properties of PMCV are not known, and the authors are not aware of any studies on the effect of disinfection on the virus thus far, mainly because there is no suitable protocol for cultivating PMCV for example in cell culture (Garseth et al., 2018). Thus, no attempts were made to ascertain whether the viral material detected by PCR in the present study was infective or not. The disinfection routine that was used is the one most commonly used in Norwegian salmon farming. The reduction in prevalence could be merely due to the mechanical rinsing off of the biological material present on the outside of the eggs. Based on the findings, we suggest that more thorough studies should be carried out to ascertain

TABLE 4 Results from testing of egg batches from cohort Y at different time points. CI = 95% confidence interval based on sample size

Cohort Y	Eyed eggs			Yolksac fry			Fry (6 g)		
Egg batch	No of samples	Prevalence (%)	CI	No of samples	Prevalence (%)	CI	No of samples	Prevalence (%)	CI
D1	30	3.0	0.1–17.2		–				
D2	30	6.7	0.8–22.1		–		90	23.3	15.1–33.4
D3	30	10.0	2.1–26.5	60	5.0	1.0–13.9			
D4	30	20.0	7.7–38.6		–				
E1	30	20.0	7.7–38.6		–				
E2	30	23.3	9.9–42.3	60	8.3	2.8–18.4	90	10.0	4.7–18.1
E3	30	10.0	2.1–26.5		–				
F1	60	10.0	3.8–20.5		–				
F2	60	1.7	0–8.9	60	3.3	0.4–11.5	90	0	0–4.0
F3	60	10.0	3.8–20.5		–				
G1	60	10.0	3.8–20.5		–				
G2	60	10.0	3.8–20.5		–		90	1.1	0–6.0
G3	60	11.7	4.8–22.6	60	11.7	4.8–22.6			

what disinfection measures should be used to reduce the risk of vertical transmission of PMCV.

Piscine myocarditis virus was detected in all batches at the eyed egg and shocked egg stages, of both cohorts. After hatching, PMCV was detected in a majority of the batches up until and including the 40 g stage. At the final sampling point, in presmolt, PMCV could no longer be detected in any of the batches we were following. However, PMCV was detected in fish sampled for tissue tropism from another batch in cohort X. With the sample size of 60 that we used in this sampling point, the detection limit is 4.9% prevalence (95% confidence), thus indicating that the PMCV could have been present even in the study batches, but at a lower level than we were able to detect.

The reduction in prevalence of PMCV with time in cohort X might suggest that the virus is not actively replicating within the progeny, and thus that they are either not actually infected but the PMCV detected is only from the surface of the fish, or that PMCV does not replicate in any considerable amount in these juvenile life stages of Atlantic salmon. In cohort Y, the prevalence of PMCV was relatively high at the final sampling point in two of the groups sampled.

The provided data of detection of PMCV in eggs and fry do not give any evidence for a true infection with replicating virus. In this study, we did not sequence the virus or measure early immune response markers for viral infection and at the 60 g sample stage we could not detect virus in the fish. In the PMCV disease challenge study by Timmerhaus et al. (Timmerhaus et al., 2011), the authors detected activation in six gene sets associated with immune responses, 2 weeks after infection of 50 g fish. However, in this study the fish were infected with a high dose of virus in post-smolts and it may be that lower infection doses do not trigger an immune response before the virus level reach a trigger level. Although we did not find virus after 60 g in our study, we know from field reports that PMCV is detected in a limited number of smolt groups prior to sea-transfer in hatcheries using only fresh water. This suggests that the virus can be retained in the population throughout the entire hatchery phase. Nevertheless, there have been no reports of any CMS outbreaks in hatcheries, neither any CMS development in challenge tests conducted on fry nor presmolts. This may imply either that (a) the virus replicates only at a very low level in juvenile fish; (b) the fish lack the ability to promote an immune response against PMCV in juvenile stages; or (c) the virus detected is incomplete, and the PCR results only detect fragments of the virus.

Infection of live virus at high levels is known to trigger an immune response, which may give protection later in life. To our knowledge, there has been no field or experimental studies that have investigated the relation between presence of PMCV in juveniles and protection against CMS at later stages. Unpublished studies on field data on heart and skeletal muscle inflammation (HSMI) strongly suggest that even high presence of the associated piscine reovirus (PRV) in juveniles does not give any protection against HSMI after sea-transfer (H. Takle, *personal communication*). Therefore, we suggest that presence of PMCV at low titre likely will not give any natural immune protection against CMS. Smolts transferred to sea with PMCV virus

would rather have an increased likelihood of developing CMS later in life. Nevertheless, this topic needs to be studied explicitly to make any conclusions as it is known that CMS spreads horizontally (Bang Jensen et al., 2013; Fritsvold et al., 2009; Haugland et al., 2011).

In order to assess whether the transmission really did occur from the individual broodstock to their respective progeny through vertical transmission, sequences of PMCV from the broodstock and the fry could have been compared. However, in the present study, the samples were generally too small, so that there was not enough material left to perform sequencing after the RT-PCR was done. In addition, the studies performed so far on phylogeny of PMCV have shown little potential for molecular tracing (Wiik-Nielsen, Alarcón, Fineid, Rode & Haugland, 2013; Xu, Mikalsen, Munang'andu & Evensen, 2018). In planning of the study, we aimed to include a negative control, in order to make sure that any findings of PMCV in progeny were not simply due to cross-contamination from the broodstock or from viral residues in the environment or equipment. Unfortunately, no such negative control was available from the population of broodstock tested. A possible solution would be to repeat the study in a laboratory facility where it is possible to control for these things. As the egg batches were disinfected immediately after fertilization and then kept in single incubators, we believe we have minimized the risk for such cross-contaminations.

For this transmission route to be of any consequence in the production, it is imperative to determine how a potential vertical transmission of PMCV occurs. One way to investigate this could be to perform infection trials with homogenate from organs that have been aseptically extracted from the fish. Using homogenated, disinfected eggs could also help determine whether PMCV is transmitted on the outside or the inside of the eggs, and whether proper disinfection could reduce the risk of transmission. The one aquatic viral disease that has been studied most extensively when it comes to its potential for vertical transmission is *infectious pancreatic necrosis* (IPN). Since 1963, a large number of diverse studies have tried to ascertain how this virus is transmitted vertically, but there is still no consensus as to whether it occurs as an embryonal infection, through contaminated water entering the egg, or plainly by resistance to disinfection with iodophores (Munro & Midtlyng, 2011). For PMCV, any of these means of vertical transmission could be valid.

We tested eggs and progeny from different combinations of PMCV positive and negative males and females, in order to investigate whether any transmission occurred from the males or the females. In our study, we could not see any substantial difference between the batches originating from the different combinations. However, those groups from both cohorts, where no PMCV was found in milt, were the ones with the lowest prevalences after hatching. Whether this is just a coincidence, or actually an indication that transmission from the males is more important than from the females should be investigated. A lot less males than females are needed for production, so that it could be feasible to only use PMCV-negative males to reduce the risk of transmission.

In our study, we applied a "worst-case" approach, selecting the batches with highest prevalence in the previous testing for each following sampling point, since we wanted to assess whether it was at all possible to continue detecting PMCV. Therefore, the prevalence of PMCV in the regular hatcheries is probably lower than what we have demonstrated here.

The present study provides a valuable input into the ongoing investigation of whether PMCV can be transmitted vertically, in addition to the well-known horizontal transmission pathway. Further studies are needed in order to ascertain whether the PCR-products detected in progeny is viable and infective PMCV or just non-infective viral fragments. This could for example be done in a study using PCR-positive material from eggs or fry in a challenge trial.

A recent study performed within the same project has shown that PMCV might be much more widespread in the Norwegian population of farmed salmon than previously thought (Svendsen et al., 2019). In this study, PMCV was found in farms with no or little prior history of CMS. Some of these farms were situated in an area where the overall prevalence of CMS until very recently has been low (Hjeltnes et al., 2018). The virus was detected soon after transfer to the sea, thus indicating that either the infection pressure of PMCV in the marine environment is high, or that the smolt were already infected at the time of sea-transfer. Thus, we suggest that further studies should try and ascertain the prevalence of PMCV in hatcheries and especially in presmolt.

In the meantime, farmers have several options for minimizing the risk of transfer of PMCV from broodstock to progeny, including screening of broodstock and aiming to use only those that are negative for PMCV or have low levels of virus. Additionally, better routines for disinfection of milt and roe should be explored, and smolt could be screened for PMCV before transfer to the sea.

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CONFLICT OF INTEREST

Author Stian Nylund is affiliated with Pharmaq Analytiq which offers screening for salmon viruses included PMCV.

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KUNNSKAP OM FISKEHELSE
I denne spalten vil Veterinærinstituttet i hvert nummer bidra med oppdatert kunnskap om fiskehelse. Ansvarlig for spalten er fiskehelseansvarlig Anne-Gerd Gjevre
anne-gerd.gjevre@vetinst.no

Av plasshensyn har vi valgt å utelate kildehenvisninger. Ta kontakt med spalteansvarlig dersom du ønsker opplysninger om dette.

Veterinærinstituttet
Norwegian Veterinary Institute

PMCV – mer utbredt enn tidligere antatt?

I desembernummeret av Norsk fiskeoppdrett skrev vi at infeksjon med piscint myokarditt virus (PMCV) hos stamfisk kan gi hjerteproblemer for smolten. Viruset ble første gang påvist og knyttet til utvikling av «hjertesprekk» eller kardiomyopatisyndrom (CMS) hos laks i 2010. Sykdommen har vært sett hos oppdrettslaks siden 1980 tallet og har tidvis medført store tap. Nye funn tyder på at PMCV kan være mer utbredt i norsk lakseoppdrett enn tidligere antatt.

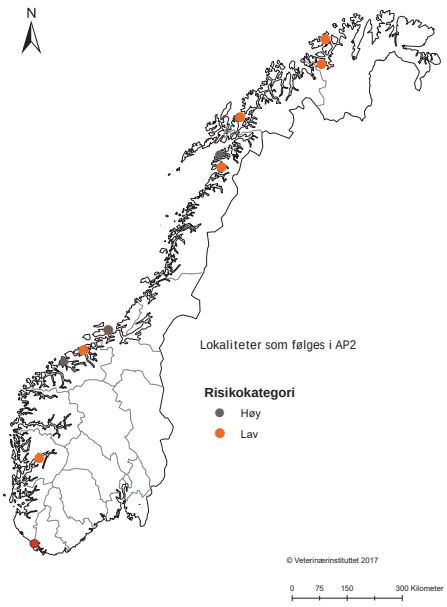
Britt Bang Jensen og Julie Christine Svendsen

Store tap, store kunnskapshull

CMS er et av de større sykdomsproblemene innenfor norsk lakseoppdrett. Sykdommen fører til redusert fiskevelferd og dødelighet for et høyt antall fisk hvert år, samt betydelige økonomiske tap for næringen. Selv om viruset som fører til sykdommen har blitt påvist, er det fremdeles mye en ikke vet om PMCV, smitteveier og risikofaktorer for sykdomsutbrudd. CMS er ikke en meldepliktig sykdom. Dette medfører at det er vanskelig å få sikre tall på hvor stor forekomsten av viruset egentlig er, og hvor mange sykdomsutbrudd en faktisk har i felt.

Epidemiologisk studie av CMS

For å lære mer om forekomsten av både virus og klinisk sykdom har vi fulgt oppdrettslaks i sjøfasen. Vi har tatt ut



Figur 1. Kartet viser lokalitetene som følges i prosjektet.

PMCV

- PMCV er et dobbelttrådet, nakent RNA-virus.
- Viruset har strukturelle likheter med Totiviridae – familien.
- Andre virus i Totiviridae-familien infiserer sopp og parasitter, og PMCV skiller seg således ut ved å infisere et virveldyr.
- Det viktigste kjente reservoaret for PMCV er laksen selv. Viruset er også påvist hos villaks, men forekomsten er så lav at dette ikke regnes som en betydelig smittekilde. Det er funnet et liknende virus hos vassild, men dette isolatet var ikke er nært beslektet med PMCV.
- Viruset kan påvises gjennom RT-PCR, og CMS bekreftes gjennom klinikk og histopatologiske funn.
- Det er utfordrende å dyrke viruset i cellekultur, noe som vanskeliggjør arbeid med vaksineutvikling.

CMS

- CMS har vært kjent i Norge siden 1980-tallet, og har også blitt påvist Færøyene, Skottland og Irland.
- I Norge har det historisk sett vært rapportert flest tilfeller fra Midt-Norge, men i senere tid har en observert et lite skifte med en økning i antall utbrudd fra de tre nordligste fylkene.
- I 2016 ble det rapportert inn 90 tilfeller av sykdommen til Veterinærinstituttet, i tillegg til 108 tilfeller som ble diagnostisert ved eksterne laboratorier. Det kan være overlapp i disse tallene.
- Klinisk karakteriseres sykdommen av typiske tegn på sirkulasjonsforstyrrelse. Syk fisk får utstående øyne, utspilt buk, samt punktformede blødninger i huden særlig på buksiden. Innvendig kan en bl.a. se forstørret hjertekammer, blodkoagel i hjertesekken, rikelig med væske i bukhulen og misfarget lever dekket av et fibrinslør.

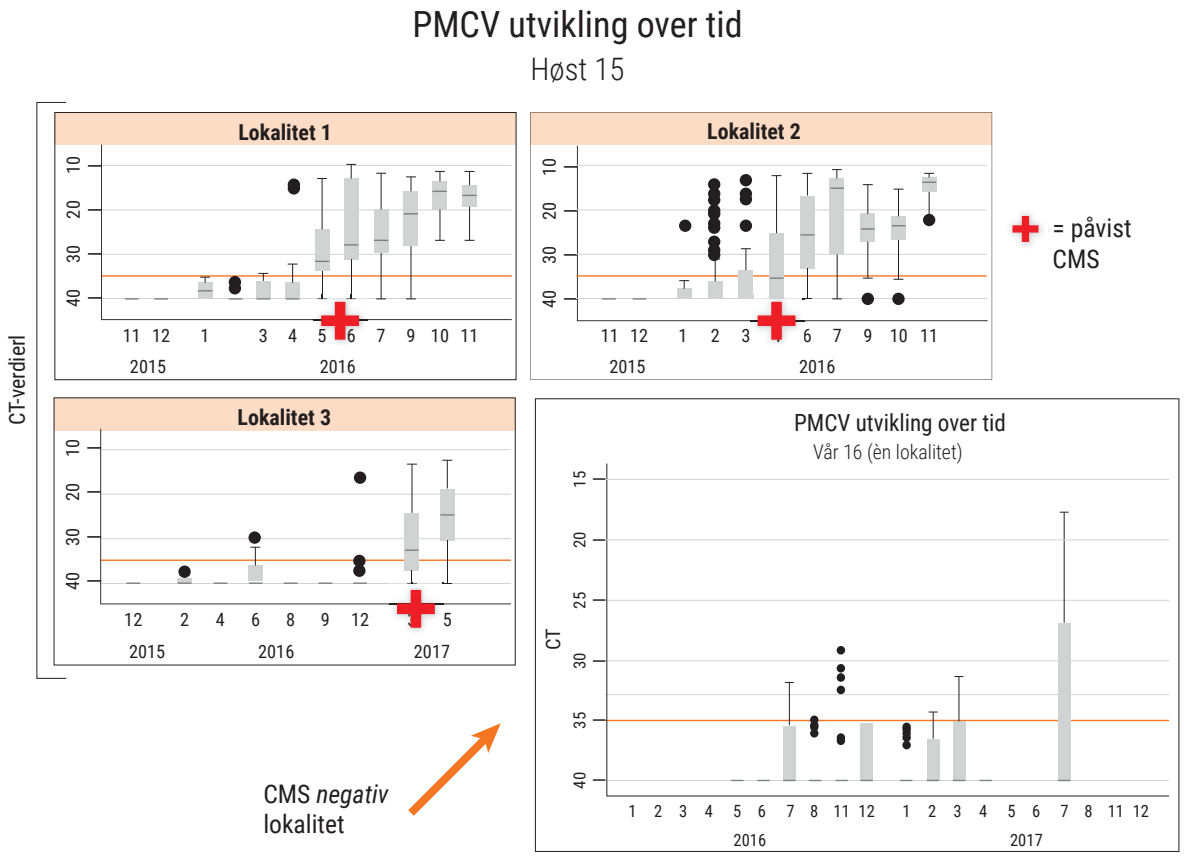
prøver regelmessig og gjennomført diagnostiske undersøkelser ved mistanke om sykdom. Hensikten har vært å lære mer om når fisken blir infisert med viruset, hvor lang tid det går fra infeksjon til klinisk sykdom og om screening kan brukes som et verktøy i arbeidet med sykdomsbekjempelse.

Vi har fulgt fisk på 12 matfisklokaliteter. H16 er den siste generasjonen som er inkludert i undersøkelsen og fremdeles følges i sjø. Lokalitetene er spredt fra Vest-Agder i sør til Finnmark i nord. Lokalitetene er inndelt høy – og lavrisiko lokaliteter for CMS. Dette er basert på oppdretternes erfaringer med tidligere CMS utbrudd. På lokaliteter med høy risiko for CMS har vi gjennomført prøvetakinger hver måned, mens lokaliteter med lav risiko for CMS har blitt prøvetatt annenhver måned. Ved hvert prøveuttak har vi tatt ut minst 20 fisk fra to merder, og vi har fulgt de samme to merdene fra sjøutsett og helt frem til slakt. Dette er et delprosjekt av en større, pågående epidemiologisk undersøkelse av CMS (CMS-Epi), hvor hovedmålet er å øke kunnskapen om spredning av PMCV og faktorer som påvirker utviklingen av klinisk CMS. Et viktig mål i dette prosjektet er å finne ut om viruset overføres fra stamfisk til avkom. Resultatet fra denne undersøkelsen kan du lese mer om i forrige nummer av Norsk fiskeoppdrett.

Resultater

Det vil være laks i sjø som følges frem til mai i 2018. Resultatene som oppsummeres her er dermed ikke endelige. Hittil har vi påvist viruset på 11 av de 12 lokalitetene som inngår i undesøkelsen. På seks av disse lokalitetene har fisken utviklet CMS. Resultatene tyder enten på at viruset er mer utbredt enn vi tidligere har antatt, eller at forekomsten har økt i senere år.

Videre viser resultatene at fisken kan bære på viruset uten at dette nødvendigvis leder til kliniske sykdomsutbrudd på lokalitetene. Screening vil derfor være et verktøy som må brukes med varsomhet. En positiv påvisning av PMCV med lavt virusnivå, er ikke nødvendigvis et tegn på forestående sykdom. Det kan derimot være hensiktsmessig å følge helhetsbildet på lokaliteten. Ved en utvikling med stigende andel infiserte individ og samtidig økende virusmengde på individnivå, vil det være fornuftig å vurdere iverksetting av tiltak som kan forebygge utbrudd av CMS.



Figur 2. Figuren viser virusutvikling på tre CMS positive lokaliteter høsten 2015, samt på én CMS negativ lokalitet vår 2016. Den røde streken angir at prøver med Ct-verdi lavere enn 35 anses for positive for PMCV, mens prøver med høyere verdi anses som negative. Rødt kryss angir tidspunkt for klinisk diagnose av CMS basert på histopatologiske prøver. Ved hvert prøveuttak ble det tatt prøver av 20 fisk fra to merder.

Vedlegg 4

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ORIGINAL ARTICLE

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Monitoring infection with *Piscine myocarditis virus* and development of cardiomyopathy syndrome in farmed Atlantic salmon (*Salmo salar* L.) in Norway

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Abstract

An epidemiological study was carried out in Norway in 2015–2018, investigating the development of infection with *Piscine myocarditis virus* (PMCV) and development of cardiomyopathy syndrome (CMS) in farmed Atlantic salmon. Cohorts from 12 sites were followed and sampled every month or every other month from sea transfer to slaughter. PMCV was detected at all sites and in all sampled cages, and fish in six sites developed clinical CMS. The initial infection happened between 1 and 7 months post-sea transfer, and the median time from infection with PMCV until outbreak of CMS was 6.5 months. Generally, fish from sites with CMS had higher viral titre and a higher prevalence of PMCV, compared to sites that did not develop clinical CMS. The virus persisted until the point of slaughter at most (11 out of 12) of the sites. The detection of PMCV in all sites suggests that PMCV is more widespread than previously known. Screening for PMCV as a tool to monitor impending outbreaks of CMS must be supported by observations of the health status of the fish and risk factors for development of disease.

KEYWORDS

Atlantic salmon, cardiomyopathy syndrome, cohort study, epidemiology, field investigation, piscine myocarditis, *Piscine myocarditis virus*

1 | INTRODUCTION

Cardiomyopathy syndrome (CMS) is a disease that affects farmed Atlantic salmon (*Salmo salar* L.). It is one of the most serious diseases in Norwegian aquaculture, resulting in reduced fish welfare, increased mortality and substantial economic loss for the industry (Brun, Poppe, Skrudland, & Jarp, 2003). The disease was first detected in Norway in the 1980s, then of unknown aetiology (Amin & Trasti, 1988). Since then, the disease has made its appearance in the Faroe Islands (Poppe & Sande, 1994; Poppe & Seierstad, 2003), Scotland (Rodger & Turnbull, 2000) and Ireland (Rodger, Mccleary, & Ruane, 2014). CMS has typically been known to affect fish in the second year of the grow-out phase. However, there is an increasing number of field reports of disease on younger fish, and scientific reports describe the disease occurring on fish groups five or six months post-sea transfer (Fritsvold et al., 2015; Wiik-Nielsen, Alarcón, Jensen, Haugland, & Mikalsen, 2016). Histological findings include inflammatory changes primarily in the spongious part of the atrium and ventricle, whilst the compact myocardium usually remains unaffected (Bruno, Noguera, & Poppe, 2013; Ferguson,

Poppe, & Speare, 1990). In severe cases, the changes can lead to a rupture of the cardiac wall, with a rapid, fatal outcome (Ferguson et al., 1990).

Cardiomyopathy syndrome was shown to transmit horizontally between fish in 2009 (Bruno & Noguera, 2009; Fritsvold et al., 2009), and the causative agent was identified in the two subsequent years, when a novel, totilike virus named *Piscine myocarditis virus* (PMCV) was detected and linked to the disease (Haugland et al., 2011; Løvoll et al., 2010). PMCV is a naked, double-stranded RNA virus with a relatively small genome. It differs from other members of the *Totiviridae*—family in several ways, amongst them through its choice of a vertebrate host and its manner of extracellular transmission (Haugland et al., 2011). Studies using RT-PCR analysis have shown that there is a good correlation between levels of virus found in heart, and the severity of cardiac lesions (Haugland et al., 2011; Timmerhaus et al., 2011). Little is known about the biophysical properties of PMCV, and it has proven difficult to grow in cell culture. The latter has made the development of a vaccine challenging, and as of now, there is none on the market.

Cardiomyopathy syndrome is not a notifiable disease, neither in Norway nor for the World Organization for Animal Health (OIE), and there is no official control programme for CMS in Norway. As such, the exact number of annual cases is uncertain. The Norwegian Veterinary Institute has diagnosed CMS in approximately 100 seasites every year since 2013, and additional cases are identified at other laboratories (Hjeltnes, Jensen, Borno, Haukaas, & Walde, 2018). On affected sites, the disease may manifest itself acutely with an abrupt increase in mortality, or as a more prolonged phase with moderate losses over time.

Diagnosis of CMS is based on histopathology. PCR for PMCV is suggestive of CMS, but finding of the virus alone without any pathological findings does not support a diagnosis of CMS.

In the years following the detection of PMCV, several studies have investigated the epidemiology of the disease, including other transmission pathways, reservoirs and risk factors for disease. Vertical transmission of CMS is suspected and is a focus of current research. Two separate studies have detected PMCV in heart samples from brood fish as well as in roe and milt, fertilized eggs and yolksac fry (Jensen, 2017; Wiik-Nielsen, Ski, Aunsmo, & Løvoll,

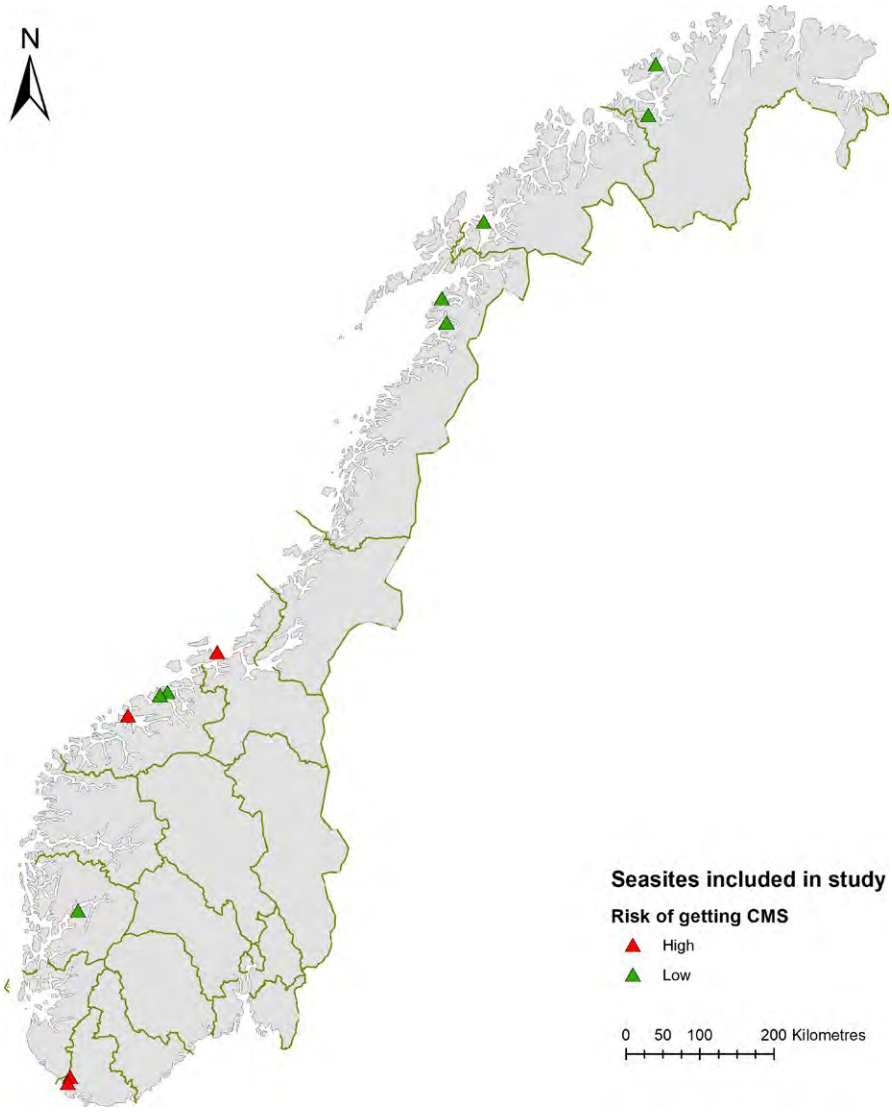

FIGURE 1 Map of Norway showing the position of the 12 seasites included in the study. Red triangle indicates that the seasite is defined as a high-CMS risk site, green triangle indicates that the seasite is defined as a low-CMS risk site

2012). There are no known occurrences of clinical disease in the freshwater phase. Findings indicate that the most important, known reservoir for the virus is farmed salmon. An overview of known and investigated sources is presented in the review recently published by Garseth and colleagues (Garseth, Fritsvold, Svendsen, Bang Jensen, & Mikalsen, 2018).

In a study of risk factors for CMS in Norway, the median time from sea transfer to a CMS diagnosis was shown to be 16 months (Jensen, Brun, Fineid, Larssen, & Kristoffersen, 2013). The same study concluded that the likelihood of developing disease increased with length of time in the grow-out phase, infectious pressure from neighbouring sites, as well as CMS in previous generations (Jensen et al., 2013). However, there is a lack of knowledge of when fish cohorts are infected, how infection is related to clinical outbreaks and for how long the infection persists.

The main aim of this study was to gain more knowledge about time of infection with PMCV, length of the interval between infection with PMCV and clinical outbreak of CMS, and how long the virus persists in the population after infection. An additional aim was to use findings to discuss whether screening for PMCV can be used as a predictor for outbreaks of CMS.

2 | MATERIALS AND METHODS

2.1 | Study population

The monitored population was farmed Atlantic salmon, stocked on seasites along the Norwegian coastline (Figure 1). Three sites were stocked in the fall of 2015, four were stocked in the spring of 2016 and five in the fall of 2016. Four fish farming companies participated in the study, and the fish originated from different hatcheries. In this study, a cohort is defined as a group of fish in a separate cage, originating from the same hatchery and stocked at the same time. Two cohorts were followed on each site, with the exception of one site where three cohorts were followed. The farmers themselves made the selection of sites and cohorts to follow.

2.2 | Study design

Groups of Atlantic salmon were followed throughout the marine grow-out phase in a prospective, longitudinal cohort study. Selected sites were defined as high-, or low risk by the farmers, based on previous CMS history (Figure 1). Sampling for PMCV-PCR (as described below) was done approximately every month on high-risk sites, and every other month on low-risk sites. Approximately 30 individuals were sampled from each cage on every sampling occasion, that is 60 fish from each site.[1] This included both recently deceased and moribund fish, as well as individuals with no external clinical signs of disease. Sampling personnel were instructed to collect 20 recently deceased and 10 live fish per cage, if possible.

Upon suspicion of clinical disease, an extended sampling was performed. The purpose of this was to investigate the spread of the virus within sites, as well as to be able to make a disease diagnosis. In addition to collecting samples from the cages already included in the study, 60 fish from each of two additional cages (i.e., 120 extra fish) were sampled on these occasions.[2] Tissue samples for histopathological assessments were also collected. The decision on whether to perform an extended sampling was based on development in cycle threshold (Ct) levels and prevalence of PMCV, together with information from the fish health service regarding mortality and the general health status on the sites. On two additional sites where clinical disease was suspected, tissue samples for histological assessment and RT-PCR analysis were collected only from the cages that were followed throughout the study due to time limitations. These are not defined as extended sampling events.

A site was categorized as CMS-positive based on results from two laboratory tests, that is RT-PCR and histopathology. The start date for an outbreak was set as the date of the sampling which rendered the positive test results.

2.3 | Tissue sampling

All samples were collected either by fish health personnel (fish health veterinarians or biologists) or by site workers given practical education on sample collection by the fish health service.

Live fish were captured by convenience sampling, whilst recently deceased fish were selected from the accumulated dead fish pool at the bottom of the net pen.

Prior to tissue sampling, all living fish were euthanized either by an overdose of anaesthetic or by a blow to the head. Samples were collected from the tip of the ventricle, in a size measuring approx. 2 mm*2 mm*2 mm. These were then stored on RNA-later and shipped for RT-PCR analysis performed by Pharmaq Analytiq. If possible, samples were shipped overnight on ice. If kept longer, samples were refrigerated for the first 24 hr and then stored in a frozen state until they were shipped.

For histopathological assessment, tissue samples measuring approx. 1 cm*1 cm*0.4 cm were collected from the heart, spleen, liver, head kidney, pancreas and skeletal muscle and fixed in 10% buffered phosphate formalin.

2.4 | Laboratory methods

2.4.1 | RNA extraction

Tissue samples were processed using a Qiagen's Universal Biorobot, with the compatible RNA purification kit (RNeasy 96 Universal Tissue Kit), according to the manufacturer's recommendation. Extracted total RNA was eluted in a final volume of 100 µl of the supplied kit elution buffer.

[1]Ninety fish from the site where three cohorts were followed.

[2]Except on two sites, one where 30 fish were collected from each of the two additional cages, and one where 60 fish were collected from one additional cage only.

TABLE 1 Information about the participating sites

Site number	Company	Region	Risk of CMS	Generation	No of. sampling events	Time of slaughter
1	A	North	Low	Spring 16	7	Jan 18
2	A	North	Low	Spring 16	8	Feb 18
3	A	North	Low	Spring 16	10	Oct/Dec 17
4	A	North	Low	Fall 16	9	April 18
5	B	North	Low	Fall 16	7	April 18
6	B	Mid	High	Spring 16	12	Aug/Sept 17
7	C	Mid	Low	Fall 16	6	Oct 17
8	C	Mid	Low	Fall 16	7	Nov 17
9	C	Mid	High	Fall 16	16	March 18
10	D	South	High	Fall 15	10	Dec 16
11	D	South	Low	Fall 15	5	May 17
12	D	South	High	Fall 15	12	Dec 16

Note. Risk of cardiomyopathy syndrome (CMS) is based on the farmers experience, as described in the text.

2.4.2 | Real-time RT-PCR

Extracted RNA from tissues of salmon was tested by Taqman real-time RT-PCR (qScript XLT-1 1-Step RT- qPCR ToughMix, QUANTAbio). During the real-time RT-PCR screening a housekeeping gene, elongation factor 1 alpha (EF1A), was used as an internal control (Olsvik, Lie, Jordal, Nilsen, & Hordvik, 2005), and a specific assay was used for detection of PMCV (Nylund et al., 2018). The primer and probe concentrations had been optimized and found to be 900 nM for all primers used and 225 nM for the corresponding probes. The samples were run in simplex for the internal control and triplicates for detection of PMCV in standard 384-well plates. All assays were run in a total volume of 10 µl in each well, with 2.5 µl of isolated total RNA as the template. Plates were analysed in an Applied Biosystems 7900 HT real-time machine under standard conditions. Each run consisted of 40 cycles, and the samples were considered positive when the fluorescence signal increased above a set threshold of 0.09. The PMCV assay has a repeatable Ct at 34.7. This level was set based on 10 replicates of ten-fold dilutions of a DNA stock and denotes the mean Ct-value of the highest dilution for which the 10 replicates were positive. Although Ct-values above this threshold are possible, they are likely to not be reproducible under the same reaction conditions. A standard curve was generated using a 10-fold serial dilution of DNA in three parallels. Regression analysis, standard curve slopes *s* of Ct versus log quantity DNA and amplification efficiency *E* where $E = [10^{1/(-\text{slope})}] - 1$ were calculated. The coefficient of determination, R^2, was 0.99. The slope for the assay was −3.44, and the amplification efficiency *E* was 0.95. For the EF1Ab assay, the corresponding values were 0.99, −3.98 and 0.78.

The formalin-fixated samples were processed, embedded in paraffin, sectioned 4–5 µm thin and stained with haematoxylin and eosin in accordance with routine procedures. Sections were scanned at 400x and scored in accordance with Table 1 in Wessel et al. (2018).

2.5 | Data collection and analysis

The field samples were collected from October 2015 to April 2018. The screening results for all individual sampling events were compiled and sorted into categories in Microsoft Excel (version 14.4.4). This included Ct-values, date of sampling, cage number and comments regarding health status of the individual fish. Based on the latter, all samples where categorized into four categories with regard to health status of the fish: "Normal" means that the fish did not display any external signs of disease; "Dead/moribund" means a recently deceased or gravely ill individual of otherwise normal size and condition; "Loser" refers to thin, weak individuals typically suffering from long term effects of prior diseases such as PD, or other ongoing challenges affecting growth and general health status; and,"unknown" indicates that we do not know the health status of the fish, that is this has not been commented upon sampling. Further details about the individual sites on geographical location, risk category, hatchery origin, time of stocking, number of cages on the sites, generation (fall or spring) and eventually time of slaughter were collected from the participating companies. The general information about the sites is summarized in Table 1.

Descriptive data analysis was carried out using the statistical software Stata (version 13.1), and a statistical comparison between the categories of health status was performed using the Wilcoxon test in R (version 3.4.4).

3 | RESULTS

3.1 | Onset of infection and disease

Viral RNA was detected through RT-qPCR on all the participating sites and in all sampled cages. Fish in 6 of the 12 monitored sites developed clinical CMS (Figure 2a), whilst fish in four of the sites did not develop CMS (Figure 2b). On two sites, there was an increase in

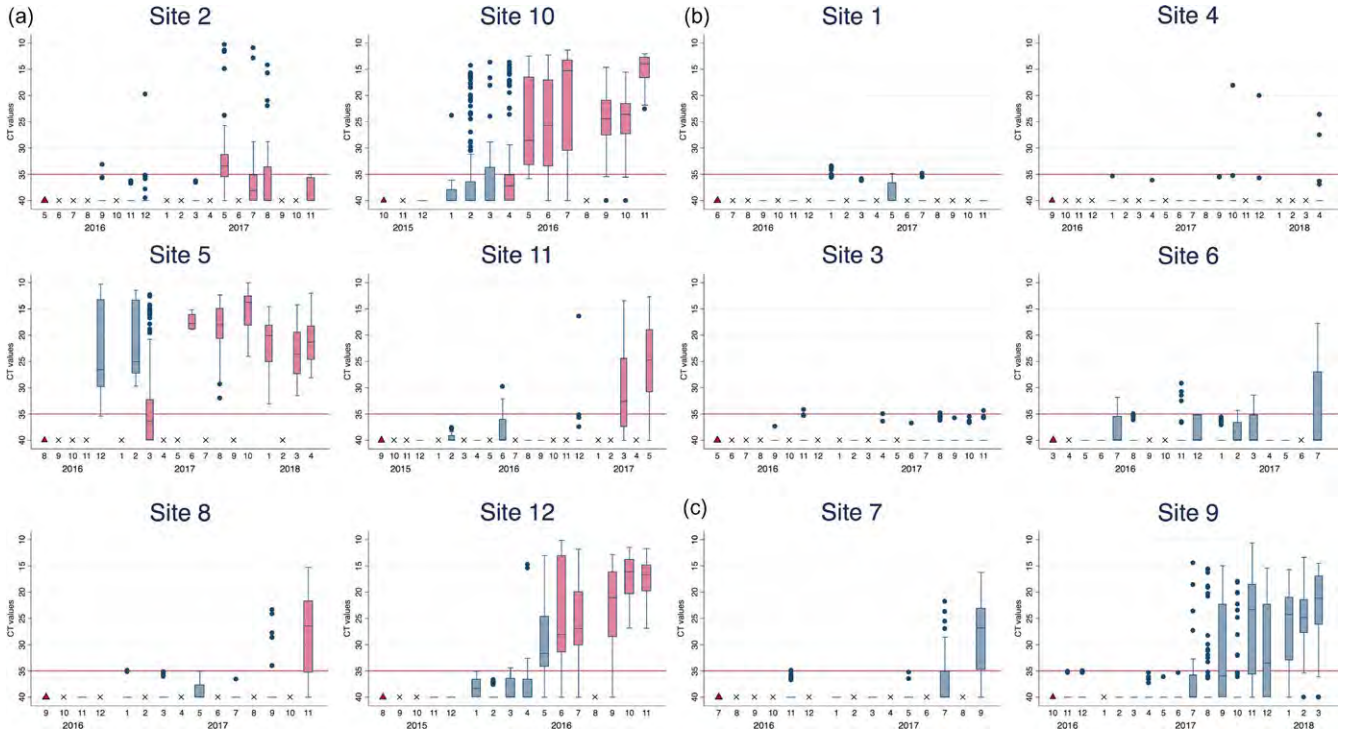

FIGURE 2 Boxplots displaying the development of PMCV in individual cohorts. The box represents the interquartile range (IQR) where the middle 50% of the data are contained. The line in the box illustrates the median, representing the middle of the dataset (50th percentile). Whiskers are drawn from the upper and lower quartile, ±1.5 IQR. Outliers mark points beyond either whisker. The red line indicates the cut-off point (35) that is commercially used for the RT-qPCR test. The triangle marks the month when the fish were stocked at the site, and an x denotes a month with no sampling. The first red box marks the time of the CMS diagnosis, and boxes remain red for the rest of the time at sea. (a) Development in CT levels on sites with clinical CMS. (b) Development in CT levels on sites without clinical CMS. (c) Development in CT levels on sites with suspicion of CMS

TABLE 2 Time (in months) from sea transfer until PMCV infection and from infection until development of clinical disease

Disease status	Months from sea transfer → PMCV (+) (median (range))	Months from PMCV (+) → CMS (+) (median (range))	Months from sea transfer → CMS (+) (median (range))
CMS-positive (*n* = 6)	4 (3–5)	6.5 (3–13)	11 (6–18)
CMS negative (*n* = 4)	4 (4–7)	n/a	n/a
CMS suspect (*n* = 2)	2.5 (1–4)	n/a	n/a

Note. CMS: cardiomyopathy syndrome; PMCV: *Piscine myocarditis virus*.

viral prevalence and a drop in Ct levels; however, a CMS diagnosis could not be made based on histopathology (Figure 2c). These sites were defined as suspected clinical cases.

The time of primary detection of PMCV varied from 1 to 4 months post-sea transfer in the two CMS suspect sites, whilst the median time was 4 months in both the sites that did not develop CMS and in those that did (Table 2). Further, the median time from sea transfer to first PMCV detection was 4 months for both spring locations and fall locations. Overall, the time from sea transfer to primary detection of PMCV varied from 1 to 7 months post-sea transfer, between the 12 sites. There was no difference in the time from sea transfer to PMCV infection between the three geographical regions (north, mid and south).

The time from sea transfer until clinical disease in the CMS-positive sites ranged from 6 to 18 months. The median time from PMCV infection to clinical outbreak was 6.5 months, with a range of 3–13 months (Table 2).

There was a higher proportion of sites with clinical disease amongst the fall sites (5/8), compared to the spring sites (1/4). Both the suspected clinical sites had fall fish.

Extended samplings were performed on seven sites (sites 1, 2, 5, 6, 7, 10 and 11; Figure 2), between 4 and 18 months post-sea transfer. On the background of this extended sampling, four of the seven sites were classified as CMS-positive, two remained CMS negative, and one was CMS suspect. PMCV was detected in fish from all additionally sampled cages, including those on sites that remained CMS negative. On two additional sites (site 8 and 12), samples of fish were collected for RT-PCR and histopathological assessment only from the two cages followed throughout the study. Results classified both sites as CMS-positive.

3.2 | Development of infection and disease

On the six sites with clinical CMS, PMCV was detected after 3–5 months at sea. On site 2, 8, 11 and 12, the detection of PMCV was followed by some months with high Ct-values, before a marked decrease in Ct value corresponding to increase in viral load (Figure 2a). The CMS diagnosis was made either concurrent with or shortly after this increase, and the viral loads remained elevated for the rest of the production cycle.

On the four sites without clinical CMS, the image is quite different: PMCV was detected but remained at generally low levels. Viral RNA was detected on three of the sites until harvest. On site 1, there was no detection of PMCV at the final sampling; however, the prevalence of PMCV in preceding sampling events was so low that a negative result does not exclude viral presence (Figure 2b).

In the two sites where CMS was suspected, but not confirmed, the infection patterns were very similar to that of the sites which developed clinical CMS (Figure 2c).

At the first detection of PMCV, the prevalence varied from 2% to 55%, with the majority of detections having high Ct-values, corresponding to low viral loads. On the last sampling before slaughter, the prevalence was highest on the CMS-positive (33.3%–100%) and CMS suspect (82% and 90%) sites. In the CMS negative sites, the prevalence ranged from 0% to 46.7%. On this last sampling event, the Ct-values were predominantly below 35 in the CMS-positive and CMS suspect sites, but still high in the CMS negative sites.

3.3 | Ct-values by state of fish

When comparing Ct-values from all sampled fish categorized into groups of "unknown,""Normal," "Dead/moribund" and "Loser," the group with "Dead/moribund" fish generally had significantly lower Ct-values ($p < 0.001$; Figure 3). The compiled results do not meet the desire in the design of the study (with 20 recently deceased and 10 live individuals per sampling event). This is both due to practical challenges in the field, where sampling personnel had to deviate from the plan, as well as missing notations describing the health status of the fish.

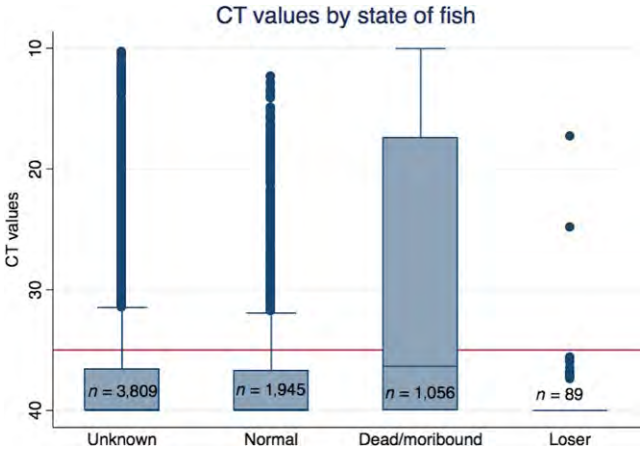

FIGURE 3 Comparison of CT-values from sampled fish of different health status. Results shown in boxplots as explained for Figure 2. For explanation of the state of fish, see text under Data collection and analysis

4 | DISCUSSION

Piscine myocarditis virus was detected at all sites and in all sampled cages at different time points of the grow-out phase, and fish on six sites developed clinical CMS. There was a higher proportion of sites with clinical CMS (five of eight) amongst the fall-stocked sites compared to the spring-stocked sites (one of four). However, three of the four spring-stocked sites were in the North, where the prevalence of CMS is lower, so it is possible that the occurrence of CMS had nothing to do with stocking time, but rather geography. Fish from sites with CMS had overall lower Ct-values, and the prevalence was higher, compared to sites without the diagnosis. Dead or moribund fish generally had lower Ct-values compared to live fish with no external clinical signs. The virus persisted until the point of slaughter at most (11 out of 12) of the sites.

At the initialization of the study, the farmers had categorized the farms into either high-CMS risk or low-CMS risk, based on previous experiences with CMS. The low-CMS risk farms were not expected to be neither infected with PMCV nor to develop CMS. This was especially true for the farms in northern Norway, where the prevalence of CMS historically has been low (Hjeltnes et al., 2018). However, we found that PMCV was present in all sites, also in the northern region. Furthermore, four of the six sites that developed CMS were low-risk sites, according to the farmers. This suggests that there is a disagreement between the perceived risk of CMS and the true risk. It also suggests that CMS is overlooked and/or underreported, since it is likely that the reason it was detected in the present study was because we were actively looking for it.

Several of the CMS-positive sites follow the same pattern with initial detections of PMCV with high Ct-values, which persists for a few months, until a sudden increase in prevalence and a decrease in Ct-values happens concurrent with an outbreak of CMS. However, on site 2 the Ct levels did not undergo a marked development. On this site, a CMS diagnosis was made in May in 2017, and the fish remained in sea for another half year. It was reported that the disease took a clinically mild form on the site. This site was situated in northern Norway, an area in which reports of CMS have historically been low, although increasing over the past two years (Hjeltnes et al., 2018). It was also stocked with spring fish. Both are factors that could direct to explain why some sites experience severe disease and great losses, whilst others seem to make it through with much less severe consequences.

At the first PMCV detection, there was some variation in prevalence between the sites, but in general, the initial prevalence was low. Quite a lot of the detections had Ct-values above the diagnostic cut-off value. This means that on a regular screening report, they would be regarded as negative results. Values above the diagnostic

cut-off value have been included in the data set to ensure coverage of the early stages of infection, where virus levels were expected to be low, even though they are considered to be non-reproducible.

At the last sampling before harvest, the picture was quite different. At all the CMS-positive sites, the prevalence of PMCV was very high, except at the one with a less severe outbreak. On the CMS-positive sites, the general image is also dominated by low Ct-values. This was also the case for the two sites with suspected disease. The CMS negative sites, however, generally had a lower prevalence and the Ct-values were higher. These results concur with the previous consensus of there being a good correlation between virus levels and clinical disease (Haugland et al., 2011; Timmerhaus et al., 2011).

The virus seems to be widespread and has been found on four sites where there was no subsequent development of clinical CMS. This indicates that the virus may exist in populations without ensuing clinical disease. Detecting PMCV does not necessarily indicate an impending disease outbreak, and the low prevalence and viral loads does not make it a suitable tool for early prediction of disease. However, screening can be purposeful to get an overview of the greater picture on a site. A development with increasing prevalence, as well as elevated viral loads, may be a warning signal. Used together with fish health assessments, this can form a basis for the decision to implement preventative measures.

In the protocol for sampling, we requested samples from 20 recently diseased fish supplied with 10 healthy fish, in order to ensure that the fish most likely to have infection or clinical disease were sampled. However, the compiled results revealed that only about 1/3 of the fish where the health status was known were dead or moribund. This could be because there sometimes simply was not as many as 20 dead fish at the time of sampling. In more than half of the samples, the state of fish was not listed when the samples were submitted. But when comparing Ct-values from the Dead/moribund with the healthy fish, Ct-values were lowest in the "Dead/moribund" group, indicating that this could be the best group to target when screening for the virus. Thus, we might have been able to detect infection at an earlier time or amongst a larger part of the fish if a greater part of the samples had been from dead or moribund fish. Another aspect of the sampling is the choice of sampling material. Studies have shown that PMCV is primarily found in areas affected with lesions (Wiik-Nielsen, Lovoll et al., 2012). The lesions are believed to develop sequentially first in the atrium and subsequently in the ventricle (Haugland et al., 2011). Therefore, it might be assumed that the best tissue to sample for early detection of PMCV would be the atrium. For screening purposes, however, it is often more practical to use the tip of the ventricle, since this is easier to sample, especially for personnel who might not be so experienced in sampling and have little time to do so. In this project, we have used the protocol recommended by Pharmaq Analytiq who offers analysis of screening samples for PMCV for commercial use, and thus, we believe that this reflects well how the results of screening would be in real life.

The detection of PMCV in all sampled cages in this study could indicate either that the virus spreads efficiently between cages on a site or that there is an element of vertical transmission. The study was not designed to look at the transmission patterns between cages. It is known from other viral diseases, and also from reports from the field, that there can be major differences with regards to prevalence and clinical signs between cages in a site. The cages to be followed within the present study were chosen by the farmers, and in most cases, they chose cages with smolt from different suppliers, since they had a personal interest in finding out if there was a difference in susceptibility to infection between different smolt groups. In order to gain a thorough understanding of factors that influence disease transmission, a study including production data from more than 1,500 fish groups has been performed concurrent with this one by the authors, and the results will be published soon.

In another study performed as part of the present project, we have looked at vertical transmission as a possible additional route of infection. The results indicate that this could indeed be a possible transmission pathway under the current production systems (Jensen, 2017). A support for this is the recent findings of CMS in the Faroe Islands which have been linked to introduction with eggs imported from Norway (Garseth et al., 2018), and a reportedly high prevalence of PMCV amongst broodfish. In our study, we have also found PMCV early after sea transfer in several sites, further suggesting that they could have been carrying the infection from the freshwater phase.

In the present study, the median time from sea transfer to clinical CMS was 10 months, which is remarkably less than the 17 months that has previously been reported (Jensen et al., 2013). This gives support to the reports of a shift in the occurrence of CMS towards younger fish. However, some of the diagnoses in the study might have been forced earlier, as there was an increased awareness of CMS on participating sites.

The median time from infection with PMCV to clinical outbreaks of CMS was four months, but with a large range from 3 to 10 months. This makes it difficult to understand transmission pathways, when investigating reasons for clinical outbreaks. In the only published study on risk factors for CMS, infection pressure based on the number of neighbouring sites with CMS adjusted by seaway distance was found to be an important risk factor for development of CMS (Jensen et al., 2013). In that study, there was no information on the actual occurrence of PMCV, and only sites with clinical CMS were included in the calculations of infection pressure. Since infection pressure was found to be significant anyway, this could indicate that shedding of sufficient amounts of virus to cause transmission of disease only happens from fish that are clinically affected by infection. Thus, an option for control is to try and mitigate disease outbreaks, even if virus has been detected. This will of course also be beneficial for the farms already infected.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

Author Stian Nylund is affiliated with Pharmaq Analytiq which offers screening for salmon viruses included PMCV.

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Vedlegg 5

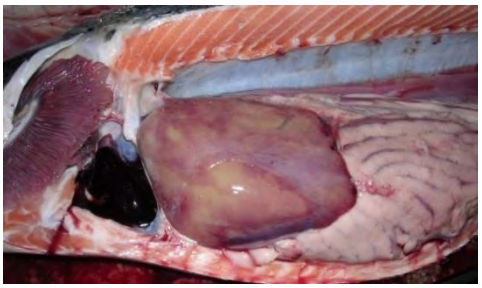
Kardiomyopatisyndrom (CMS) hos laks
Sykdomsutvikling - Agens- Epidemiologi

Sykdommen kardiomyopatisyndrom (CMS) ble beskrevet hos oppdrettslaks midt på 1980-tallet. Årsaken var lenge ukjent, men det ble tidlig mistenkt at et virus var ansvarlig. I 2010 ble dette bekreftet da piscint myokarditt virus (PMCV) ble beskrevet og knyttet til sykdommen. Det ble vist en klar sammenheng mellom tilstedeværelse og mengde av PMCV og utvikling av de karakteristiske CMS-relaterte patologiske endringene i hjerte. CMS opptrer langs hele norskekysten med Midt-Norge som tyngdepunkt. Det registreres i overkant av 100 CMS tilfeller per år, men det reelle antallet ligger trolig høyere siden sykdommen ikke er meldepliktig. I tillegg til Norge, er CMS påvist i Irland, Skottland og på Færøyene. CMS-lignende patologi er også beskrevet hos laks i Canada. CMS gir redusert fiskevelferd og betydelige økonomiske tap for næringen.

Kardiomyopatisyndrom (CMS)

CMS er en kronisk sykdom i hjertet hos atlantisk laks. Sykdommen opptrer oftest hos fisk med god vekst og i normalt hold. Dødeligheten utvikler seg langsomt, men med episoder av forhøyet dødelighet i forbindelse med stressende håndtering, ugunstige miljøforhold eller generelt sykdomsstress. Diagnosen CMS stilles på grunnlag av kliniske symptomer, funn ved obduksjon og histopatologisk undersøkelse. Obduksjonsfunnene er sirkulasjonsforstyrrelser og blødning til hjertesekken. Histopatologiske funn er betennelse og nekrose i hjertemuskelceller og i hjertets indre, svampaktige muskellag (spongiøst myokard). Skadene opptrer først i forkammeret, før de brer seg videre til hjertekammeret.

Laks med CMS og sirkulasjonsforstyrrelser; utstående øyne, blødninger i hud og ødem i skjellomme. Foto: Per Anton Sæther, MarinHelse AS



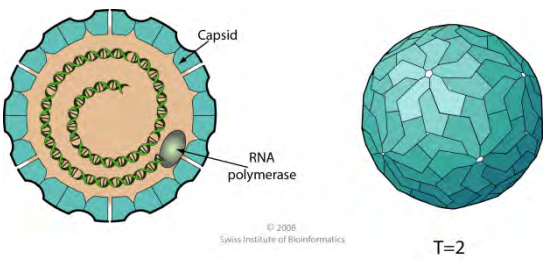
Laks med CMS og sirkulasjonsforstyrrelser; utstående øyne, blødninger i hud og ødem i skjellomme. Foto: Brit Tørud, Veterinærinstituttet

Piscint myocarditt virus (PMCV)

PMCV er et relativt lite og enkelt oppbygget virus med et kappeprotein som omslutter et RNA arvestoff. Det viktigste målorgan for infeksjonen er laksens hjertemuskelceller. Det er mulig å detektere både virusets arvestoff og dets kappeprotein i infisert vev og celler. Viruset finnes hovedsakelig i CMS-syk laks i oppdrett. De genetiske forskjellene mellom virusisolater er små.

Reservoar for PMCV

Laks i oppdrett er det viktigste kjente reservoaret for PMCV. I tillegg er viruset påvist hos en liten andel villaks og hos vassild. PMCV fra vassild er ulikt virus fra vill og oppdrettet laks (86 % likhet på nukleotide nivå). I 2017 ble det rapportert om funn av PMCV og CMS relatert patologi hos rensefisk i Irland.



PMCV har klare likhetstrekk med virus i familien *Totiviridae*. Illustrasjon: Swiss Institute of Bioinformatics.

Smitteveier

PMCV smitter horisontalt mellom fisk. Vertikalt smitte, fra stamfisk til avkom, er så langt ikke utelukket og undersøkes i CMS Epi prosjektet. Virus i små mengder er utbredt hos stamfisk, og kan påvises i både melke og rognvæske. I tillegg er virus-RNA gjenfunnet i befruktet rogn og yngel fra PMCV-positiv stamfisk.

Risikofaktorer

Risikoen for sykdomsutbrudd i oppdrettslaks øker med tiden fisken har stått i sjø, med økende størrelse på utsett, samt med økt smittepress i form av smitte i og nærhet til omkringliggende anlegg. CMS i tidligere utsett er også identifisert som en risikofaktor, det samme er tidligere tilfeller med HSMB. Det er usikkert om faktoren CMS i tidligere utsett er en risiko på grunn av reell overlevelse av virus i miljø og villfisk, eller om risikoen representerer likheter i driftsrutiner, som samme settefiskleverandør, kontaktnett osv.

Dødeligheten under et CMS-utbrudd kan øke dersom fisken blir utsatt for stress. Andre faktorer som rask vekst, miljøfaktorer, ernæring og mangel på fysisk aktivitet har også blitt utpekt som mulige risikofaktorer.

CMS kan gi betydelig dødelighet, enten over tid eller som her i episoder med akutt dødelighet. Foto: Per Anton Sæther, MarinHelse AS

Sykdomskontroll

CMS er en smittsom sykdom og forebygges primært ved å blokkere virusets smitteveier. Horisontal smitteoverføring med fisk og gjennom vann er de kvantitativt viktigste smitteveiene. Tidlig utslakting reduserer smittepresset lokalt. Viruset er nakent og trolig relativt stabilt i miljø slik at det også kan overføres med utstyr, personell og skadedyr. Viktige tiltak er dermed å bryte smitteveier i tid og rom mellom generasjoner og fiskegrupper. PMCV-screening benyttes for å få kjennskap til smittestatus i fiskegrupper. QTL-selektert rogn med økt motstandskraft mot CMS er på markedet og det arbeides med å få på plass en vaksine. I tillegg er det utviklet funksjonelle fôr som skal sikre næringsopptak og redusere skadeomfanget av sykdommen. CMS syk laks bør ikke utsettes for stressende påkjenninger.

Produksjonstap

I 2003 ble de årlige direkte kostnadene ved CMS beregnet til å ligge mellom 33,5 og 66,3 millioner norske kroner for industrien som helhet. I 2007 beregnet Marine Harvest at CMS kostet næringen mer enn 200 millioner. I tillegg til tapt biomasse, vil utbrudd av CMS medføre ekstraordinære arbeidsoperasjoner med reallokering av mannskap og utstyr. Dødelighet i avlskjernen og stamfisk-populasjoner kan medføre tap av verdifull genetikk. Etter 2007 har både den totale produksjonskostnaden og lakseprisen økt og antall CMS tilfeller registrert hos Veterinærinstituttet og private laboratorier har gått fra under 70 til over 100 per år. CMS er derfor uomtvistelig en av de største tapsfaktorene i norsk oppdrettsnæring.

Dette fakta-arket er utarbeidet i forbindelse med FHF prosjektet «Epidemiologisk studie av kardiomyopatisyndrom (CMS): Spredning, risikofaktorer og sykdomsforløp i norsk lakseoppdrett» (FHF prosjekt 901118).
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REVIEW

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Cardiomyopathy syndrome in Atlantic salmon *Salmo salar* L.: A review of the current state of knowledge

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Abstract

Cardiomyopathy syndrome (CMS) is a severe cardiac disease affecting Atlantic salmon *Salmo salar* L. The disease was first recognized in farmed Atlantic salmon in Norway in 1985 and subsequently in farmed salmon in the Faroe Islands, Scotland and Ireland. CMS has also been described in wild Atlantic salmon in Norway. The demonstration of CMS as a transmissible disease in 2009, and the subsequent detection and initial characterization of piscine myocarditis virus (PMCV) in 2010 and 2011 were significant discoveries that gave new impetus to the CMS research. In Norway, CMS usually causes mortality in large salmon in ongrowing and broodfish farms, resulting in reduced fish welfare, significant management-related challenges and substantial economic losses. The disease thus has a significant impact on the Atlantic salmon farming industry. There is a need to gain further basic knowledge about the virus, the disease and its epidemiology, but also applied knowledge from the industry to enable the generation and implementation of effective prevention and control measures. This review summarizes the currently available, scientific information on CMS and PMCV with special focus on epidemiology and factors influencing the development of CMS.

KEYWORDS
Atlantic salmon (*Salmo salar* L.), cardiomyopathy syndrome, piscine myocarditis virus, PMCV, CMS

1 | INTRODUCTION

The establishment of large-scale intensive farming of Atlantic salmon *Salmo salar* L. facilitated a dramatic change in conditions for pathogen transmission and growth. This has led to emergence and widespread distribution of several infectious diseases within the industry (Rimstad, 2011).

Cardiomyopathy syndrome (CMS), a severe cardiac disease of Atlantic salmon, made its entry in Norwegian salmon farming in the mid-1980s (Amin & Trasti, 1988) and was subsequently detected in the Faroe Islands (Poppe & Sande, 1994; Poppe & Seierstad, 2003), Scotland (Rodger & Turnbull, 2000) and Ireland (Rodger, McCleary, & Ruane, 2014). A disease resembling CMS has also been detected in Canada (Brocklebank & Raverty, 2002). Due to the late onset of disease during the production cycle and a large number of outbreaks, CMS has significant economic impact at both company and industry levels in Norway (Brun, Poppe, Skrudland, & Jarp, 2003). In 2009, it was demonstrated that CMS is a transmissible disease (Bruno & Noguera, 2009; Fritsvold et al., 2009), and subsequently in 2010 and 2011, two separate research groups linked CMS to a virus resembling viruses of the *Totiviridae* family (Haugland et al., 2011; Lovoll et al., 2010). The discovery of piscine myocarditis virus (PMCV) had a significant impact on the development of new diagnostic, research and monitoring tools and has consequently increased our knowledge about the

disease. New tools and knowledge provide new perspectives and better opportunities to continue the search for a more complete understanding of the epidemiology and pathogenesis of CMS.

In 2015, the Norwegian Seafood Research Fund-FHF launched a 3-year research project on CMS and PMCV: "An Epidemiological study of Cardiomyopathy Syndrome (CMS): Transmission, risk factors and disease development in Norwegian salmon farming" (CMS-Epi) (http://www.fhf.no/prosjektdetaljer/?projectNumber=901118). The goal of the project was to increase knowledge about transmission of PMCV and factors influencing the development of CMS by epidemiologic studies and this literature review. The review aimed to summarize the current state of knowledge on both disease and causative agent, with special emphasis on disease development and epidemiology. The authors have reviewed scientifically published articles, as well as grey literature including reports, non-technical and popular science publications, marketing materials, patents and handouts from seminars and scientific symposia.

2 | CARDIOMYOPATHY SYNDROME

CMS primarily affects Atlantic salmon during their second year at sea, but has recently also been recorded shortly after sea transfer (Hjeltnes, Walde, Bang Jensen, & Haukaas, 2016). The disease may appear as an outbreak with sudden mortality without prior clinical signs, or have a chronic manifestation with prolonged moderately increased mortality (Brun et al., 2003; Ferguson, Poppe, & Speare, 1990). Diseased fish have normal to high condition factor and can display both macro- and microscopic signs of severe circulatory disturbances (Bruno, Noguera, & Poppe, 2013). Typical external findings are exophthalmia, ventral skin haemorrhages and raised scales due to oedema (Figure 1).

Common internal signs are ascites and dark coloured liver with fibrinous casts. The *atrium* and *sinus venosus* are usually enlarged, sometimes ruptured, and blood or blood clots often fill the pericardial cavity (Bruno & Poppe, 1996). Some dead fish may be without macroscopic changes, but still present with severe cardiac histopathological lesions.

Histopathologically, CMS is characterized by subendocardial inflammation, myocarditis and in severe cases, degeneration and necrosis of spongious myocardium. Cellular infiltration of mainly mononuclear cells, often lymphocytes and macrophages, initially occurs in the subendocardium, before progressing to the spongious myocardium (Bruno et al., 2013) (Figure 2). Lesions are usually first observed in the atrium, subsequently in the ventricle. The compact myocardium is usually not affected, but epicardial cell infiltrates may extend into the compact layer along branches of coronary vessels (Ferguson et al., 1990). Lesions may progress to such a state that the wall of the *atrium* or *sinus venosus* weakens or ruptures, with resultant haemopericardium and sudden death (Ferguson et al., 1990). Atrial inflammatory lesions may be more severe than the ventricular lesions and due to heart failure and severe congestion; there may be secondary lesions in other internal organs, for instance liver and spleen.

In a study of late-stage ventricular CMS lesions (30 and 33 weeks post-injection (wpi)) from an intraperitoneal (i.p.) challenge trial, laser capture microdissection combined with real-time PCR and immunohistochemistry revealed that the inflammatory cells were a mixture of T cells, IgM antibody-producing cells of the B-cell lineage (plasmablasts and plasma cells), and MHCII+ antigen-presenting cells, such as monocytes, macrophages, activated macrophages ($CD83^{+}$), B cells and possibly granulocytes (Wiik-Nielsen, Ski, Aunsmo, & Lovoll, 2012). In severe cases, a cellular epicarditis can be seen.

Cardiac lesions induced by CMS may resemble those of the most important differential diagnoses: pancreas disease (PD) and heart and skeletal muscle inflammation (HSMI). Although the three diseases are distinguishable in typical cases by histopathology (Kongtorp, Halse, Taksdal, & Falk, 2006; Kongtorp, Taksdal, & Lyngoy, 2004; Mcloughlin & Graham, 2007), a histopathological diagnosis can be challenging if two or all three diseases occur in the same individual, or the fish is in the late recovery phase of a disease (Wiik-Nielsen, Alarcon, Jensen, Haugland, & Mikalsen, 2016).

3 | AETIOLOGY—PISCINE MYOCARDITIS VIRUS

Although a viral aetiology was suggested in the initial description of CMS (Amin & Trasti, 1988), several hypotheses of non-infectious nature, including immunological, physiological and environmental

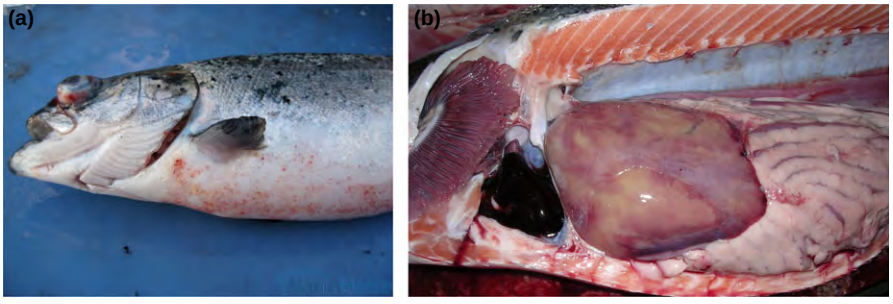

FIGURE 1 Gross pathological conditions in farmed salmon diagnosed with CMS. (a) Salmon showing exophthalmia, ventral skin haemorrhages and raised scales due to oedema. Photograph: Per Anton Sæther, MarinHelse AS. (b) Salmon at autopsy showing ascites, blood clot in the pericardial cavity and discoloured liver with fibrinous casts. Photograph: Brit Tørud, Norwegian Veterinary Institute

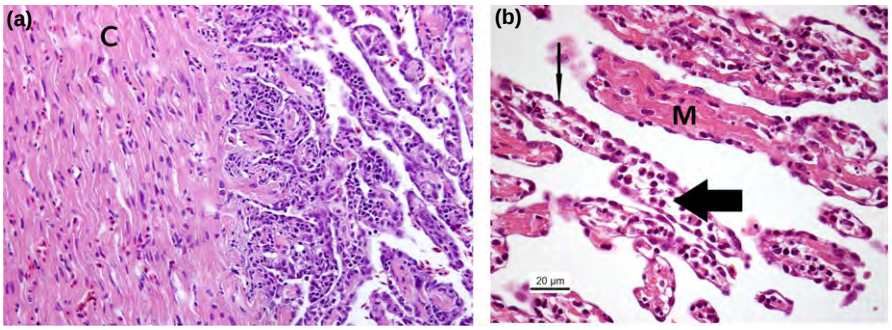

FIGURE 2 Histopathology CMS. (a) Typical histopathological findings of moderate to severe CMS: a distinct border separates severe inflammation of ventricular spongious tissue to the right from normal ventricular compact tissue to the left (C) (H&E staining, 200× magnification). Photograph: Trygve Poppe (b) Typical histopathological findings of severe CMS of the atrium. Large amounts of various inflammatory cells (large arrow) have replaced normal, eosinophilic myocardium (M). The endocardial cells are hypertrophic and hyperplastic (small arrow) (H&E staining, 400× magnification). Photograph: Torunn Taksdal, Norwegian Veterinary Institute

aetiologies, were put forward during the first decades after discovery (Kongtorp, Taksdal, & Lillehaug, 2005).

In 2009, both Fritsvold et al. and Bruno and Noguera demonstrated that CMS is a transmissible disease by reproducing characteristic lesions in smolts injected with tissue homogenate from CMS-diagnosed fish (Bruno & Noguera, 2009; Fritsvold et al., 2009). In 2010, a virus was identified in fish suffering from CMS. The presence and load of piscine myocarditis virus (PMCV) in heart samples from both field and experimental challenges correlated well with diagnosis of CMS and severity of lesions in the heart (Haugland et al., 2011; Lovoll et al., 2010).

3.1 | Classification of PMCV

PMCV share genomic characteristics with members of the family *Totiviridae*, a family that includes viruses that persistently infect protozoan parasites and fungi in five registered genera (Anonymous, 2017). Recently, several other viruses with similarities to *Totiviridae* have been identified. The genomes of these viruses include characteristics indicating a higher complexity than the registered totiviruses. Four of them infect arthropods, namely shrimp, mosquito, fruit fly and ants (Koyama et al., 2015; Poulos, Tang, Pantoja, Bonami, & Lightner, 2006; Wu et al., 2010; Zhai et al., 2010), while PMCV is the first one found to infect a vertebrate host. In 2016, two new viruses with similarities to the *Totiviridae* were found in Golden shiner *Notemigonus crysoleucas* (Mitchill) baitfish from commercial outlets. One of them had closest genomic similarities to PMCV (Mor & Phelps, 2016a), while the other was closer to arthropod-infecting toti-like viruses (Mor & Phelps, 2016b). None of these arthropod- or fish-infecting viruses are yet officially assigned to the virus family.

In general, the viruses registered in the totivirus genera are transmitted to new cells during cell division, sporogenesis or cell fusion. PMCV and the other recently discovered toti-like viruses are either known or presumed to transmit extracellularly, and have extra protein-coding sequences that have been suggested to encompass all or some of their cell entry machineries (Dantas, Cavalcante, Oliveira, & Lanza, 2016; Haugland et al., 2011). This is a feature not shared with the official *Totiviridae* members in general. *Giardia lamblia virus* (GLV) is the only official member which is transmitted extracellularly (Miller, Wang, & Wang, 1988) and is also the totivirus with the closest relationship to PMCV and the other unassigned viruses (Haugland et al., 2011). It is suggested that PMCV, the arthropod-infecting viruses and GLV represent a discrete clade of toti-like viruses that carry components for cell entry and might deserve the definition as a separate virus family or subfamily (Nibert & Takagi, 2013), or a new genera belonging to *Totiviridae* (Dantas et al., 2016).

3.2 | Structure and composition of PMCV

PMCV virions are spherical with a diameter of approximately 50 nm (Figure 3). Similar to members of the family *Totiviridae*, the particles seem to be simple, consisting of a non-enveloped protein shell surrounding the RNA genome. No details of the virion have been revealed, but there are indications of a structural symmetry on the viral surface. The buoyant density of the viral particle is 1.3842 g/mL (Haugland et al., 2011). Based on genome characterization and genetic similarity with other *Totiviridae* members, the protein shell of PMCV is tentatively composed of multiple copies of a coat protein, similar to the icosahedral structures of *Totiviridae* members and other dsRNA viruses (Janssen et al., 2015).

The viral particle encapsidates a non-segmented double-stranded (ds) RNA genome, with a size of 6,688 base pairs. The positive-sense strand has three open reading frames (ORF1, 2 and 3) (Haugland et al., 2011) (Figure 4). ORF1 encodes a protein of 861 amino acids (aa) with a predicted molecular mass of 91.8 kilodalton (kDa). The protein is believed to represent the coat protein, based on comparison with the organization of the genomes of *Totiviridae* (Haugland et al., 2011). ORF2 encodes a protein of 726 aa, which has a predicted molecular mass of 83.1 kDa. This protein is believed to represent a RNA-dependent RNA polymerase (RdRp), based on its position in the genome and similarity to amino acid sequences coding for RdRps found in the *Totiviridae* (Haugland et al., 2011). The

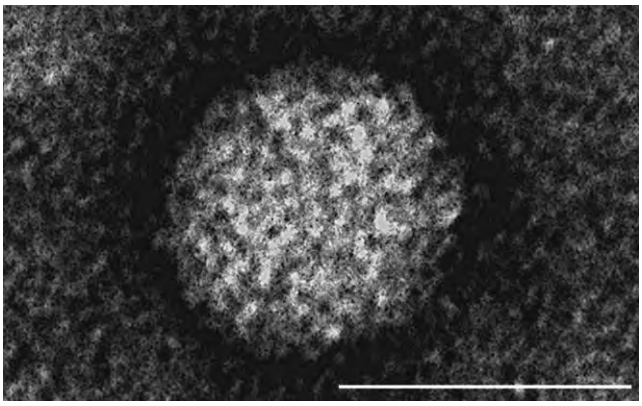

FIGURE 3 EM image of PMCV from Haugland et al., 2011©

presence of a third ORF in the genome has been a feature exclusively seen for PMCV, but it has recently also been found in the PMCV-like virus found in Golden shiner (Mor & Phelps, 2016a). The PMCV ORF3 encoded protein is 302 aa long, with a predicted molecular mass of 33.4 kDa. It shares no sequence homology with proteins of known totiviruses or other viruses/organisms, although BLAST analysis and conserved domain search show some similarity to a chemokine superfamily motif at the N-terminal end (Haugland et al., 2011).

The role of the ORF3-encoded protein in virus particles and infected cells remains elusive, although research on this is ongoing. It seems to be a common feature of GLV and toti-like viruses that infect multicellular organisms using extracellular environments, and they all have additional sequences in the genome. The sequences are mainly related to the capsid, or a polyprotein including the capsid, or an additional protein encoded in an additional ORF, like ORF3 in PMCV. It is suggested that these additional genomic sequences are expressing products needed in the more advanced infection routes than those seen for the simple totiviruses (Nibert & Takagi, 2013). A role of the ORF3-encoded protein in the formation of surface structures of the viral particle was proposed at an early stage, as the predicted molecular mass is similar to the predicted mass of one unit of a trimer-forming fibre-like protrusion on the infectious myonecrosis virus (IMNV) virion. Such fibre-like protrusions are suggested to participate in the extracellular transmission of the virus and thus to play a role in viral pathogenesis (Tang et al., 2008). Yet another possibility is that the encoded protein is non-structural and only present in the infected cell. Recent unpublished *in vitro* research supports this and points towards a cell lytic mechanism related to expression of the protein in the cell (Mikalsen, Haugland, & Evensen, 2014; Mikalsen, Kim, & Evensen, 2016, 2017). Several mechanisms are possible; for instance, the protein promotes the release of viral particles from infected cells and this lytic action might also be related to the necrosed cardiomyocytes characteristic of lesions in severe CMS-affected fish. Others have also suggested a role in enhancing or modulating the inflammation observed in CMS, related to the putative chemokine superfamily motif in the N-terminus of the ORF3 gene product (Haugland et al., 2011).

3.3 | Replication of PMCV

Details of the intracellular replication mechanisms of PMCV are not known. Similarities in the genome characteristics (ORF1 and 2) with other totiviruses indicate similar steps in the replication of the virus. This also includes the organization of these two ORFs, which resemble two overlapping frames with a site for -1 ribosomal frameshift found in the overlapping region (Figure 4) (Haugland et al., 2011). As for the *Totiviridae* members, this might direct the translation of a major capsid protein from ORF1 and a minor fusion protein of capsid protein and the following RdRP from ORF1 combined with ORF2, but this has not yet been confirmed experimentally.

Mature totivirus virions are transmitted to new cells either during cell division, by sporogenesis, during cell fusion or are released from the host cell like GLV (Janssen et al., 2015). PMCV might share some transmission mechanisms with the totiviruses. Still, it has been shown both *in vitro* and *in vivo* that the virus uses an extracellular transmission route (Haugland et al., 2011) and there is also general reason to believe that PMCV uses a more advanced replication and transmission mechanism than the totiviruses, due to the more advanced multicellular organism hosting the virus.

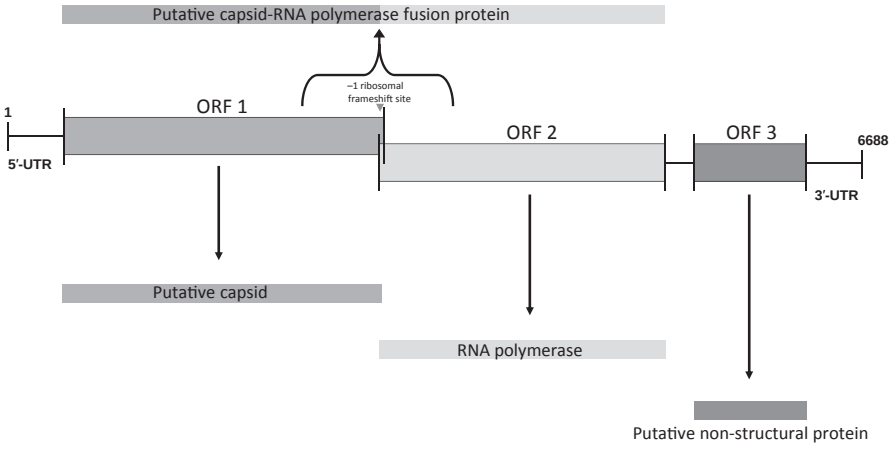

FIGURE 4 Overview of PMCV genome. Aase B. Mikalsen, NMBU

3.4 | Genetic variation and virulence factors

A comprehensive study addressing the genetic variation in the PMCV genome showed that PMCV infecting farmed salmon in Norway is genetically homogenous and seems to belong to a single genogroup (Wiik-Nielsen, Alarcon, Fineid, Rode, & Haugland, 2013). The most divergent isolates in this study shared 98.6% nucleotide identity. The sequences clustered to some extent geographically, for example, isolates from the three most northerly sites grouped together, as did all isolates from the counties of Rogaland and Hordaland. Still, highly similar isolates were also found despite considerable distance between sampling sites. A relatively high variability among within-site isolates was also found in some farms (Wiik-Nielsen et al., 2013).

In a smaller study, Irish isolates were found to be similar to the Norwegian isolates and also presented the same within-farm variation (Rodger et al., 2014). PMCV isolates from wild Atlantic salmon in Norway (Garseth, Biering, & Tengs, 2012) are similar to the isolates from farmed Atlantic salmon (Garseth, Sindre, Karlsson, & Biering, 2016) (Figure 5). A virus sequenced from Atlantic argentine *Argentina silus* (Ascanius) represents the most divergent PMCV isolate. Partial sequence data (1128 nt) of the RdRp gene revealed 86% nucleotide identity, but the majority of the difference was related to different codon usage and the sequence-encoded amino acid sequence with 97% identity to the salmonid isolates (Bockerman, Wiik-Nielsen, Sindre, Johansen, & Tengs, 2011; Tengs & Bockerman, 2012).

The amino acid sequence diversity was higher within the ORF3-encoded protein compared to the putative capsid (ORF1), in both the Norwegian and Irish isolates (Rodger et al., 2014; Wiik-Nielsen et al., 2013). The combination of amino acids in the ORF3-encoded protein, positions 84, 87 and 97, has been suggested as positions for a putative virulence motif, as the combination of amino acids IKR or VQQ has been found exclusively in these positions (Rodger et al., 2014; Wiik-Nielsen et al., 2013). It has not been possible to relate these putative virulence motifs to severity of the disease, due to the lack of reliable data on mortality and severity of disease in individual fish from the farms.

3.5 | Pathogenesis and tissue tropism

The route of viral entry to the fish has not been identified. Several challenge studies, both including i.p. injection and cohabitant challenge, showed that the virus infects the fish and replicates to increasing viral load over time causing a systemic infection with presence in heart, kidney, liver, spleen, gill, muscle, peripheral blood leucocytes, red blood cells and also in serum samples (Hansen et al., 2011; Haugland et al., 2011; Timmerhaus et al., 2011). Virus has been detected in kidney and spleen as early as 1 week after injection, and these organs, together with gills, also had the highest viral levels after 2 weeks and reached the levels found in the hearts after 4 weeks (Hansen et al., 2011). BIn general, heart, spleen and kidney show highest viral loads at both early infection and peak pathology stages (Hansen et al., 2011; Timmerhaus et al., 2011). The heart is considered the target organ for virus replication (Haugland et al., 2011; Timmerhaus et al., 2011). A comparison of microdissected inflamed ventricular tissue with adjacent non-inflamed tissue demonstrated that PMCV was almost exclusively present in the lesions (Wiik-Nielsen, Ski et al., 2012), and high amounts of viral genome are found in the sarcoplasm of degenerated and necrotic cardiomyocytes (Haugland et al., 2011).

4 | DIAGNOSTIC METHODS

The diagnosis of CMS is based on a combination of clinical observations, necropsy and histopathological findings.

The histological assessment of suspected heart lesions by light microscopy can be combined with real-time PCR analysis, preferentially of heart samples, for an aetiological diagnosis (Haugland et al., 2011). There is good correlation between virus genome levels in heart samples detected by real-time PCR methods and histopathological scores of cardiac ventricular lesions (Haugland et al., 2011; Timmerhaus et al., 2011), and as earlier mentioned, the virus has also been found in several other organs and blood components.

Comparison of viral loads between blood, liver, heart, spleen and kidney at 4 and 8 wpi in an experimental challenge showed highest and equal viral loads in heart, spleen and kidney (Timmerhaus et al., 2011), indicating that these tissues are the most suitable for diagnostics and screening of viral presence. In typical cases of clinical CMS, PMCV can be present in extraordinarily high amounts in the tissue samples, compared to many other common viral fish pathogens; Ct values below 10 are not unusual (Fritsvold et al., 2015). This makes careful handling and strict sterile routines at sampling and tissue preparation for PCR, which is crucial to avoid contamination of other sample material and equipment.

The type of cardiac tissue chosen during sampling can be important for several reasons. The development of viral load is highly correlated with the development of lesions (Haugland et al., 2011), and lesions develop sequentially first in the atrium, subsequently in the ventricle. In addition, ongoing studies indicate that PMCV may be unevenly distributed within the atrium, spongious ventricle, compact ventricle and bulbous arteriosus of the heart (personal communication Camilla Fritsvold, Norwegian Veterinary Institute).

Virus-specific nucleic acids have also been detected in fish tissue with histopathological changes typical of PMCV infection, using *in situ* hybridization (Haugland et al., 2011), but this method is not used for routine diagnostics. Detection of PMCV-specific proteins using immunohistochemistry (IHC) has not been established as a routine diagnostic tool due to the limited availability of reliable antibodies against the viral proteins. Theoretically, antibodies against the capsid (ORF1) and ORF3-encoded protein would be good candidates for IHC, but this has proven difficult to obtain, in particular to the ORF3-encoded protein (personal observation, Aase B. Mikalsen, Norwegian University of Life Science). Still, preliminary studies show that IHC on cardiac tissue can serve as a supplement to the

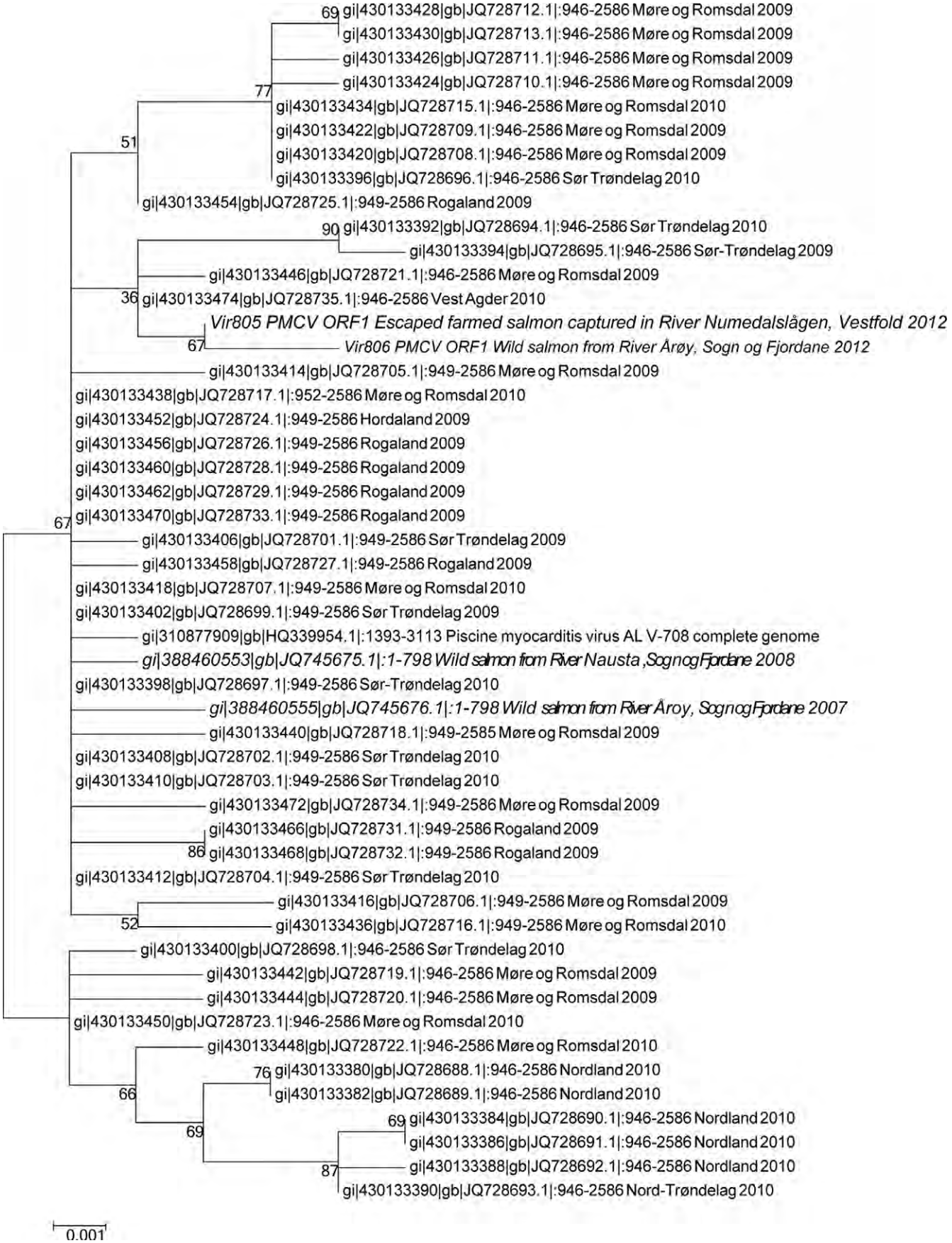

FIGURE 5 Phylogenetic tree displaying genetic variation between PMCV isolates. Åse Helen Garseth, Norwegian Veterinary Institute (Garseth et al., 2016)

diagnostic methods currently available (Gulla, Negard, & Nørstebø, 2012) (Figure 6). Both *in situ* hybridization and IHC have the advantage over different PCR methods: they not only detect specific viral antigens of PMCV, but also visualize their distribution and localization in the examined samples, linking PMCV to the pathological lesions seen in cardiac tissue with CMS.

Several attempts have been made to grow the virus in different cell cultures: PMCV replicates in cultured fish cells, although at low levels, with release of infectious virus to the culture supernatant (Haugland et al., 2011). Still, an efficient enrichment of the virus in the cells has not been achieved and the replication only induces weak signs of a cytopathogenic effect to the cells (Haugland et al., 2011); hence, virus detection and isolation in cell culture is at present not a suitable tool for diagnostics or research.

5 | HOST RESPONSES

Immune and general host responses to PMCV infection and CMS have been studied in experimentally i.p. challenged salmon (Timmerhaus, 2012; Timmerhaus et al., 2011; Wiik-Nielsen, Ski, et al., 2012).

Using microarray-based transcriptome analysis, six gene sets related to early antiviral and interferon response, complement response, B-cell response, MHC antigen presentation, T-cell response and apoptosis were studied over time at early and clinical stages after infection (Timmerhaus et al., 2011). A strong and systemic induction of antiviral and IFN-dependent genes of the innate immune system was shown as early as 2 wpi. While this levelled off during the infection, it was followed by a biphasic upregulation of B-cell genes and genes involved in major histocompatibility complex (MHC) antigen presentation with first peak at 4 wpi and main peak approximately a month later. This late main peak coincided with a high upregulation of genes related to T-cell response including induction of both CD4 and CD8 genes, possible signs of cytotoxic T-cell and helper T-cell activation (Figure 7). At the same time point, typical CMS pathological condition, including cardiac lesions with heavy leucocyte infiltration, was seen in the fish. This also coincided with a peak/plateau phase of viral load in the fish.

A heavy upregulation of complement-response genes in the heart was seen preceding this main peak of the adaptive response, which could suggest a complement-dependent activation of the humoral antibody responses. During the last week of the trial, the viral load was heavily reduced in the fish and severity of the cardiac lesions gradually levelled off and it was suggested that these responses were important for viral clearance and recovery. Still, a study using laser capture microdissection of myocardial lesions in fish sampled at a very late phase of CMS (30 and 33 wpi) shows that the viral genome persists in the lesions despite massive infiltration of leucocytes (Wiik-Nielsen, Ski, et al., 2012).

Despite a strong correlation between viral load and severity of cardiac lesions, Timmerhaus and coworkers noticed that not all infected fish developed significant cardiac pathological condition during their challenge (Timmerhaus et al., 2011). In a second study of the sample set, fish that had developed severe cardiac lesions were grouped as high responders (HR), while fish without significant cardiac lesions were grouped as low responders (LR) (Timmerhaus et al., 2012). The challenged fish mounted similar antiviral and innate immune responses the first weeks post-challenge (Timmerhaus et al., 2011, 2012). From about 6 wpi, the fish group diverged in the two main directions. Alongside with the differences in the development

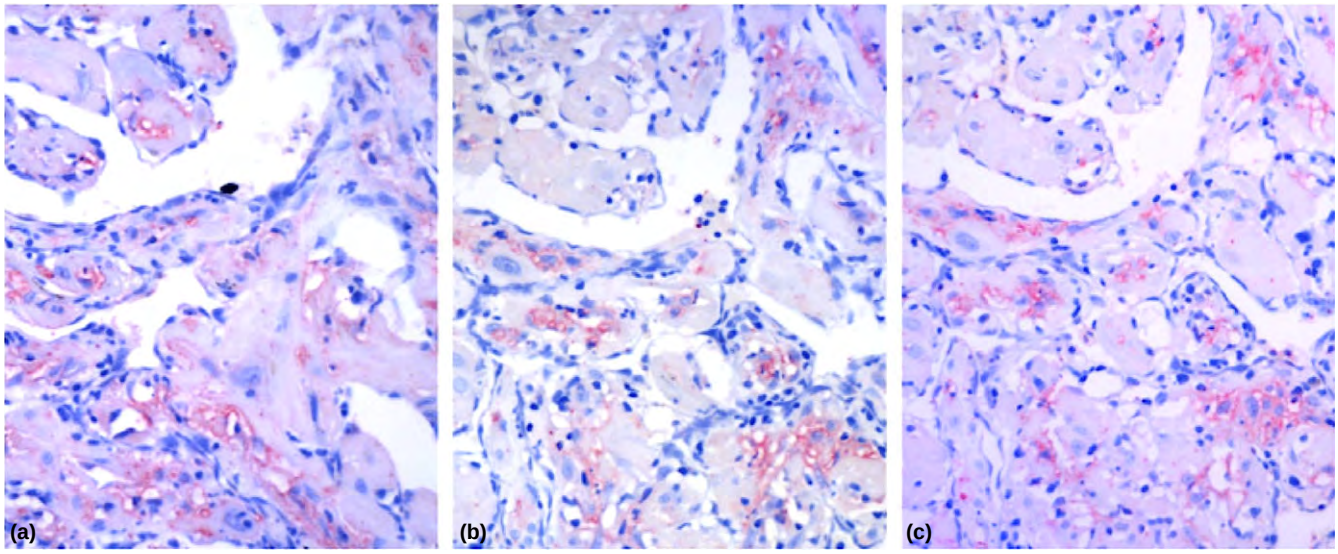

FIGURE 6 Immunohistochemistry of heart samples from Atlantic salmon with a histopathological diagnosis of CMS. Red coloration indicates the presence of PMCV proteins after detection using polyclonal antibodies originally made against recombinant proteins from ORF1- and ORF3-encoded proteins (kindly provided by PHARMAQ AS). (a) Anti-ΔORF1 (truncated variant), (b) anti-ORF1 and (c) anti-ORF3. All antibodies were raised in rabbits and diluted 1:500 and detection subsequently developed using a streptavidin–alkaline phosphatase method (Gulla et al., 2012)

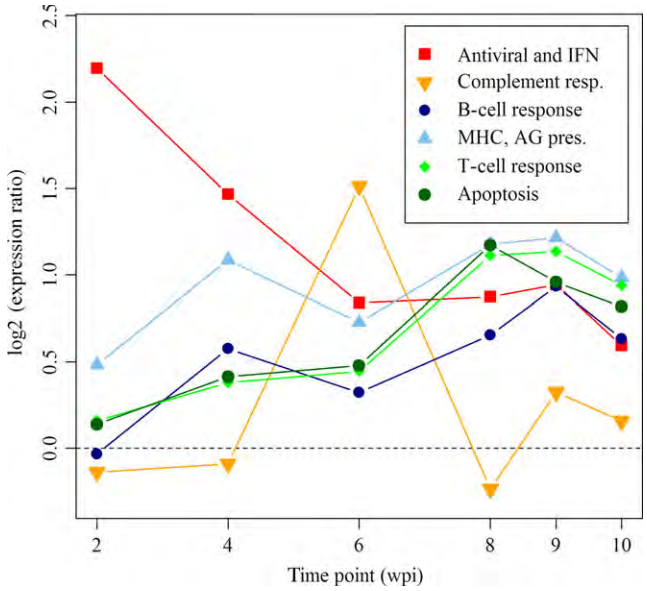

FIGURE 7 Host response: Simplified figure of the immune response of CMS in Atlantic salmon, infected with PMCV in a challenge trial (Timmerhaus et al., 2011). The medians of the expression ratios of the six gene sets are plotted against the sampling time points (printed with permission, Gerrit Timmerhaus, Nofima)

of cardiac pathological condition, the two groups differed both by type and by strength of immune response. A continuous increase in viral load and cardiac pathological condition was observed in the HR fish, coincident with the induction of genes related to apoptosis and cell death mechanisms, suggested to be related to lymphocyte regulation and survival. Subsequently, at late infection phase, a broad activation of genes involved in adaptive response, and particularly T-cell responses accompanied by the increased pathology, has shown to reflect the increased infiltration of virus-specific T cells in the infected heart. In contrast, the LR fish mounted an earlier activation of natural killer cell-mediated cytotoxicity and nucleotide-binding oligomerization domain-like receptor (NOD-like receptor) signalling pathway and later, in sharp contrast, a significantly reduced transcription of the adaptive response and instead activation of genes involved in energy metabolism. It was thus suggested that these LR fish handled the infection by immune responses in the preceding stages and/or by a different composition/regulation of the late responses. Following this, the fish could manage to activate cardiac energy metabolism for recovery and regeneration of infected tissue in the late stage.

Using laser microdissection on formalin-fixed hearts, originating from an i.p. challenge study at 30 and 33 wpi (Wiik-Nielsen, Ski, et al., 2012), tissue samples of typical ventricular CMS lesions were compared to adjacent normal cardiac tissue. Transcript levels of PMCV and immune genes were analysed, and cell populations in the lesions were characterized (Wiik-Nielsen, Ski, et al., 2012). The results showed a strong correlation demonstrating that the leucocyte infiltration of the CMS lesions occurred in response to the PMCV infection, supporting the results of Timmerhaus et al. (2011, 2012). In this study, correlation analysis indicated that activated ($CD83^+$) macrophages ($MHC\ II^+$) may have a coordinating role in CMS lesion development. The simultaneous presence of large amounts of PMCV and various inflammatory cells in the cardiac lesions strongly indicates that the salmon immune response might be insufficient in elimination of the virus (Timmerhaus et al., 2011, 2012). Based on mammalian models of viral infections, it was suggested that innate and adaptive immune effectors may, as an adverse effect, contribute to or actually cause, the myocardial damage seen in severe CMS, perhaps in combination with, and maintained by, development of autoimmunity (Blauwet & Cooper, 2010). Hence, the fish immune system may play a double-faced role in the late phases of CMS: virus-specific immune cells such as B and T cells seem to be important in the clearance of virus, but somehow this activity may actually run out of control in fish with severe pathological lesions, increasing cardiac tissue damage and resulting in immunopathology instead of immunity (Wiik-Nielsen, Ski, et al., 2012).

Correspondingly, Yousaf and coworkers investigated cardiac neuropathy in pacemaker tissue in CMS-, PD- and HSMI-affected Atlantic salmon (Yousaf, 2012; Yousaf, Amin, & Koppang, 2012) and found extensive lymphocytic infiltration of the cardiac conduction system in both CMS- and HSMI-affected hearts. Furthermore, necrosis of cardiomyocytes was observed in close vicinity of the pacemaker tissue in CMS-affected hearts, and immunohistochemistry demonstrated neurogenesis by the identification of proliferative cell nuclear antigen (PCNA). The authors suggest that the extensive lymphocytic infiltrations likely lead to fatal arrhythmias (Yousaf, 2012; Yousaf et al., 2012).

6 | EPIDEMIOLOGY

6.1 | Occurrence and distribution of CMS and PMCV

The first account of CMS dates back to 1985, describing occurrences of an endomyocarditis of unknown, but suspected viral aetiology in Atlantic salmon in mid-Norway (Amin & Trasti, 1988). In the following decade, the disease was reported from surrounding areas and then spread both south and north. Thus, since 2001, CMS has been reported from all salmon-producing areas in Norway every year, even though mid-Norway remains a hotspot (Figure 8) (Hjeltnes, Bornø, Jansen, Haukaas, & Walde, 2017). In 1992, CMS was recorded in Ireland and the Faroe Islands (Rodger et al., 2014), and in 1995 in Scotland and Ireland (Poppe & Sande, 1994; Rodger & Turnbull, 2000). Currently, CMS is rarely observed at the Faeroe Islands (personal communication Debes H. Christensen, Faroese Food and Veterinary Authority). There are some indications that CMS might have been found in Canada, but apart from that, it seems to be confined to salmon-producing areas in the north-eastern Atlantic Ocean.

Thus far, CMS outbreaks have only been observed in Atlantic salmon after transfer to sea, typically occurring during the second year of the seawater phase. In a study including all Norwegian

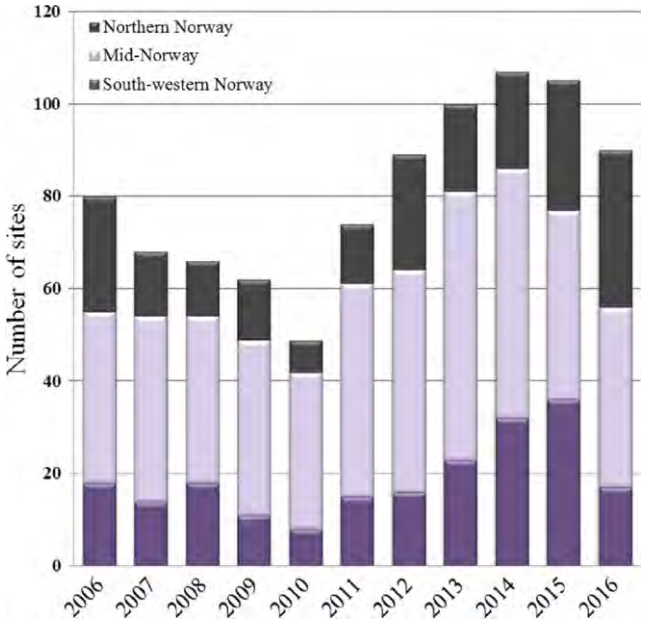

FIGURE 8 An overview of the occurrence of CMS outbreaks along the Norwegian coast, subdivided into regions, from 2006 to 2016. Northern Norway includes counties Finnmark, Troms and Nordland; mid-Norway includes counties Nord- and Sør-Trøndelag and Møre & Romsdal; and finally, south-western Norway includes counties Sogn & Fjordane, Hordaland, Rogaland, Aust-og Vest Agder (Source: Norwegian Veterinary Institute)

salmon cohorts from 2004 to 2012, the median time from sea transfer to diagnoses of CMS was 16 months, with an interquartile range of 13–19 months and an average fish weight of 3.6 kg (Bang Jensen, Brun, Fineid, Larssen, & Kristoffersen, 2013). Cases of CMS have also been reported from fish groups as early as five to 6 months after sea transfer (Fritsvold et al., 2015; Wiik-Nielsen et al., 2016).

In the above-mentioned study (Bang Jensen et al., 2013), altogether 371 (16%) of the 2285 registered cohorts were diagnosed with CMS, and a study from 2003 found CMS registered in 14.6% of the spring smolt and 13.3% of the fall smolt groups, which were followed from sea entry until slaughter (Brun et al., 2003). As for seasonal variations, these are reported to be slight, although with an increase in cases in fall and spring (Kongtorp et al., 2005).

CMS outbreaks and CMS-related pathological lesions have not been described in hatcheries, but PMCV has been found in low quantities at this stage of the production (Wiik-Nielsen, Lovoll, et al., 2012).

6.2 | Transmission routes and in-field disease spread

In vivo experiments have shown that PMCV is transmitted from Atlantic salmon injected with the virus to cohabitating fish. The virus shows increased replication over time in the cohabitants, who also develop cardiac changes typical of CMS (Haugland et al., 2011). In a field study from 2014, infection pressure was found to be one of the most important risk factors for disease diagnosis, underlining that CMS is usually spread horizontally, from farm to farm in sea water (Bang Jensen et al., 2013).

Vertical transmission of PMCV has been suspected and is currently investigated in the CMS-Epi Project. In this project, heart samples from 128 of 132 broodfish were PMCV positive, and viral RNA was also detected by real-time PCR in 60% of milt samples and 69% of the roe samples, although only at levels close to cut-off for the method at Ct value of 35 (Bang Jensen, 2017; Nylund, 2015). Furthermore, PMCV was detected by real-time PCR in all stages of the progeny, including smolts both before and after sea transfer. The prevalence of PMCV-positive fish was >25%, and Ct values were close to the cut-off value of the method (Bang Jensen, 2017).

A very interesting question is whether the low levels of PMCV seen in freshwater phase can be found in the fish group throughout the production phase and have a significant impact on morbidity in the sea phase compared to infection pressure from neighbouring farms and other external factors linked to infection and disease.

In a previous study, the prevalence of viral RNA from both piscine orthoreovirus (PRV) and PMCV in Atlantic salmon broodfish and progeny was investigated by real-time PCR (Wiik-Nielsen, Lovoll, et al., 2012). RNA sequences from PMCV were detected in heart (Ct 24) and spleen (Ct 22) of approximately 80 % of the broodfish before stripping. In the progeny of these fish, viral RNA was detected in seven of 40 fertilized eggs (Ct 38) and five of 20 yolk-sac fry (Ct 38). Upon commencement of feeding, viral RNA was not detected in any of 20 tested fry (Wiik-Nielsen, Lovoll, et al., 2012). However, at this sample size, the minimum detectable prevalence is 14%, which means that prevalence below this level could have avoided observation (Cameron & Baldock, 1998). Whether these detections can be attributed to infective viral particles or simply fragments of viral RNA is currently unknown.

PMCV screening of Atlantic salmon has been performed in Chile since 2013, and so far, no positive samples have been reported (Lara, 2014). Accordingly, PMCV has not been introduced to Chile despite large-scale import of eggs from Norway, a strong contradiction of vertical transmission of PMCV. On the other hand, the latest CMS outbreak at the Faroe Island was in a fish group originating from eggs imported from Norway (personal communication Debes H. Christensen, Faroese Food and Veterinary Authority, and Peter S. Østergård, Aquamed).

In conclusion, the primary route for transmission of CMS is horizontal (Bang Jensen et al., 2013; Timmerhaus, 2012), but vertical transmission of PMCV cannot be excluded (Wiik-Nielsen, Lovoll, et al., 2012) and is therefore a focus of ongoing research.

6.3 | Reservoirs

Based on published studies, the only certain source of PMCV infecting Atlantic salmon is the salmon itself, but further investigation of marine reservoirs including cleaner fish should be initiated. Several potential reservoirs have to some extent been investigated, including spawners of wild Atlantic salmon, some marine species and biota in the environment surrounding fish farms.

CMS-like lesions have been recorded in wild salmon (Poppe & Seierstad, 2003), and two studies have detected PMCV in approximately 0.25% of wild salmon spawners (Ct values of 25–30) (Biering & Garseth, 2013; Garseth et al., 2012). Although exchange of PMCV between wild and farmed salmon is plausible based on phylogenetic analyses (Garseth et al., 2016), the low prevalence in wild salmon indicates that this reservoir is of minor importance for farmed fish. The presence of PMCV in an escaped farmed salmon captured in River Numedalslågen (Ct value of 15) suggests that the virus can be spread by farmed salmon escapees (Biering & Garseth, 2013).

In a real-time PCR-based survey of 32 marine fish species, a strain of PMCV with a separate genotype was found in 11 of 38 pools of tested Atlantic argentine. Nine of 30 tested individuals within the pools were positive (Bockerman et al., 2011; Tengs & Bockerman, 2012). PMCV was not found in the other tested species, but the sample sizes were limited, and definite conclusions about the absence of PMCV could not be made.

Two species of cleaner fish, corkwing wrasse *Symphodus melops* L. and ballan wrasse *Labrus bergylta* (Ascanius), from an Irish Atlantic salmon farm, have been reported with PMCV (Ct values of 29–33) and unspecific cardiac pathological condition (Scholz et al., 2017). Atlantic salmon at the farm were diagnosed with CMS 3 months earlier and mortality due to CMS persisted during sampling of wrasse. The partial PMCV sequences obtained from wrasse were very similar to PMCV sequences from Irish Atlantic salmon, including isolates obtained from the same and a neighbouring farm. The wrasse in question were recruited from wild stocks in the vicinity of the farm and stocked continuously, and according to the authors, annual PMCV screenings of different wrasse species in a number of bays in Ireland had so far been negative. It is therefore likely that cohabiting salmon were the source of the PMCV infection in wrasse. Hence, the authors conclude that ballan and corkwing wrasse are susceptible to PMCV infection under aquaculture conditions (Scholz et al., 2017).

Rainbow trout *Oncorhynchus mykiss* (Walbaum) has been farmed alongside Atlantic salmon since before the CMS was first described, without developing CMS.

In 2014, the presence of PMCV was investigated in sediments, plankton, biofilm and bottom-living organisms, as well as organisms found around the margins of a fish cage on a single fish farm with an outbreak of CMS. PMCV was not detected in any of the environmental samples. However, the virus was found in samples of mucus, faeces and salmon lice from the fish (Hellebø, Stene, & Asphaug, 2014).

6.4 | Risk factors for agent introduction and disease outbreaks

As for other infectious salmon diseases, the probability of developing CMS increases with the length of time in the sea, increasing cohort size and infection pressure. CMS in previous cohorts is also identified as a risk factor (Bang Jensen et al., 2013). The latter factor could be caused by survival of the virus in the environment, for instance in wild or escaped farmed salmon (Biering & Garseth, 2013; Garseth et al., 2012), or it could reflect factors associated with the particular sea site, such as management and environmental conditions (Bang Jensen et al., 2013). The presence of PMCV in cleaner fish, as recently reported from an Irish salmon sea site, represents another potential risk factor especially if the cleaner fish are reused or moved between sites or cages (Scholz et al., 2017).

Diagnosis of HSMI in the same cohorts has been identified as a risk factor (Bang Jensen et al., 2013). This apparent link between HSMI and CMS could reflect similarities in conditions contributing to the development of disease other than the viral pathogen itself, for instance environmental conditions, management or unspecific cardiac responses related to physiology.

The mortality during a CMS outbreak seems to increase if diseased fish are exposed to stress (Skrudland, Poppe, Jarp, & Brun, 2002). Other factors such as fast growth, environmental factors, nutrition and lack of exercise have also been pointed out as potential risk factors that should be further investigated (Lovoll et al., 2010).

6.5 | CMS and other virus infections

CMS can occur in a combined infection with other viral agents. A recent study investigating coinfections of PMCV, SAV, PRV and Atlantic salmon calicivirus (ASCV) found a lack of correlation between levels of PRV, PMCV and ASCV, and a negative correlation between levels of PMCV and SAV. The study material was limited, but the authors suggested that the negative correlation between PMCV and SAV may be attributable to one infection suppressing the other (Wiik-Nielsen et al., 2016). However, it is also pointed out that non-specific immune responses could be involved and should be the focus of further studies. PRV is ubiquitous and a common finding alongside PMCV.

A connection between CMS outbreaks and previous outbreaks of IPNV has been suggested. In one study, a previous outbreak of IPNV was found to occur four times as often in fish groups with CMS compared with CMS-free fish groups (Brun et al., 2003). However, in another study, no associations between previous IPN outbreaks and CMS was found (Bang Jensen et al., 2013), and fish challenged with tissue homogenate originating from fish suffering from CMS developed CMS despite testing negative for IPNV (Bruno & Noguera, 2009).

7 | PREVENTION AND CONTROL

CMS is a transmissible viral disease; hence, the principal preventive measure is to block virus introduction to aquaculture facilities. When PMCV is introduced to a fish group or facility, the outcome of infection is largely influenced by husbandry-, environmental- and host-related factors. Prevention and control of CMS is thus a multifaceted task consisting of biosecurity and husbandry measures in addition to actions aimed at modulating the host response or otherwise

alleviating the course of infection (Aunsmo, Garseth, & Midtlyng, 2006; Pettersen, Rich, Jensen, & Aunsmo, 2015).

7.1 | Biosecurity measures

Thus far, the most important known reservoir of PMCV is farmed Atlantic salmon (Bockerman et al., 2011; Hjeltnes et al., 2016; Wiik-Nielsen, Lovoll, et al., 2012). Accordingly, introduction of Atlantic salmon to a facility represents a risk of introducing PMCV, and in general, keeping both number of introductions and sources of origin of fish low will reduce this risk (Jarp, Gjevre, Olsen, & Bruheim, 1995; Jarp & Karlsen, 1997). A PCR-based screening for PMCV can provide information about infection status of different fish groups such that risks pertaining to introduction, moving or other handling of fish groups can be assessed.

Knowledge about an infectious agent's resistance to disinfectants, UV radiation, organic matter, suboptimal salinity and temperatures is crucial in the assessment of biosecurity risks. So far, the biophysical properties of PMCV are not known and research has been impeded by the lack of viable cell cultures. However, biosecurity assessments should be taken into account that PMCV is a naked virus and therefore anticipated to be fairly robust. This includes an increased likelihood of PMCV transmission by fomites and personnel, but not least transmission through water. In hatcheries, the use of sea water thus constitutes a risk of PMCV introduction, and although the effect of compulsory water disinfection could be beneficial, it has currently not been documented.

The majority of salmon in Norway are produced in open net pens during the sea phase, with fish contained in pens, while water, effluents and pathogens are allowed to pass out and in. Farmed fish at sea sites are therefore constantly interacting with the environment and exposed to waterborne infectious agents from neighbouring farms and wild fauna (Pettersen, Rich, et al., 2015). Generally, temporal and spatial biosecurity measures include employing the "all in-all out" principle combined with fallowing, and furthermore, strategic location of the farm in terms of distance to neighbouring farms, current conditions and thoroughfare of wellboats. The most important measure to prevent spread of PMCV between pens and farms is to reduce the overall infection pressure. This can be performed either by stamping-out of infected farms or by preslaughter of infected pens (Bang Jensen et al., 2013).

A set of measures are used to block vertical transmission. The effect of standard egg disinfection procedure utilized in Norway (100 ppm iodophore for 10 min) against PMCV is currently unknown. Pathogen screening and subsequently discarding gametes from test-positive broodfish is a frequently used measure for vertically transmitted agents. PMCV screening of broodfish is not standard procedure but one of the Norwegian breeding companies, Salmobreed, reports that they, on request from the customer, can offer eggs from brood fish screened for PMCV (personal communication Rudi Ripman Seim, Salmobreed). The practical value of this measure is limited when the prevalence among broodfish is high, for example, after disease outbreak in brood stocks. The establishment of specific pathogen-free brood stock could resolve this challenge.

The salmon farming industry at the Faroe Islands is now nearly free from CMS and PMCV. The disease was practically eradicated from the Faroese industry during the early 2000s when the industry was reorganized in the wake of a serious infectious salmon anaemia (ISA) epizootic. Today, the Faroese salmon farming industry continues to practise this high level of biosecurity, including "all in-all out" principle on site, and strict area based synchronized fallowing. Broodfish are kept in land-based facilities, and detection of PMCV or CMS in a fish group has so far been met with voluntary stamping-out (personal communication Debes H. Christensen, Faroese Food and Veterinary Authority, and Peter S. Østergård, Aquamed).

7.2 | Husbandry

CMS-induced heart lesions are not necessarily fatal per se, but they reduce the cardiovascular capacity and leave affected fish fragile (Brun et al., 2003; Hjeltnes, 2014; Johansen, 2013; Skrudland et al., 2002). Affected fish will thus not be able to withstand even minor stress or physical strain. CMS represents an important fish welfare issue, and accordingly, it is recommended to keep all handling and stress to a minimum until slaughter, to reduce both suffering and losses. Early slaughter, and stun and bleed at site, is frequently applied to reduce losses (personal communication Harald Takle, Marine Harvest). Stress reduction, and in particular early slaughtering, has the added benefit of reducing the total time and amount of virus shedding and thus the infection pressure at site.

Recently, the absence of effective chemotherapeutics against the ectoparasitic copepod salmon louse *Lepeophtheirus salmonis* L. has led to the development of a range of non-medicinal treatments. These comprise crowding and pumping, in addition to exposure to stressors such as elevated temperatures or flushing alone, or in combination with brushing. Mortality due to circulatory failure in fish affected by CMS or other cardiovascular diseases is not uncommon and can be considerable during and after such treatments (Hjeltnes et al., 2016), and again, this represents an important fish welfare problem. Also, impaired gill health, for instance due to infections, can potentially influence the outcome in CMS-affected fish negatively. CMS-associated mortality was thus reduced by introducing routine formaldehyde treatments against gill parasites and fungi upon transfer of brood stock from sea water to freshwater (personal communication Brit Tørud, Norwegian Veterinary Institute). An assessment of health status should always be carried out before a fish group is exposed to stressful handling or treatment.

Various forms of aerobic exercise increase the cardiac capacity and overall robustness of salmonids (Claireaux et al., 2005) and has also resulted in lower mortality in salmonid alphavirus (SAV) transmission trials (Castro et al., 2013). Whether exercise is beneficial for the outcome of PMCV infection is currently unknown.

7.3 | Modulating host response

7.3.1 | Selective breeding

In two separate generations, AquaGen recorded a substantial between-family variation in CMS-induced mortality and subsequently identified a genetic marker (quantitative trait loci—QTL) for CMS resistance (http://aquagen.no/wp-content/uploads/2015/07/qtl-innova-cms-2015-english.pdf). QTL-selected salmon are expected to have lower viral load and morbidity, with less severe cardiac lesions. This results in lower mortality during CMS outbreaks and also increased ability to withstand transportation and handling in the final stages of production. The documentation is supported by beneficial health economic calculations. QTL-selected eggs with CMS resistance have been available for farmers since 2013, and after 2016, two of three AquaGen products have this characteristic. Taking the market situation into account, it is estimated that one quarter of smolt transferred to sea autumn 2017 and spring 2018 will be based on eggs QTL-selected for CMS resistance (personal communication Torkjel Bruheim, AquaGen).

Cardiovascular health and capacity in general has also been prioritized by several other breeding companies (personal communication Rudi Ripman Seim, Salmobreed), and in the next few years, it is anticipated that CMS-specific resistance will be included in the breeding programme of several other companies as well (personal communication Harald Takle, Marine Harvest).

7.3.2 | Vaccination

An effort to develop a vaccine against PMCV is ongoing, but has so far been hampered by the lack of a cell line for *in vivo* virus replication (personal communication Øyvind Haugland, Pharmaq). However, the fact that there is little genetic variation in the Norwegian virus isolates studied to date is considered promising for the ongoing vaccine development.

7.3.3 | Functional feed and clinical diets

Functional feeds are feeds that beyond their nutritional composition are formulated with health-promoting features (Martinez-Rubio et al., 2014). The health-promoting function is typically gained either by altering the quantity or ratios of existing ingredients or by adding new ingredients. A study published in 2014 concluded that salmon fed trial diets with lower lipid content (~18% versus ~31%), and higher Ω-3/Ω-6 ratio (PUFAs) (~4 to ~1.4) than in a reference diet, performed better after intramuscular challenge with PMCV. In one trial diet, histidine was added as this amino acid plays important roles as buffer and antioxidant in muscle cells (Martinez-Rubio et al., 2014). The study concluded that lipid content and composition may have an immunomodulatory effect, resulting in a milder and delayed immune response after PMCV infection, and significant reduction in tissue damage during CMS outbreaks. Adding histidine to the diet had no effect on CMS-related lesions.

Currently, several commercial feed companies are offering functional feeds aimed at strengthening the cardiovascular health, increasing the tolerance to stress and promoting dietary uptake during CMS, PD and HSMI. Farmers are in general recommended by the manufacturers to start feeding as early as possible during the course of disease, or even before expected risk periods.

7.4 | Legislative control

CMS is not and has never been a notifiable disease in Norway, Faroe Island, Scotland nor in the OIE. The considerable time-lag between the first appearance of CMS and the discovery of the aetiological agent has made legislative control measures challenging. The Norwegian FSA latest assessments concerning inclusion of CMS on the disease list was in 2008. The disease remained unlisted (personal communication Stian Johnsen, Norwegian Food Safety Authority). Despite its infectious appearance and significant economic impact, the implementation of control strategies would be impeded by the lack of knowledge about the aetiological agent.

Listing of a disease has the benefit of providing an overview of the disease situation, something that is not available for CMS as it is. The yearly published Fish Health Report presents the number of farms in Norway diagnosed with CMS by the Norwegian Veterinary Institute. However, in recent years, an increasing number of private laboratories are offering various diagnostic services, making this overview less exhaustive than earlier. Thus, the feasibility of maintaining an overview of the disease situation will depend on new collaborative agreements between the diagnostic laboratories and the willingness of the industry to supply and publish information on disease occurrence, also for non-listed diseases.

8 | PRODUCTION LOSS AND THE ECONOMIC PERSPECTIVE

CMS may have different manifestations and thus different loss profiles in various farms, but will in general strike late in the production cycle and affect fish in good condition. The potential for economical loss is therefore considerable. In 2002, the direct annual financial losses of CMS was estimated to € 4.5–8.8 million for the Norwegian salmon industry (Brun et al., 2003).

In 2007, Marine Harvest Norway (MHN) reported a biological loss of 1200 tonnes of salmon due to CMS during a 6-month period. Assuming that this was representative for the industry and that MHN accounted for 25% of the total Norwegian production, the overall CMS-related loss for the Norwegian salmon industry was estimated to more than € 25 million (NOK 200 million) that year (http://www.fhf.no/prosjektdetaljer/?projectNumber=900261).

The two estimates were based solely on output losses (biological loss), while cost of prevention and extraordinary costs, for instance, due to higher labour cost, potential reductions in growth rate and feed utilization were not considered.

Since 2007, the number of CMS cases reported by NVI and private laboratories has increased (Hjeltnes et al., 2016), and more fish are probably affected per case today as the number of fish per location has more than doubled. In addition, both production expenditures and sales prices of salmon have risen (Olafsen, Winter, Olsen, & Og Skjermo, 2012) (http://www.fiskeridir.no/English/Aquaculture/Statistics). Accordingly, the economic impact of CMS remains undisputable, and the potential for financial benefit through the introduction of efficient control measures is considerable.

9 | NEW INSIGHTS AND FUTURE INVESTIGATIONS

Unfortunately, the lack of knowledge about PMCV, the causative agent of CMS, has hampered the ability of industries and authorities to implement effective control measures. PMCV has therefore been able to spread throughout the industry. In a Norwegian survey, respondents representing the salmon farming industry and fish health services regard CMS as one of the most serious disease problems. The disease results in reduced fish welfare, significant management-related challenges and mortality in ongrowing and broodfish farms (Hjeltnes et al., 2016).

PMCV has just recently been described, and although tools to detect the virus in a practical and diagnostic setting are available, the virus is not fully characterized. The structure of the viral protein shell is unknown, and the function of proteins encoded by the genome (ORF1-ORF3) has not been fully understood; especially, ORF 3 has intrigued the researchers as it is not present in the registered totiviruses. The replication mechanism of the virus is currently investigated, as are characteristics pertaining to virulence and antigenicity.

A suitable and available cell line for *in vivo* virus replication is necessary to enable evaluation of the biophysical properties of the virus, including resistance to disinfectants, to increase knowledge of the pathogenesis of CMS, to refine experimental trial models and to move forward in the development of vaccines and diagnostic methods. For diagnostic use, more efficient production and improved quality of antibodies towards the viral proteins are needed.

The route of entry of PMCV is not known; neither are the target tissues and cells, how the virus is transmitted, how it behaves in the host during infection, which factors are important or even necessary for the development of disease in the host, nor how and when the virus is released from the host.

Reservoirs of PMCV have only to a limited degree been investigated. It is therefore necessary to identify possible reservoirs and implement targeted measures to prevent restocking of virus from these reservoirs. Interesting in this context is the detection of PMCV in cleaner fish (Scholz et al., 2017).

Regardless of origin of the virus, the occurrence and significance of CMS indicate that the virus is endemic in parts of the Norwegian industry. Control and mitigation of the disease will therefore require a coordinated effort from farmers and competent authorities. It is thus imperative to gain sufficient knowledge to be able to implement effective industry-level control measures. In this context, it is noteworthy that animal health economics studies have increasingly been applied to estimate the economic impact of disease and the value of control measures (Aunsmo, Valle, Sandberg, Midtlyng, & Bruheim, 2010; Pettersen, Osmundsen, Aunsmo, Mardones, & Rich, 2015; Pettersen et al., 2016). Such models could be valuable tools in future management of CMS in farmed salmon.

Setting the economic impact of CMS aside, it is evident that this disease has a significant negative impact on the welfare of farmed salmon. There is also a potential for harmful effects on wild conspecifics. The competent authorities should therefore safeguard the health and welfare of farmed salmon and prevent potential adverse effect on wild stock through regulations.

The aquaculture industry is constantly seeking to optimize their production, and some of the new production methods may have a beneficial effect on diseases. For example, some egg producers have begun to keep cohorts of broodfish on land for their entire life cycle. Used in combination with screening and selection of pathogen-free broodfish, this approach could be a successful way of mitigating CMS, as a vertical transmission route has not yet been excluded. Growing smolt to a larger size before sea transfer will make them more robust towards infections in general and in addition potentially reduce the total farmed biomass and production time in the sea. More effective management of the salmon louse *Lepeophtheirus salmonis* L. and amoebic gill disease (AGD) is required to reduce the number of stressful treatments that cause mortality in fish today. Floating enclosures will decrease interaction with, and transfer of parasites to and from, the environment (Nilsen, Nielsen, Biering, & Bergheim, 2016).

More knowledge about factors pertaining to the prevention and control, and thus the epidemiology of PMCV and development of cardiomyopathy syndrome, is needed. Gained knowledge on both topics can be translated into industry-level control measures and improved husbandry practices, thus improving fish welfare, preventing potential impacts on wild stocks and not least reducing mortality and costs for the industry.

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CONFLICT OF INTERESTS

No conflict of interest has been identified for any of the authors, although Aase B. Mikalsen is listed as inventor in the PMCV-patent. A patent owned by Pharmaq.

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Vedlegg 7

Workshop om vertikal overføring av virus i lakseoppdrett.

Scandic Hotel Ørnen, 1.februar 2017 kl 9-11. Møterom «Kuling 2»

Presentasjon av workshoppen:

Det er i laksenæringen stor interesse for muligheten for vertikal overføring av agens, dvs overføring av smitte fra stamfisk til sjøsatt smolt via settefiskfasen. Om slik smittetransmission er mulig, gir det store konsekvenser for smittespredning og kontroll. I FHF-prosjektet «Epidemiologisk studie av Kardiomyopatisyndrom (CMS): Utredning av spredning, risikofaktorer og sykdomsforløp i felt», har vi undersøkt muligheten for overføring av PMCV fra stamfisk til sjøsatt smolt under gjeldende produksjonsforhold, og vi ønsker nå å presentere og diskutere resultatene på en workshop der vi tar for oss flere aspekter rundt dette med mulig vertikal overføring, inklusive muligheter for kontroll.

Program:

- Vertikal overføring av virus: hvordan skjer dette rent biologisk, og hva skal til for å si at det er snakk om «ekte» vertikal overføring. V/ Espen Rimstad, Professor, NMBU
- Status vedr kunnskap om vertikal overføring for ulike virus. V/ Are Nylund, Professor UIB
- Biosikkerhet i genbank for vill laks –en strategi for å beskytte stamfisk mot smitte. V/ Åse Garseth, Forsker, Veterinærinstituttet
- Presentasjon av metode og funn fra CMS-prosjektets arbeide med vertikal overføring av PMCV. V/ Britt Bang Jensen, Seniorforsker, Veterinærinstituttet
- Diskusjonsrunde. Moderator: Harald Takle, Marine Harvest
    - Hvordan komme nærmer en avklaring på vertikal overføring eller ikke
    - Muligheter for kontroll og forebygging
    - Identifikasjon av kunnskapshull

Deltakere på workshop om vertikal overføring av virus i lakseoppdrett 01.02.2017

- Torkjel Bruheim
- Bård Skjelstad
- Kari Olli Helgesen
- Arne Guttvik
- Stian Nylund
- Anne Berit Olsen
- Brit Hjeltnes
- Harald Takle
- Torunn Taksdal
- Julia Fossberg
- Anne-Gerd Gjevre
- Inger Helene Meyer
- Eirik Sigstadstø
- Sven Martin Jørgensen
- Henrik Duesund
- Øyvind Breivik
- Patricia Apablaza
- Britt Bang Jensen
- Julie Svendsen
- Espen Rimstad
- Are Nylund
- Lars Qviller
- Åse Helen Garseth
- Hetron Mweemba Munangandu
- Siri Sakaya
- Miriam Furne
- Anette Mjanger

Referat fra workshop om vertikal overføring av virus i lakseoppdrett

Hotell Scandic Ørnen, 1.februar 2017

Vertikal overføring av virus: hvordan skjer dette rent biologisk, og hva skal til for å si at det er snakk om «ekte» vertikal overføring. V/ Espen Rimstad, Professor, NMBU:

Espen la ut med å forklare, at OIE's Aquatic Animal Health Code (2008) har følgende definisjon på vertikal overføring: "Vertical transmission means the transmission of a pathogen from a parent aquatic animal to its progeny via its sexual products". Vertikal overføring av smitte hos fisk vil si at foreldrefisk smitter avkom via rogn og melke. Begrepet er uavhengig av om smittestoffet befinner seg på innsiden eller utsiden av egget."Vertical transmission" er ikke definert i OIE's Aquatic Animal Health Code (2016).

Når en ser på RNA virus' biofysiske egenskaper, er det åpenbart at virus ikke er laget for å ligge inaktiv lenge i en celle, siden RNA har høy turnover i cellen. Infeksjon med RNA virus medfører heller ikke toleranse. Noen DNA virus kan dog gi latente (sovende) infeksjoner, fordi det ikke brytes ned på samme måten som RNA virus. De eneste kjente virus som nedarves hos vertebrater er de hvor genometer integrert i kjønnscellers DNA, det vil si endogene retrovirus. Espen mener ikke at det er beskrevet noe tilsvarende hos fisk.

Når en påviser virus ved PCR-metoder, må en huske på at slike metoder, som ikke innebærer dyrkning av agens, ikke nødvendigvis påviser et smittsomt agens. Derfor er det en vurderingssak hva betydningen er ved PCR-funn av virus i for eksempel kjønnsprodukter og befruktede egg.

Om virus skal kunne overføres vertikalt, må det tåle overflatedesinfeksjon av egget, være svært motstandsdyktig i miljøet, forbli assosiert med rognkorn helt til klekking, smittes fra rognkorn til yngel etter klekking og forårsake sykdom hos yngel. Det kan også hende, at det heller er snak om mekanisk overføring av virus etter desinfeksjon.

Hvordan bør en gå frem for å finne ut om virus overføres vertikalt: Rogn må være desinfisert. Dette materialet må deretter males opp og injiseres i frisk fisk for å se om det kan smitte.

Espen avsluttet med å stille forsamlingen spørsmålet om hvorfor vi egentlig er så interessert i å finne ut om det foregår vertikal overføring av ulike virus?

Status vedr kunnskap om vertikal overføring for ulike virus. V/ Are Nylund, Professor UIB

Are åpnet med å si, at spredning av laksevirus alltid vil reflektere biologien til laksen. Virus bør være lavvirulent for å kunne spre seg. Det finns eksempler på såkalte «fossile» virus som er inkorporert i vertens genom, og disse sprer seg trolig vertikalt.

Deretter gjennomgikk han forskjellige fiskevirus med tanke på vertikal overføring:

- SSSV (swimbladder sarcoma virus) er et endogent retrovirus beskrevet av Paul et al 2006, som også er funnet i genomet hos norsk laks. Are påpekte, at det bare er ILA som replikeres i cellekjernen, de andre replikeres i cytoplasma.

- Poxvirus (SGPV): vanlig hos både vill og oppdrettslaks, i både sjø og ferskvann. Stor genetisk variasjon tyder på at det opprinnelig er et laksevirus. Men det er også funnet hos noen rekearter.
- Paramyxovirus er ikke undersøkt, og det er ingenting som tyder på vertikal overføring.
- PMCV: Virus er funnet i yngel, men med lav prevalens og fra en liten prøvemengde (Wiik-Nielsen et al)
- PRV: Er funnet i Chile og USA, og har trolig vært i USA lenge før vi begynte med oppdrett i Norge
- SAV: Castric & Cabon utførte smitteforsøk i 2006. Yngel ble holdt i kar i 5 mdr. (Stamfisken 20 hunner og 20 hanner) ble smittet med SAV1. Vanlig desinfeksjon hindret ikke vertikal overføring. Men dette er tydeligvis kontroversielt, og fortsatt uavklart
  I eget forsøk publisert 2009, ble det funnet veldig lav prevalens i yngel, lavere enn det som vil bli detektert v. screening. Kongtorp et al 2010 fant SAV i hjerte, men ikke i ova og sperm. Karlsen sin artikkel (2014) sier at PD ble introdusert fra et villt reservoir. Mener at det er mulig at PD er vertikal overført. Grunnen til det ikke er i Chile kan være fordi egg er blitt kjøpt fra nordmøre før 2007 (da det ikke var PD der).
- ILA: I et prosjekt fantes 83% prevalens av ILA HPR0 i stamfisk, og fant det igjen i egg etter desinfeksjon, samt i yngel og smolt. Fortsatt veldig delte meninger om ILA kan være vertikal overført eller ikke.

Are sluttet med å minne om Førevar prinsippet og at en derfor bør fokusere på å finne ut mer om vertikal overføring og kanskje tenke som om de fleste virus er vertikal overførbare.

Biosikkerhet i genbank for vill laks –en strategi for å beskytte stamfisk mot smitte. V/ Åse Garseth, Forsker, Veterinærinstituttet

Åse fortalte om genbanken, som består av både en frossen genbank med cryopreservert melke initiert i 1986, og en levende genbank med vill laks holdt i anlegg. I genbanken er det fokus på å hindre introduksjon, oppformering og spredning av vertikal overført sykdom. Stamfisken screenes for IPNV, BKD og PMCV, og egg fra positive stamfisk kasseres. Fisken går i landbaserte anlegg, i ferskvann hele livet, fra elver uten anadrome arter. Alle vannenheter har seperat vannintak, og det utføres vanlig helsekontroll 12 ganger i året, inklusive obdusering av fisk.

Det er gjort studier på vertikal overføring av PRV, SGPV og PMCV, der det ble påvist 0.25% prevalens av PMCV i foreldre, mens det var 20% for PRV og 85% for SGPV. Virus ble ikke påvist i avkom, men resultater fra PMCV testing foreligger ikke.

Presentasjon av metode og funn fra CMS-prosjektets arbeide med vertikal overføring av PMCV. V/ Britt Bang Jensen, Seniorforsker, Veterinærinstituttet

Britt inledte med å introdusere målet med denne delen av CMS-Epi prosjektet, som er å finne ut om PMCV overføres vertikalt fra stamfisk til settefisk og om PMCV føres med smolten videre i fra settefiskfasen frem til sjøsetting, under faktiske produksjonsforhold.

I prosjektet har vi fulgt to grupper, fra to forskjellige aktører, der vi har testet hjerter fra stamfisk, og så valgt ut grupper av hannerxhunner som vi har fulgt. Der har vi testet kjønnsprodukter, egg, larver, yngel og frem til smolt. Prevalensen var veldig høy i stamfiskhjerte, og høy i kjønnsproduktene. Det

var mulig å finne virus i egg og yngel helt frem til smoltstadiet, men med høye Ct-verdier (lav virusmengde). Vi fant, at prevalensen i egg falt drastisk etter desinfeksjon, men at virus ikke nødvendigvis var helt borte.

Videre er det planlagt sekvensering av prøvene fra ulike stadier, samt å sammenholde med resultater fra resten av prosjektet. Vi må diskutere funnene internt og med andre for å skjønne betydningen.

Diskusjon (kommentarer samlet som kulepunkter):

- For å finne ut om PMCV overføres vertikalt, bør vi prøve å male opp eggene etter desinfeksjon og injicere dette i friske fisk for å se om det er infektivt. Altså et levende assay.
- Marine Harvest nevnte, at de tapte over 1 mio fisk pga CMS i 2016.
- Det ble spurt om vi kanskje burde ha sett høyere mengde virus, siden virus replicerer seg, for eksempel i yngel.
- Det er et problem at vi mangler gode cellekulturer for å kunne dyrke virus.
- Det er rart at det ikke er sett stor spredning av PMCV i settefisk i produksjonen.
- Det ble nevnt et studie (upublisert), der en brukte dyrket virus, og der en fant en virus i milt og nyre før en fant det i hjerte.
- Er PMCV virkelig så homogen? Og hvilken betydning har det? Det er små variasjoner i ORF3, men vi vet ikke så mye om hva det betyr. Vi må forvente at variasjoner finnes i capsidproteinene.
- En bør kanskje være førevar og fokusere på å screene stamfisken, og da bare bruke PMCV-fri stamfisk, selv om dette kan være vanskelig når prevalensen av PMCV i stamfisk generelt er meget høy.
- En må heller ikke utelukke at det kan forekomme subkliniske infeksjoner.
- Det er rart at det ikke ses CMS rett etter sjøsetting, fordi det er der fisken har vært utsatt for aller mest stress (transport, blanding osv).
- De har ikke CMS på Færøerne der en bare bruker virus fri stamfisk. Nå er en begynt å finne klinisk CMS i Irland og Skotland.
- En driftsleder fortalte, at hans opplevelse av CMS er at sykdom holder seg til smoltgrupper. Fisken dør i tusenvis, men dette er knyttet til smoltleverandør. Fisken dør brått, og det er den fineste fisken som dør. De dør vanligvis under foringsstress, vanligvis i forbinnelse med hurtigvoksperioder.

Vedlegg 8

Synopsis/Summary of CMS-workshop held in Bergen March 2018

Contents:

Attachment: List of participants.

Listing of knowledge gaps:

PMCV:

- Characterize proteins that are coded for by the virus RNA
- Understand mechanisms for infection and replication in the host
- Develop cell culture
- Biophysical properties
- Identification of virulence markers
- Implications of quasi-species

CMS:

- Pathogenesis
- Factors that trigger disease development in infected fish
    - Consequences of de-lousing
    - Why the difference between S0 and S1?
- What is the importance of coinfections with CMS?
- Can infected fish get rid of virus?
    - Regeneration or repair of the heart?
- Refinement of diagnostic methods
-

Transmission, prevention and control:

- Can PMCV be transmitted vertically?
    - Is virus fond in eggs infective?
    - How does the virus survive the freshwater phase?
- Virus reservoir (beyond the farmed salmon)
- The importance of smolt from freshwater phase?
- What are possible industry management strategies? Can we apply the same as for PD…?
- How do we prevent CMS, and how do we minimize impact of infection?
- What is potential for vaccine development?

Program:

Wednesday, March 14th	**CMS-workshop**	
13.00-15.15	**Welcome & Situation updates**	Chair: Britt Bang Jensen
13.00-13.15	Welcome and introduction	Britt Bang Jensen, NVI
13.15-13.30	Overview of the CMS-situation in Ireland	Susie Mitchell, FishVetGroup
13.30-13.45	Update on the situation in Norway	Camilla Fritsvold, NVI
13.45-14.30	CMS from an industry perspective (15 min presentations)	Sandra Sclittenhardt, Marine Harvest Ireland Arne Guttvik, Salmar

Thursday, March 15th	**CMS-workshop**	
9.00	**New research V: Transmission and development of CMS**	Chair: Sven Martin Jørgensen, FHF
9.00-9.20	Review of current knowledge & Update on CMS in wild salmon	Åse H. Garseth, NVI
9.20-9.40	CMS in the Faroe Islands	Debes Christiansen, Faroese Food and Veterinary Authority
9.40-10.00	The case for and against vertical transmission of PMCV	Britt Bang Jensen, NVI
10.30-12.00	**New research VI: Infection dynamics and diagnostics**	Chair: Neil Ruane, Marine Intitute
10.30-10.50	Development of infection in the field on a herd level	Julie C. Svendsen, NVI
10.50-11.10	Risk factors for CMS	Arthur Mårtensson, NVI
11.10-11.40	Screening for PMCV	Stian Nylund, Pharmaq Analytiq
11.40-12.00	PMCV: Improved diagnostics and investigating the prevalence in farmed and wild fish in Ireland	Andrew Tighe, Marine Intitute
	New research VI: Infection dynamics and diagnostics	
13.00-13.20	Infection dynamics and genetic variability of the virus over the course of infection:	Øystein Evensen, Norwegian University of Life Sciences
13.20-13.40	Development of CMS challenge models	Makoto Inami, VESO Vikan
13.40-15.00	**Discussion: -Options for control -Research gaps**	Facilitator: Sven Martin Jørgensen, FHF

Participants:

A total of 121 persons had signed up for the PD-meeting and CMS-wokshop. The final number of participants was 123. The list of participants is attached.

Minutes:

Most of the presentations from the meeting has been published at www.trination.org, but some presenters have reserved their presentations for publication, due to sensitive information or because they plan to publish in a peer-reviewed journal.
Below are some comments and questions for each presentation, and minutes from the discussion.

Individual presentations:

Overview of the CMS-situation in Ireland (Susie Mitchell, FishVetGroup):
-CMS is an increasing problem
-The first case was in 2012, in 3.5kg fish with low mortality but dramatic presentation (Published by Rodger et al 2014). Next case was in 2014. In 2016 and 2017 there were 4 cases each year
-A prevalence survey of 2016/2017 stock revealed that all have PMCV, but with high ct-values except for clinical outbreaks.
-Typical clinical presentation:
    -rise in mortality in 1-3 pens, dramatic clinical signs. Other pens might become diseased
    -viral load appears to be directly correlated with severity of pathology
    -virus can persist for the remainder of the generation, but it can also disappear
    -Initially the outbreaks came after 14-18 mths, but later it is in 4-8 months
    -CMS is diagnosed in a lot of cases where there is already other diagnoses
-PMCV has been detected in cleaner fish that were cohabitating with salmon with clinical CMS
Knowledge gaps from the presenter:
-What are possible industry management strategies? Can we apply the same as for PD…?
-How do we prevent CMS, and how do we minimize impact of infection?
-What is potential for vaccine development?
-What is the importance of coinfections with CMS?
Comments from the audience: The heart from the infected wrasse looks like a VHS heart –important to think about differential diagnoses.

Update on the situation in Norway (Camilla Fristsvold, Norwegian Veterinary Institute)
-The presentations is based on Fish Health report from 2017 (available at www.vetinst.no)
-Clinical presentation of CMS:
    -Either acute outbreak or more prolonged, chronic.
-In 2017, CMS was diagnosed in 96 farms at NVI, and 100 farms in private labs, but there is most likely an overlap of these reportings.
-CMS is distributed over the whole country, but there are some hotspots in Møre & Romsdal and in Hordaland
-CMS was ranged as the second most important problem by the fish health personnal, at the same level as mechanic injuries due to delousing, and second only to salmon lice (so ranged more important than PD)
-Increasing importance most likely due to increased handling (because of for example mechanical delousing).
Knowledge gaps from the presenter:
-Cell culture method
-Refinement of diagnostic methods
-Knowledge of pathogenesis
-Good vaccine
-Biophysical properties

CMS from an industry perspective (Sandra Sclittenhardt, Marine Harvest Ireland)
-Before 2016, CMS was no big problem in Ireland.
-In one case, only the two mid pens in a site was infected and had disease, but not the rest.
-Disease presents itself differently from site to site
-In 2017 new smolt was introduced into an area where there was already CMS, and these got CMS after 3 mths.
-There was no pattern in which pen got disease –even if they shared wellboat or came from different or same hatcheries. Some pens were fine, but got sick later in the year when they were handled and there was algae bloom etc. The cage difference is also observed for PD and IPN.
-CMS seems to have had an effect on the fish all the way through to slaughter.
-Retrospective testing of broodstock, showed nothing before 2009, but all broodstock from 2009 to now were positive for PMCV.
-Screening before seatransfer also showed a high prevalence in recent years, and PMCV is now detected in all farming areas.

CMS from an industry perspective (Arne Guttvik, Salmar)
-The PD border has been moved further and further north, but SAV3 is contained below Stadt
- CMS causes more loss in biomass than SAV (almost double)
- CMS is mostly a problem in the Mid region
-Large difference between fish groups at the same site
-For S0 fish, CMS is mainly a problem in the second year at sea
-Mortality due to SAV is probably hiding some of the CMS
-Compared to PD, CMS is a lot worse problem, but there are regulations for PD and not for CMS
<u>Knowledge gaps from presenter:</u>
-Why do we focus more on PD and legislation on that?

Review of current knowledge (Åse Helen Garseth, Norwegian Veterinary Institute)
-Review of current knowledge on CMS and PMCV is available at:
http://onlinelibrary.wiley.com/doi/10.1111/jfd.12735/epdf
-The increased mechanical delousing is causing stress and more disease
-Listing of CMS was last evaluated in 2008, when the agent was not yet identified
-Economic impact in 2007 was estimated to 25mio€
Update on CMS in wild salmon:
-CMS on wild salmon described in 2001-2002
-In review: 3 of 1250 broodfish from 2007-2012 was PMCV positive
-PMCV included in the test program for wild salmon from 2016. In 2016, 7 positive of 125 tested, all positive from Hardanger (CT 20.5-34.1). Tested offspring (60 samples) were all negative (The offspring that were tested were parr, and there is no information regarding ct-values above 37). In 2017: 1 of 146 was positive (also from Hardanger)
-Institute of Marine Researhc found PMCV in fingerlings in Hardanger –are these from wild, farmed or hybrids?
-Sequencing showed that there are differences in isolates from two salmon from the same river
<u>Comments from the audience:</u>
-CMS is the nicest disease to die of, since there is sudden mortality, so it is not a big welfare problem
Reply: Many fish can have liver damage or heart damage long before and suffer

CMS in the Faroe Islands (Debes Christiansen, Faroese Food and Veterinary Authority)

-Before 2000, aquaculture was not regulated, and there were high stocking density and short distances. In 2005 almost total fallowing of the industry, because of ISA. Now there are 22-25 marine sites owned by 3 big companies, and 8-10 freshwater farms.
-From 2005-2013 there were no diseases (and intensive screening).
-There were outbreaks of CMS in the 90'es, but since there has been no, or only occasional findings of CMS.
-Screened for PMCV from 2011
-In 2012, PMCV was found in returning wild salmon
-In 2013: One clinical outbreak of CMS
-In 2017: 5 outbreaks, plus 5 farms with detected PMCV
-In 2014: outbreak was in fish imported eggs from Norway:
    -cages in this farm were infected one after the other
    -The negative cage had the lowest accumulated mortality.
- Has performed a pilot study to test the effect of treatment with Optilicer on CMS
-Testing of new broodstock showed PMCV in all of the fish, and they later got a clinical outbreak
-302 females and 131 males were stripped and tested. In egg fluid, only 2 was negative. In males most were also positive
-PMCV in eggs 4-8 weeks post fertilization were tested: ~800 eggs, no positives

The case for and against vertical transmission of PMCV (Britt Bang Jensen, Norwegian Veterinary institute)

-Limited studies on vertical transmission of PMCV.
- CMS has recently been observed shortly after sea-transfer and there is a high prevalence of PMCV in broodstock, which both suggests a possible transfer from the broodstock throught the freshwater phase
- If CMS is transmitted vertically it has implications for trade, but also offers possibility for control/break of infection chain.
-Performed a study in field, tested broodstock and progeny from two cohorts.
    -Broodstock: 98% prevalence in hearts, 69% in roe, 59% in milt. Low ct-values in heart, high in roe and milt
    -Eggs: Disinfection reduced prevalence from 18-43% to 1-4%
    -PMCV was detected until week 43
    -ct-values in progeny (incl eggs) were above/around cut-off
-Don't know if virus detected is infective.
-Arguments against vertical transmission: low prevalence in gonads, survival in fresh water, not found in Chile despite imports of eggs.
-Thinks that PMCV can be transmitted from the broodstock because of study results, and findings in the Faroe Islands, and high prevalence in broodstock

<u>Comments from the audience:</u>

-Aquagen previously didn't think it was an issue, but might revise
-Seems like a propagating epidemic, where infection builds op in the population over time, and eventually in broodstock and offspring, but horizontal transmission is still the most important

Development of infection in the field on a herd level (Julie Svendsen, Norwegian Veterinary Institute)

-Has followed 25 cages in 12 seasites from the entire coast.
    -Tested 2*30 fish every month or every other month for PMCV
    -Found PMCV in all, but only clinical outbreaks in 6 sites
-Virus seems to be widespread, and can exist in populations which never get disease
-Detecting PMVCV does not necessarily indicate and impending outbreak of PMCV
-The virus seems to persist in the herds from first detection to slaughter
-The cases where diagnosis was uncertain, should take into consideration the status on the farm (feeding, mortality etc). If no clinic on the farm, then classify as CMS

Risk factors for CMS (Arthur Mårtensson, Norwegian Veterinary Institute)

-Production data from 6 generations, from spring 12 to fall 14.
    -Includes causes of death. CMS-cases were identified from this
    -Includes data on disease, treatments, feed, movement, smolt supplier, diseases in freshwater etc.
    -Descriptive data and modelling of risk factors

Preliminary results:

-The fall cohorts have a much higher risk of CMS: 58.6% against 22.3% (still valid when controlling for geography and hatchery)

Geography: In south and mid, 50-65% develop CMS, in North only 5%

-Hatcheries deliver often exclusively within their zones. There are large differences in the prevalence from different hatcheries, but this has to be controlled for geography
-At farms with more than one positive group: 90% have registered on another group within one week.
-In 50% of farms with CMS, all groups will be positive
-A model has been developed and it so far includes ponding season, time at sea and will include more.
-Needs to refine case-definition

Screening for PMCV -The why, what and how (Stian Nylund, Pharmaq analytiq AS)

-Why:
    -Good correlation between viral load and heart damage
    Uses of screening: for PMCV most common to use when there is suspicion of disease, but should be used for more purposes
-What have we found:
    -Data from 2016-2017: Majority of samples is from seaphase, 20% from broodfish, and <10% from juveniles and smolt
    -Prevalence in smolt less than 0,5%, in seaphase around 20% and broodstock 40%
    -Prevalence of 55% in ovarian fluid and 65% in milt
    -Prevalence of 0,4% in smolt and 2,5% in juveniles
-How:
    -Because it is a RNA virus, we detect both viral genome and viral transcripts
    -What affects sensitivity and specificity: Choosing the right gene and the size of primers and probes
    -Limit of detection is different from the cut-off. The cut-off is the lowest amount of template it is possible to detect and reproduce, whereas the limit of detection is the lowest amount of template it is possible to detect.

How should we screen:

-Combine PCR with histology
-Screen broodfish –use negative fish
-Screen smolts before transfer –pay special attention to those positive

-Screen before handling
Comments from the audience:
-The ventricle is the best part of the heart, but it is not consistent
-We have to remember that a negative test does not say that the virus is not there. One must also remember the sample size and the prevalence that can be detected.

PMCV: Improved diagnostics and investigating the prevalence in farmed and wild fish in Ireland (Andrew Tighe, Marine Intitute)
-Irish data on samples from broodstock and progeny from Teresa Morrissey confirms the findings by CMSEpi
-Phylogeny:
-Norwegian isolates group with the Irish
-Some irish have an amino acid substitution that we do not know why

Infection dynamics and genetic variability of the virus over the course of infection (Øystein Evensen, Norwegian University of Life Sciences)
-Infection starts in atrium and moves to the ventricle
-Higher level of ORF3 during infection has been seen by some
-ORF3 has a CXC motiv, which could be cytokine/inflammatory
-When the protein is expressed it has a cytotoxic effect on the cells
-Processing of protein is associated with cytotoxicity
-At each sites there are many different sequences –meaning a number of mutations happens during the infections
-The ALV708 sequence is always present
-Normal event, and leads to formation of virus clouds =quasispecies.
-Normally quasispecies supports theory of evolution, but in this case it seems ALV708 is always present, and that is strange
-Is it because this strain is the only one that can infect, and then mutations happen later?
-Is the ORF3-protein a virulence marker/associated with virulence?
-Is lack of cytotoxicity in vitro a proxy for in vivo virulence?
Approach to investigate further:
-Heart pathology associated with different OF3 variants
-Development over time –quasispecies composition
-Experimental challenge

Development of CMS challenge models (Makoto Inami, VESO Vikan)
-Have problems with contamination with PRV, even though tissue homogenate is screened for PRV and PRV antibodies are used.
-PMCV is seen at 7 -8 weeks, with ct-values around 20
-Both IM and IP administration works
-Cohabitants become positive after 9-10 weeks, but never 100% of them, and high ct-values and no clinical signs. There are never any mortalities, even after 24 weeks
-In the lab model, histopathological changes will happen in the heart, but no external changes or ascites and blodclots or discoloration of liver
-Stress treatment did not increase transmission, but increased histoscores

Discussion (Facilitated by Sven Martin Jørgensen, FHF):

-What is it that causes some fish to be infected and others not?

-Has it to do with something in the origin of the fish

-Maybe cohabitants are only infected because some infectious material is leaking from the injection point.

-It seems the virus starts in the atrium, and move to the ventricle, but in the field the changes are worse

-The heart of salmon is able to regenerate throughout life.

-Is it the division of the cells that causes the spread?

-Is there a viremic phase? –it is always found in the heart. But someone should try taking sequential blood samples

-In smaller fish under 1kg there is much more cell division in the heart, the heart has most likely more capacity to repair when they are smaller

-The lytic effect seen in the cell cultures is most likely the same as we see in the hearts –they are being lysed

-Hearts with PD or PRV regenerates, whereas those with PMCV gets scars, which is not regenerating but repair with decreased function

-What about antibodies? There is a monoclonal antibody, but we need an antigen in a purer form, so it is difficult.

-Can anybody produce antibodies –yes for research purposes.

-What are risk factors for shedding of PMCV?

-We don't know the infectious dose. What is the critical level of virus. Again a live culture model would be very useful.

-In older fish you can see high levels of virus where the pathology score does not correlate, why is that?

-We don't see so much CMS in broodstock, even though they have low ct-values and sometimes dramatic changes in the hearts

-We could also use modelling to learn more about transmission and spread, but we do not have a good enough coverage

-Why the difference between S0 and S1?

-Coinfections needs to be investigated more

-Challenge study with tissue homogenate and convalescent antiserum. Pharmaq is about to test this

Vedlegg 9

Kardiomyopatisyndrom (CMS) hos laks
Muligheter for kontroll

Sykdommen kardiomyopatisyndrom (CMS) ble beskrevet hos oppdrettslaks midt på 1980-tallet. Årsaken var lenge ukjent, men det ble tidlig mistenkt at et virus var ansvarlig. I 2010 ble dette bekreftet da piscint myokarditt virus (PMCV) ble beskrevet og knyttet til sykdommen. Det ble vist en klar sammenheng mellom tilstedeværelse og mengde av PMCV, og utvikling av de karakteristiske CMS-relaterte patologiske endringene i hjerte. CMS opptrer langs hele norskekysten med Midt-Norge som tyngdepunkt. Det registreres i overkant av 100 CMS tilfeller per år, men det reelle antallet ligger trolig høyere siden sykdommen ikke er meldepliktig. I tillegg til Norge, er CMS påvist i Irland, Skottland og på Færøyene. CMS-lignende patologi er også beskrevet hos laks i Canada. CMS gir redusert fiskevelferd og betydelige økonomiske tap for næringen.

Hvordan blir fisken smittet?

PMCV smitter gjennom vann og tas trolig opp gjennom fiskens slimhinner. Det viktigste mål for virusset er laksens hjertemuskelceller. PMCV kan påvises ved PCR-analyser av fiskens hjerte. Nyere studier tyder på at laksen smittes i løpet av de første 7 måneder i sjøen (figur 1). I starten vil bare noen få fisk være smittet, og med lave mengder virus. Det kan derfor være vanskelig å oppdage infeksjonen i denne fasen. Når en fiskegruppe først er smittet med PMCV, vil infeksjonen opprettholdes virus helt frem til slakt.

Smitteveier

PMCV smitter horisontalt mellom fisk i anlegg og fra anlegg til anlegg. Nyere studier viser at det er en mulighet for at virus kan smitte vertikalt, fra stamfisk til avkom. PMCV er utbredt i stamfisk, og små mengder virus kan påvises i melke og rognveske fra 60-70% av stamfisken. I tillegg er virus-RNA gjenfunnet i befruktet rogn og yngel fra PMCV-positiv stamfisk. Det gjenstår å undersøke om virusoverføring fra stamfisk til yngel utgjør en smittevei av betydning.

Figur 2. Figuren viser en 24-måneders klokke som illustrerer laksens tid i sjø. Klinisk utbrudd av CMS skjer mellom 6 og 18 måneder etter sjøsetting, med en median rundt 10 måneder.

Figur 1. Figuren viser en 24-måneders klokke som illustrerer laksens tid i sjø. Infeksjon med PMCV skjer innenfor de første 7 måneder i sjø, med en median rundt 4 mdr. Virusinfeksjonen opprettholdes i fisken helt frem til slakt.

Utbrudd av klinisk CMS

Studier har vist at utbrudd av klinisk CMS typisk skjer mellom 6 og 18 måneder etter sjøsetting (figur 2). Utbrudd karakteriseres ved langsom utvikling av dødelighet, med episoder av forhøyet dødelighet i forbindelse med stressende håndtering, ugunstige miljøforhold eller generelt sykdomsstress.

Det er typisk, at sykdommen bare treffer en eller noen få merder, mens de resterende merdene stort sett går fri.

Diagnosen CMS stilles på grunnlag av kliniske symptomer (figur 3), funn ved obduksjon og histopatologisk undersøkelse. Ved obduksjon ses sirkulasjonsforstyrrelser og i fisk som er død av CMS ses ofte blødning til hjertesekken. Histopatologisk sees betennelse og nekrose i hjertemuskelceller. Skadene opptrer først i forkammeret, før de brer seg videre til hjertekammeret.

Mengden av virus i hjertet korrelerer med forekomst av sykdom, slik at det under klinisk utbrudd finnes høye mengder virus. En øking i PMCV mengde samtidig med begynnende dødelighet kan ses som en forvarsel om at et CMS-utbrudd er på gang.

Risikofaktorer for utbrudd av CMS

Risikoen for sykdomsutbrudd i oppdrettslaks øker med tiden fisken har stått i sjø. Fiskegrupper som allerede har hatt PD eller HSMB er mer utsatt for å få CMS enn de som ikke har hatt disse sykdommene.

Vårutsett har lavere risiko for å utvikle CMS enn fisk satt ut på høsten. Det er dog variasjoner i om det har noen betydning om fisken er satt ut tidlig eller sent på våren. I noen områder har fisken som er satt ut sent på våren lavest risiko, mens det i andre områder er de som settes ut tidlig på våren som har lavest risiko.

Det er flere ting som peker på at det kan være en sammenheng mellom utbrudd av CMS og opveksten gjennom settefiskfasen. Fra felt har det blitt rapportert, at det ses store forskjeller i hvilke fiskegrupper som blir berørt under et CMS-utbrudd på samme lokalitet, og at det ofte er fisk fra de samme settefiskleverandørene som rammes år etter år. Et nyere studie der produksjonsdata har blitt analysert med tanke på å avdekke risikofaktorer for CMS, har bekreftet at det ses en tydelig sammenheng mellom settefiskleverandør og risikoen for utbrudd av CMS, også på tvers av lokaliteter.

Dødeligheten under et CMS-utbrudd kan øke dersom fisken blir utsatt for stress. Andre faktorer som rask vekst, miljøfaktorer, ernæring og mangel på fysisk aktivitet har også blitt utpekt som mulige risikofaktorer.

Anbefalinger for kontroll av CMS:

- Førevar prinsipp ved transport av fisk: anta at alt er infisert
- Identifisere mulige kritiske smittepunkter i produksjonen
- Seleksjon av stamfisk med lav/ingen forekomst av PMCV
- Omhyggelige desinfeksjon av egg
- Kontroll på PMCV i settefiskanlegg

Figur 2. Laks med CMS og sirkulasjonsforstyrrelser; utstående øyne, blødninger i hud og ødem i skjellomme. Foto: Per Anton Sæther, MarinHelse AS

Muligheter for kontroll

CMS er en smittsom sykdom og forebygges primært ved å hindre virussmitte. Når en fiskegruppe først er infisert, opprettholdes infeksjonen helt frem til slakt. Det ser ut til at virus kan introduseres på fler tidspunkter i produksjonsforløpet; for eksempel fra stamfisk til yngel, mellom settefisk i anlegg og mellom anlegg i sjø. Det er derfor viktig, at en identifiserer mulige kritiske smittepunkter i produksjonen og forsøker å hindre eller redusere muligheten for smitteoverføring i disse. Screening og seleksjon av stamfisk med lave nivåer eller ingen PMCV kan bidra til å hindre introduksjonen via egg. Det samme kan grundig desinfeksjon av egg og strenge biosikkerhetsrutiner for å unngå smitteoverførsel fra stamfisk til egg etter desinfeksjon.

I settefiskanlegg vil innføring av screening gi bedre kunnskap om smittestatus i anleggene. Skille av generasjoner og fiskegrupper, og grundig desinfeksjon av anlegg og ustyr imellom dem er også av stor betydning. QTL-selektert rogn med økt motstandskraft mot CMS er på markedet, og det arbeides med å få på plass en vaksine. I tillegg er det utviklet funksjonelle fôr som skal sikre næringsopptak og redusere skadeomfanget av sykdommen. CMS syk laks bør ikke utsettes for stressende påkjenninger.

Dette fakta-arket er utarbeidet i forbindelse med FHF prosjektet «Epidemiologisk studie av kardiomyopatisyndrom (CMS): Spredning, risikofaktorer og sykdomsforløp i norsk lakseoppdrett» (FHF prosjekt 901118).
http://www.fhf.no/prosjektdetaljer/?projectNumber=901118

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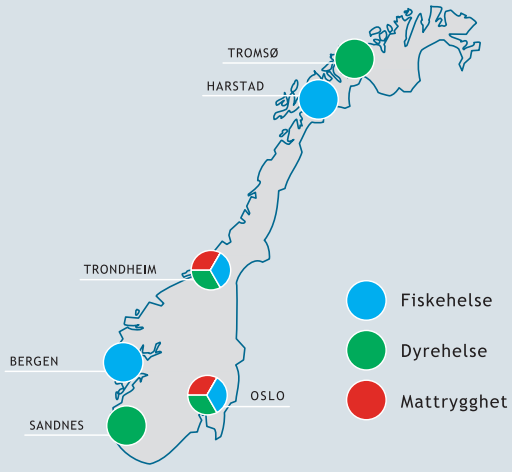

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