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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 venosu*s 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
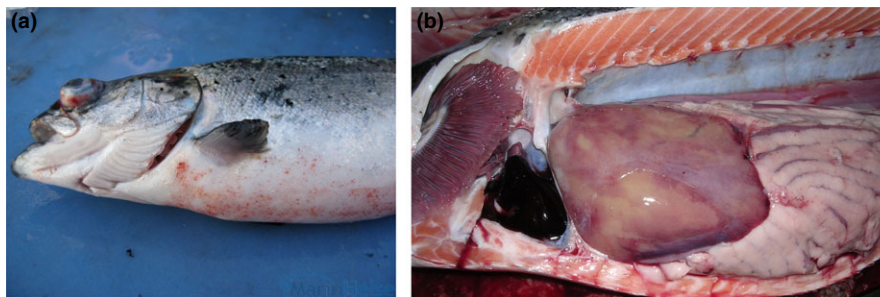

FIGURE 1 Gross pathological conditions in farmed salmon diagnosed with CMS. (a) Salmon showing exophthalmia, ventral skin haemorrhages and raised scales due to oedema. Photograph: Per Anton Sæther, MarinHelse AS. (b) Salmon at autopsy showing ascites, blood clot in the pericardial cavity and discoloured liver with fibrinous casts. Photograph: Brit Tørud, Norwegian Veterinary Institute
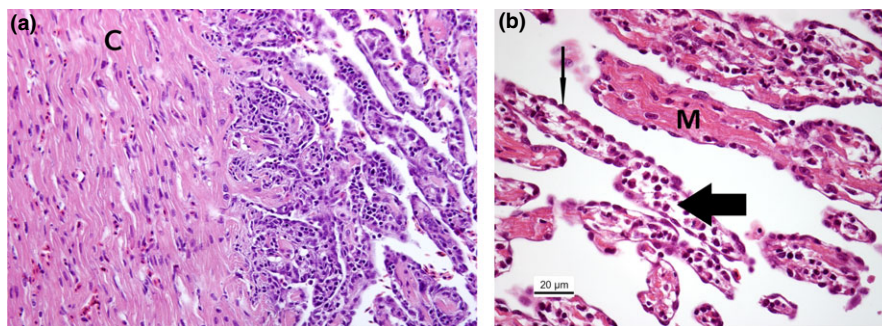
FIGURE 2 Histopathology CMS. (a) Typical histopathological findings of moderate to severe CMS: a distinct border separates severe inflammation of ventricular spongious tissue to the right from normal ventricular compact tissue to the left (C) (H&E staining, 200× magnification). Photograph: Trygve Poppe (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 machinery (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
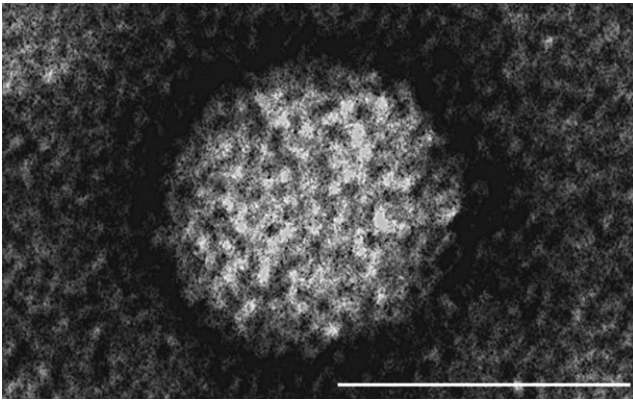
FIGURE 3 EM image of PMCV from Haugland et al., 2011©

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.
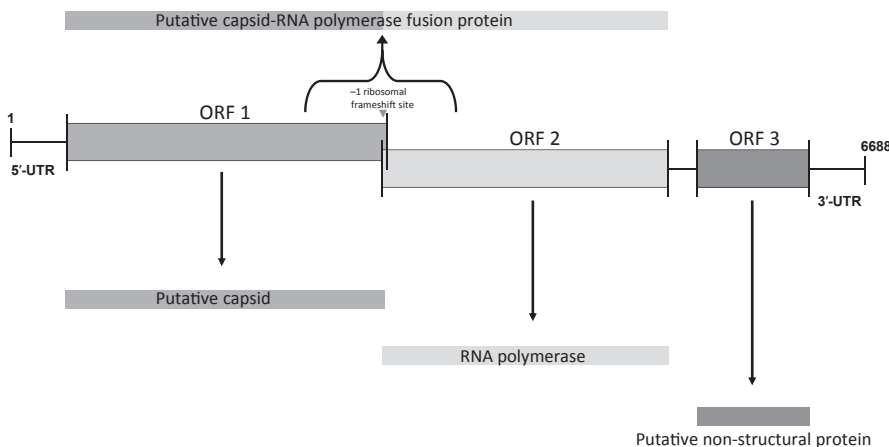

FIGURE 4 Overview of PMCV genome. Aase B. Mikalsen, NMBU

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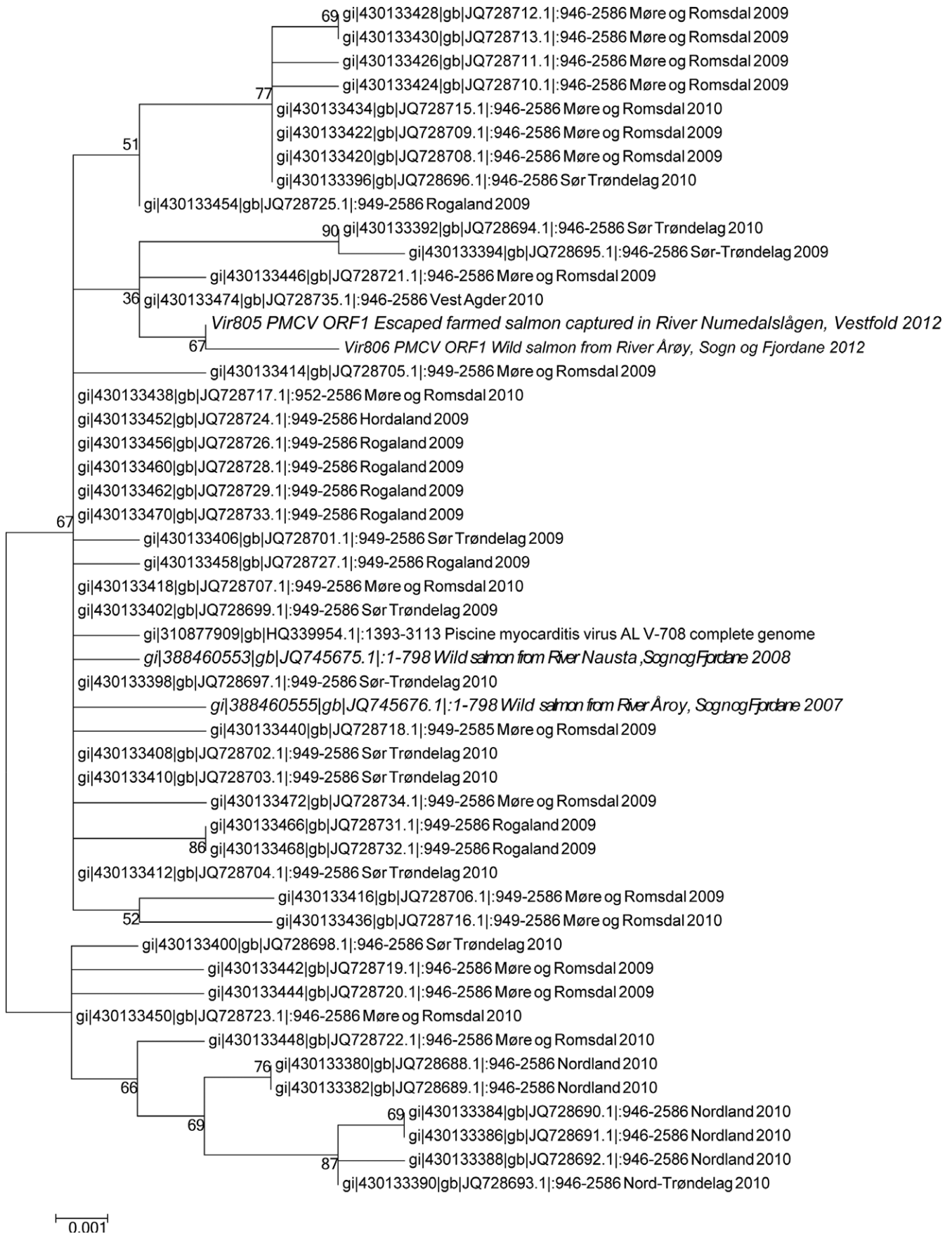
FIGURE 5 Phylogenetic tree displaying genetic variation between PMCV isolates. Åse Helen Garseth, Norwegian Veterinary Institute (Garseth et al., 2016)

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
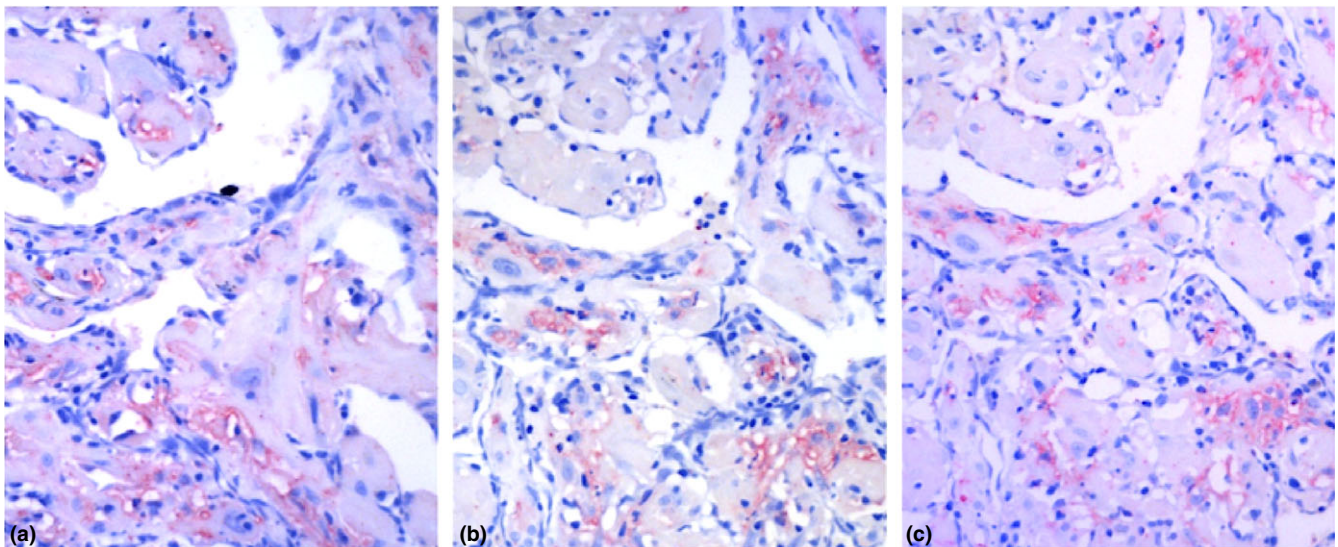

FIGURE 6 Immunohistochemistry of heart samples from Atlantic salmon with a histopathological diagnosis of CMS. Red coloration indicates the presence of PMCV proteins after detection using polyclonal antibodies originally made against recombinant proteins from ORF1- and ORF3-encoded proteins (kindly provided 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)
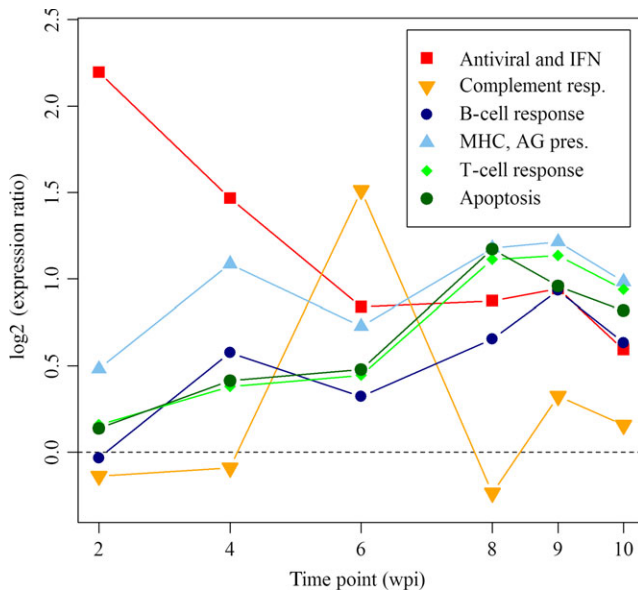
FIGURE 7 Host response: Simplified figure of the immune response of CMS in Atlantic salmon, infected with PMCV in a challenge trial (Timmerhaus et al., 2011). The medians of the expression ratios of the six gene sets are plotted against the sampling time points (printed with permission, Gerrit Timmerhaus, 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
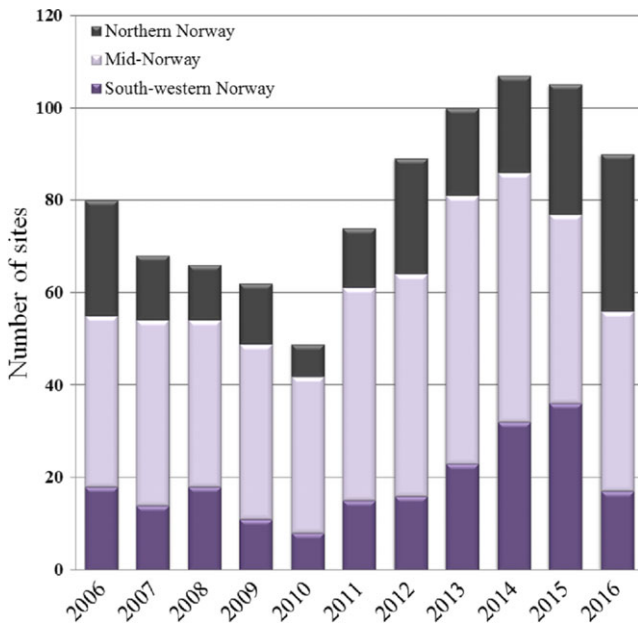
FIGURE 8 An overview of the occurrence of CMS outbreaks along the Norwegian coast, subdivided into regions, from 2006 to 2016. Northern Norway includes counties Finnmark, Troms and Nordland; mid-Norway includes counties Nord- and Sør-Trøndelag and Møre & Romsdal; and finally, south-western Norway includes 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 cohabiting 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.

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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