Abstracts
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Functional consequences of PMCV infection in Atlantic salmon

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Summary of presentation

The viral heart disease, cardiomyopathy syndrome (CMS) poses a serious challenge for salmon aquaculture industry and is associated with high mortalities. The triggering cause of the disease is infection with piscine myocarditis virus (PMCV). Cause of death from CMS is primarily atrial rupture occurring during stressful handling such as delousing. Interestingly, wild fish and experimentally infected salmon do not usually develop CMS and mortality is rarely observed. The underlying reasons for this difference between farmed and experimental/wild fish remains unknown. However, farmed salmon are exposed to an intense rearing scheme, where temperatures are elevated to increase growth and reach smoltification faster. Thus, we hypothesized that the intense rearing protocols used in aquaculture renders farmed Atlantic salmon more vulnerable to PMCV infection than experimental and wild individuals.

First, echocardiography was applied to assess heart function and shape in two experimental fish groups; one that was reared in conditions resembling more natural conditions including low water temperature (SIMNAT) and another that was reared under conditions typically used in aquaculture (SIMFARM). Both groups were followed longitudinally from hatching to harvest. At smoltification, SIMFARM exhibited increased blood flow velocities and signs of hypertrophic remodeling. Cardiac function worsened gradually over time post sea transfer and after a one-year period, cardiac output was decreased in SIMFARM compared to SIMNAT. Consequently, intense rearing protocols at high temperatures seem to induce accelerated age-related cardiac impairment.

Next, the functional consequences of PMCV infection were examined in commercial cohorts at harvest. PMCV-infected fish had increased ventricular contractility combined with lower heart rates. Presumably, the increased contractility is a compensatory mechanism to maintain stroke volume and cardiac output at rest. In addition, both systolic and diastolic intraventricular blood pressures were elevated. Collectively, these data indicate that PMCV infected hearts are working harder than non-infected hearts at rest. During stressful interventions, cardiac demand is increased in parallel with blood pressure and we believe that, especially diastolic, blood pressure becomes too high and causes atrial rupture in the already weakened heart late in the production cycle.

In summary, our data indicates that intense rearing protocols leads to impaired cardiac function later in the lifecycle. In turn, the combination of an already weakened heart and PMCV infection, leading to increased blood pressures, seem to drive mortality. Thus, less intense rearing in the freshwater stage could be beneficial for both general fish performance and health, and the ability to cope with diseases.

Describe how this is relevant to the industry

CMS is one of the biggest contributors to mortality in the aquaculture industry. Although piscine myocarditis virus (PMCV) is the triggering component of the disease, little is known about the mechanisms leading to CMS and mortality. The reasons for this are multiple, but lack of appropriate tools for assessing cardiac morphology and function is a major contributing factor. Here we show that echocardiography can be successfully applied to monitor cardiac health over time and aid in obtaining mechanistic insights to PMCV infection. We highlight the importance of freshwater rearing protocol to improve resistance against CMS. Additionally, indications of what drives mortality in PMCV infected fish is presented.

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Effect of pancreas disease vaccines on infection levels in Atlantic salmon challenged with salmonid alphavirus, genotype 2

Ragnar Thorarinsson, Elanco Animal Health
Co-authors (if relevant) Anne Ramstad, Jeffrey C. Wolf, Hilde Sindre4, Eystein Skjerve and Jose F. Rodriguez

Summary of presentation

PIT-tag marked parr were immunized with two different pancreas disease (PD) vaccines and a hexavalent oil-adjuvanted vaccine (OAV). The control groups were injected with the same hexavalent OAV lacking salmonid alphavirus (SAV) antigen, or with saline. All groups except the saline control were simultaneously immunized against yersiniosis disease to reflect the vaccination strategy commonly used in Mid-Norway. After an immunization period of ~1500 degree days at 12-13 °C, the fish were exposed to SAV, genotype 2 (SAV2) using a cohabitation challenge in seawater. Samples were taken before challenge and at 19, 54 and 84 days post challenge.

Results including growth, side effects and levels of SAV2 neutralizing antibodies before challenge will be presented. Post challenge data including mortality, viremia, levels of neutralizing antibodies, levels of pathological changes in key organs and loss of growth caused by PD through the challenge period will, in addition, be presented and discussed.

Describe how this is relevant to the industry

These results are highly relevant for salmon farmers in Scotland, Ireland and Norway, especially the SAV2 zone in mid-Norway which borders the PD-free region to the north. The recent spread of SAV2 into the PD-free region in northern Norway has to date resulted in stamp-out calls at 4 sea sites by the Norwegian Food Safety Authority ("Mattilsynet"). With the future SAV infection status of this region uncertain, discussions are ongoing amongst the local producers whether to consider zonal vaccination against PD as a risk reduction measure.

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Deciphering the molecular basis of PMCV infection and the disease, CMS.

Espen Rimstad, Norwegian University of Life Sciences
Co-authors (if relevant) Ingvild B. Nyman, NMBU; Torstein Tengs, Nofima, Ås, Norway, Øystein Wessel, NMBU

Summary of presentation

In this study we aimed to elucidate the molecular pathogenesis of PMCV infection and its role in contributing to the pathological changes found in CMS. The molecular pathogenesis of PMCV infection has been a complex and long-standing puzzle. CMS was histologically described already in the late 1980-ies but it took about twenty years before it was demonstrated to be a transmissible disease, and subsequently to be associated with Piscine myocarditis virus (PMCV). The physical structure of the PMCV particles resembles that of Totiviridae, which are non-enveloped particles with an icosahedral one-layered capsid, made of a single major polypeptide. The genome of classical totiviruses is a single molecule double-stranded RNA (dsRNA) with two open reading frames (ORFs). PMCV is distinct from the classical totiviruses by having an extra ORF3. Both field and

experimental studies indicate that natural PMCV transmission between fish can occur, although very slowly.

Classically, the totiviruses have been associated with latent infections in unicellular organisms such as yeast, protozoa, and fungi. Transmission of virus from one cell to another of latent infections of unicellular organisms is dominantly vertical by cell division, but horizontal through cell-cell fusion may occur. Hence, such viruses lack the components of the capsid that are necessary for the viral entry of cells from the extracellular environment.

However, the virus particles of certain totiviruses, such as those infecting arthropods (shrimps and mosquitoes), possess fiber protrusions that likely facilitate extracellular transmission. Interestingly, PMCV virions ion many aspects more closely resemble classical totiviruses of unicellular organisms rather than arthropod totiviruses. Both PMCV and classical totiviruses have overlapping ORF1 and ORF2, the size of the PMCV genome is within the range of the classical totiviruses, while the genome of the arthropod- infecting totiviruses is bigger.

We characterized the replication and pathogenesis of PMCV infection and describe the distribution of PMCV within the fish's heart and identified the form the of PMCV RNA present in certain fish organs. We also investigated how the PMCV genome is arranged within the virus particle. Given the noted similarities to classical and other totiviruses we have explored for eventual other possible hosts for PMCV than the fish itself.

Our research revealed that a dominating viral process in the heart is transcription of viral single-stranded RNA (ssRNA). The acute, massive transcription observed in the heart is not mirrored in the kidney. This could indicate that although kidney cells are susceptible to infection the replication is inhibited in these cells, unlike the cells of the spongiosum of the heart.

We have established RNAscope in situ hybridization assay for the detection of RNA from PMCV. Viral RNA was demonstrated as intense and punctate staining of myocardial cells in stratum spongiosum, with a sharp demarcation against the stratum compactum.

Describe how this is relevant to the industry

Implementation of efficient, targeted interventions against CMS necessitates a comprehensive understanding of the etiology and pathogenesis. If it turns out that if PMCV primarily infects an organism other than salmon, the existing strategies aimed at reducing the prevalence of CMS in salmon may prove ineffective. The current project has sought to elucidate the molecular pathogenesis of PMCV infection and its role in contributing to the pathological changes found in CMS.

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Introducing dbDNATM – the foundation for a new wave of innovation in aquaculture vaccines

Ian Thompson, Touchlight Aquaculture Ltd

Co-authors (if relevant) Sungwon Kim and Lisa Caproni, both of Touchlight.

Summary of presentation

DNA vaccines are an essential tool for infectious disease management in aquaculture. There are two outstanding success stories so far, namely Apex-IHN® for IHNV and Clynav® for PD. In addition, there are many examples in the literature of successful DNA vaccine trials against other

commercially important diseases. Current DNA vaccines are based on plasmid DNA (pDNA). These products are produced by industrial scale fermentation of E. coli, followed by alkaline lysis and some degree of chromatographic purification. In contrast, Touchlight Genetics Ltd are pioneers in the field of enzymatic DNA. Their proprietary doggybone DNA (dbDNA™) is produced using in vitro methods using enzymes. A more complete description of dbDNA™ and its advantages over pDNA will be presented. Touchlight Aquaculture Ltd aims to leverage the unique attributes of dbDNA™ to develop a new generation of DNA vaccines to reduce the impact of infectious diseases in aquaculture, with a priority focus on the three viral cardiomyopathies; PD, CMS and HSMI. Of the three, PD is the most tractable, and has been the focus of our early work. Data will be presented from two in vivo studies involving SAV3 challenge in freshwater at five hundred degree days post vaccination. In the first study, we tested two dbDNA™ SAV vaccines at 7.5, 2.5 and 1 microgram doses to answer some basic questions about the applicability of the platform for aquaculture. One dbDNA™ construct utilised native SAV sequence and the other was codon-optimised (CO) for fish. We included a pDNA group for reference purposes at label dose (6.0 – 9.4 micrograms). In this study, we demonstrated that dbDNA™ vaccines were at least as efficacious as pDNA in reducing viraemia and subsequent systemic spread to sites of secondary replication (heart) post challenge. Significant efficacy was apparent at dbDNA™ doses as low as 1 microgram. In this study, we were also surprised to observe a remarkable impact of codon optimisation on antibody responses. 100% of fish vaccinated with the CO dbDNA™ candidate produced both SAV3 and SAV2 neutralising antibodies at five hundred degree days post vaccination compared with only 50% of fish in the pDNA group and 30% of fish in the native dbDNA™ group. In the second study, we evaluated several novel construct designs intended to provide further increases in efficacy. The hypothesis behind these constructs and their molecular basis will be described. In this study, we identified a lead candidate for commercial development which out-performed our reference dbDNA™ candidate from the first study. Finally we will talk about our ambitions for the dbDNA™ SAV vaccine candidate and our future R&D aspirations.

Describe how this is relevant to the industry

DNA vaccines are an essential tool for infectious disease management in salmon aquaculture. Touchlight Aquaculture is leveraging an exciting new DNA platform, Doggybone DNA (dbDNA), to develop a new range of innovative vaccines for salmon, focusing initially on the three viral cardiomyopathies (PD, HSMI, CMS). This presentation will showcase the critical attributes and benefits of the dbDNA platform for salmon producers and provide molecular and clinical data relating to the companies PD vaccine candidate for consideration by the academic members.

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Comparison of whole blood transcriptome responses of Atlantic salmon infected by three Piscine orthoreovirus variants

Thomais Tsoulia, Norwegian Veterinary Institute

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Øystein Wessel, Jorunn Jørgensen and Maria K. Dahle

Summary of presentation

Piscine orthoreovirus (PRV) infection is common in salmonid aquaculture, and three PRV genotypes (PRV-1-3) have been identified. Among the three, PRV-1 is associated with heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon (Salmo salar), PRV-2 causes erythrocytic inclusion body syndrome (EIBS) in Japanese coho salmon (Oncorhynchus kisutch), while PRV-3 induces HSMI-like disease and anemia in farmed rainbow trout (Oncorhynchus mykiss). These PRV

variants can also cross-infect between species, however, PRV-2 and PRV-3 do not cause HSMI in A. salmon.

Instead, PRV-3 infection in A. salmon efficiently blocks PRV-1 infection and HSMI, while injection of PRV-2 or inactivated adjuvanted PRV-1 partially protects against HSMI. Red blood cells are target cells for all PRV genotypes, and here we compared transcriptome responses in blood cells of A. salmon injected with PRV-1, PRV-2 or PRV-3 at two and five weeks post injection. Transcriptional analysis of blood cells infected by PRV-1 and PRV-3 were characterized by induction of genes involved in transcriptional regulation and innate immunity, whereas PRV-3 at week two triggered stronger antiviral responses than PRV-1. Indicatively, genes related to viral dsRNA recognition (e.g. RIG-I receptor), activation of innate antiviral responses (e.g. interferon regulatory factors 1, 2 and 10) and interferon stimulated genes (e.g. ISG15 and RSAD2) showed approximately two-fold induction for PRV-1, but more than five-fold induction in PRV-3 infected fish. However, after five weeks there was no difference in the response magnitude between these groups. Interestingly, PRV-2 did not induce antiviral responses as PRV-1 and PRV-3 rather it suppressed genes involved in apoptosis signaling and protein folding. These findings propose that the protection mediated by PRV-3 in A. salmon might occur due to greater expression of viral pattern recognition receptors together with transcription factors that regulate innate antiviral immunity.

Describe how this is relevant to the industry

HSMI is one of the most prevalent viral diseases in Atlantic salmon (Salmo salar) aquaculture in Norway. In terms of profitability, HSMI has been ranked among the 10 most threatening diseases to the Norwegian fish farming industry, leading to a significant economic loss. PRV-1 is the causative agent of HSMI, whereas a related genotype, PRV-3, results in a non-pathological infection that can confer cross- protection against PRV-1 infection and heart inflammation. Hence, comparing the transcriptional responses elicited by each PRV variant may provide better insight into the virus- host interaction mechanism and signaling pathways that determine a pathological outcome. Considering the lack of effective prevention measures for HSMI, this study also supports the determination of valuable biomarkers to support development of vaccines or other protective measures against PRV-1 and HSMI in farmed A. salmon.

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Observations of differences in prevalence of SAV-induced cases between production areas in Norway over the last 3 years

Hege Spigseth Hovland, Elanco Animal Health
Co-authors (if relevant) Dag Vollstad and Ragnar Thorarinsson

Summary of presentation

Marked reduction in number of SAV-induced cases has been documented in Norway over the last 3 years, in both the SAV2 and the SAV3 zone. This improvement, however, varies considerably between the defined production areas (PAs), including neighboring PA's that are comparable with similar temperature profiles, density of sea sites, farming practices including sea lice treatment regimes, disease risk profiles and mortality levels. In the presentation, the regional variations in the prevalence of SAV- induced cases over the last 3 years will be reviewed and possible explanatory causes for these differences discussed.

Describe how this is relevant to the industry

The formalized PA structure represents a central pillar of health management within Norwegian fish farming. Understanding the disease dynamics within and between the PA's, is crucial to minimize the impact and spread of infectious diseases and thus, to safeguard fish welfare and production sustainability.

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Susceptibility of brown trout (*salmo trutta*) to piscine orthoreovirus genotype 3 (PRV-3)

Niccoló Vendramin, DTU AQUA National Institute of Aquatic Resources

Co-authors (if relevant) Juliane Sørensen (DTU AQUA), Anne Berit Olsen (Norwegian Veterinary Institute), Arvind Sundaram (Oslo University Hospital, Norwegian Veterinary Institute), Maria Dahle (Norwegian Veterinary Institute) Argelia Cuenca (DTU AQUA)

Summary of presentation

Piscine orthoreovirus (PRV) is a relevant pathogen for salmonid aquaculture worldwide. Currently, three distinct PRV genotypes are described with relatively discrete host niche. PRV-1 infects predominantly Atlantic salmon (Salmo salar) and some variants cause Heart and Skeletal Muscle Inflammation (HSMI). PRV-2 is reported to cause erythrocytic inclusion body syndrome EIBS in Coho salmon (Onchorhynchus kisutchi) in Japan. PRV-3, discovered in Norway in 2015 and detected for the first time in Denmark in 2017 in association with complex disease cases in rainbow trout (O. mykiss) in recirculating aquaculture systems (RAS). PRV-3 has also been associated in Brown trout (Salmo trutta) with Proliferative Darkening Syndrome in pre-alpine rivers. In this study, we assess the susceptibility of brown trout to infection with PRV-3. Three experimental groups of specific pathogen free brown trout were included in our challenge trial: 1) a negative control, 2) a group challenged with infected PRV-3 blood from rainbow trout, and 3) a group challenged with gradient purified PRV-3 particles. The challenge model to study infection and pathogenesis was cohabitation. Shedders were intra-peritoneal injected, tagged and maintained in tanks with non-tagged cohabitant fish. The experiment run for 10 weeks, with sampling every two weeks to assess viral load (RT- qPCR), histopathological changes, and host immune response by RNAseq. No reduced survival was observed along the experiment, but PRV-3 successfully replicated in shedders and transferred horizontally to cohabitants. After peak of viremia heart lesions consistent with those observed in HSMI in Atlantic salmon were present in both, shedders and cohabitant brown trout. Prevalence of heart lesions was higher in fish challenged with infected blood possibly due to exposure to a higher virus load. No lesions consistent with PDS were observed. Changes in gene expression were quite different between challenged and control groups at 8 weeks post challenge. ,

This study documents the susceptibility of brown trout to PRV-3 and its pathogenesis. This allow to a better understanding of the role of brown trout in the epidemiology of PRV-3.

Describe how this is relevant to the industry

PRV-3 is a widespread viral pathogen in farmed rainbow trout. The virus is associated with severe disease losses in RAS.

The role of brown trout, occasionally co-farmed with rainbow trout, in the PRV-3 epidemiology is here documented and discussed.

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Viral genomic surveillance - supporting disease control in Atlantic salmon aquaculture

Bertie Knight, University of Edinburgh

Co-authors (if relevant) Oliver Eve (University of Edinburgh), Manu Gundappa (University of Edinburgh), Elise Hjelle (Pharmaq), Mari Solhiem (Pharmaq), Ane Santrø (Pharmaq), Svein Alexandersen (Pharmaq), Marius Karlsen (Pharmaq), Daniel Macqueen (University of Edinburgh)

Summary of presentation

Viral genomic surveillance is important across a range of viruses including those that cause cardiomyopathies such salmonid alphavirus (SAV) and piscine orthoreovirus (PRV). My project aims to create Nanopore-based sequencing assays for these two viruses, and infectious pancreatic necrosis virus (IPNV) to create a database of whole-genome sequences with associated epidemiological metadata. This database will allow the characterisation of viral genetic diversity, update molecular epidemiology and the reconstruction of spatial-temporal transmission scenarios.

IPNV is a viral pathogen causing pancreas and other major organ necrosis in farmed Atlantic salmon. IPNV is classified based on the phylogenetic relationship of the partial nucleotide sequence of VP2 which contains immunodominant epitopes and determines viral antigenicity. There are eight genotypes (G1-8) with G1, G3 and G5 circulating in Scotland, G1 and G5 in Chile and G5 in Norway. Control efforts include vaccination and the adoption of a heritable quantitative trait locus (QTL) which confers resistance to IPNV into the breeding programme. This QTL has been successful in significantly reducing mortalities associated with IPNV. A novel G5 (NG5) variant has been identified in Scotland, Norway and Chile associated with higher mortality in vaccinated QTL fish. Chilean NG5 is more similarly related to currently circulating G5 strains in Chile than European NG5. This suggests convergent evolution of vaccine/QTL evading phenotypes in Europe and Chile.

I have designed and optimised an amplicon-based sequencing assay, that can capture “classic” G5 and NG5, to sequence IPNV samples (n=69) from between March 2022 and June 2023 from Norway (n=68) and Scotland (n=1) and constructed maximum-likelihood trees to assess their phylogenetic relationship. Fifty-two of these isolates cluster with reference NG5 sequences and the other seventeen sequences cluster with “classic” G5 references. These isolates were sampled randomly and reflects an unbiased representation of the current circulating genotypes of IPNV. Initial sequencing results show that NG5 was the predominant genotype in Norwegian aquaculture (in 2022-2023) comprising of ~75% of isolates sequenced. The distribution of NG5 in Norway is widespread, with isolates from all counties between Rogaland and Finnmark (excluding Møre og Romsdal). Interestingly, the majority of the “classic” G5 isolates sequenced are from the three most northernly counties, Nordland, Troms and Finnmark. The lower density of aquaculture sites has possibly delayed the introduction of NG5 to these counties and has yet to outcompete “classic” G5.

Assessing of the VP2 hypervariable domain between “classic” G5 and NG5 reveals key amino acid substitutions which has led to altered antigenicity and contributes to the vaccine and QTL evasion phenotype. Comparison of Chilean NG5 with local “classic” G5 shows limited differences and suggests mutations in other proteins may contribute to this phenotype and highlights the need for whole-genome viral sequencing to elucidate molecular changes in novel variants.

A pan-genotype amplicon-based Nanopore sequencing assay has also been designed for SAV, the causative agent for pancreas disease (PD), and applied in a trial sequencing run with samples from Scotland (n=4) and Norway (n=4). Analysis of these sequences reveals that the lineage of SAV2

transmitted from Scotland to Norway in ~2010 is still circulating in Scottish aquaculture. This isolate is the missing link in this transmission event and provides further genomic evidence for this event. Furthermore, a sequenced SAV3 isolate clusters within a monophyletic group sister to all available whole genome SAV3 sequences identified in Macqueen et al. 2021. This demonstrates a large amount of uncharacterised genetic diversity with the SAV3 subtype. Additionally, Scottish SAV5 sequences cluster with two main lineages which have been circulating since 2007.

Describe how this is relevant to the industry

Viral pathogens threaten the future sustainability and expansion of the Atlantic salmon aquaculture industry, resulting in economic losses and representing a concern for fish welfare. Viral genomic surveillance for SAV, IPNV and PRV whole genome sequence data coupled with epidemiological metadata, can be used to better understand viral evolution and transmission and can contribute to improved management of viral disease spread.

Characterising viral genetic diversity is key in identifying the emergence of novel viral strains. The genomic landscapes for these viruses are relatively unknown due to the limited number of publicly available sequences.

Greater characterisation will enable the distinction between novel variants and under sequenced currently circulating viral strains. This data can aid updates to vaccines and the development of therapeutics. Furthermore, viral genomic surveillance can help gain a better insight into the geographical distribution of different viral subtypes and their relative contribution to regional outbreaks. More detailed and updated viral molecular epidemiology can allow for more targeted infection control measures. Additionally, this approach can be used to reconstruct historical and contemporary transmission scenarios between countries and farming regions. Inferences on these transmission scenarios can inform infection control measures and limit future transmission. For IPNV and PRV, this can also be applied to provide genomic evidence for vertical transmission routes, through linking outbreaks in broodstock fish and disease in freshwater hatcheries. Standard partial sequencing approaches used for genotyping lack the resolution to be able to conduct these analyses.

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Genomic tracking of the spread of piscine myocarditis virus (PMCV) in farmed salmon.

Mingli Zhao, Royal Veterinary College

Co-authors (if relevant)Marius Karlsen, Guillaume Fournié, Helene Duault, Svein Alexandersen, Mari Solheim, Chris Matthews, Silvia Soares, Sarah C. Hill.

Summary of presentation

Piscine myocarditis virus (PMCV) is a double-stranded RNA virus that causes cardiomyopathy syndrome (CMS) in Atlantic salmon (Salmo salar). Despite the serious economic and welfare challenges caused by CMS within salmon aquaculture, we understand very little about how virus lineages are spread between farms and different generations of fish. This prevents us from efficiently focusing biosecurity activities that are intended to control disease.

Using the evolved patterns of virus genetic changes to reconstruct ancestral relationships between viruses can help demonstrate linkage between observed disease outbreaks, and can allow us to identify factors that may be increasing the risk of spread at the population-scale. Use of these methods to study PMCV has been prevented by poor currently availability of PMCV genomes. We

therefore developed two approaches that enabled us to conduct whole-genome sequencing of PMCV from over 280 samples collected from Scottish and Norwegian fish farms, and analysed these genomes using phylodynamic methods to reconstruct epidemic dynamics and spatial dispersal patterns of PMCV. In contrast to previous assumptions, we find substantial diversity present in all three genes of PMCV. This underscores the value of using whole-genome sequences in epidemiological investigations of PMCV.

Phylodynamic analysis revealed distinctive clustering of genomes from Norway and from Scotland, which is indicative of most viral lineage spread occurring within rather than between these two countries. Despite this, we observed multiple introductions of PMCV lineages into Scotland. Lineages of PMCV present in Norway likely emerged in southern Norway and disseminated from the south to the north of the country. We use national- scale data on salmon aquaculture and boat movements to test which factors can best explain the observed epidemiological patterns, and help quantify the relative importance of vertical and horizontal transmission to PMCV maintenance and spread.

Describe how this is relevant to the industry

This research addresses critical questions aimed at enhancing our understanding of how to effectively control the spread of piscine myocarditis virus in fish populations. By investigating the dynamics of transmission between fish farms at different production stages, the study aims to identify key areas where biosecurity measures could be focused, thereby reducing the risk of virus spread. Our study also significantly improves our understanding of the genetic diversity of PMCV. This will be useful to understand how PMCV evolves, and can contribute to the development of effective vaccines.

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Fish pathogens surveillance during complex disease outbreak in RAS by high-throughput microftuidic qPCR

Juliane Sørensen, DTU Aqua
Co-authors (if relevant)Argelia Cuenca, Simon Brøndgaard Madsen, Lone Madsen, Niccolò

Vendramin.

Summary of presentation

Piscine orthoreovirus genotype 3 (PRV-3) has been associated with severe disease outbreaks in rainbow trout farmed in recirculating aquaculture systems (RAS) in Denmark since 2017. In 2017 to 2019, a surveillance program showed that PRV-3 is widespread within Danish aquaculture with approx. 72% of the investigated farms testing positive for the virus.

However, only few farms had disease outbreaks associated with the detection. Upon investigating the virus genome, no differences were observed in isolates associated with and without disease. As most disease outbreaks occurred during the winter, the effect of water temperature was evaluated under experimental condition, showing elevated virus load at low temperatures but mortality was not observed.

However, field investigations have highlighted the presence of other pathogens during PRV-3 associated disease outbreaks. In order to investigate PRV-3 associated disease further, we developed a high- throughput microftuidic qPCR (HT-qPCR) method (Fluidigm/Standard BioTools) for

simultaneous detection of 22 pathogens, and field samples were collected on a monthly basis from a RAS farm with history of PRV-3 associated disease across 7 months (March to September 2022).

In order to compare the HT-qPCR method with current gold standard diagnostic methods, sample collection consisted of 1) heart, spleen and kidney in RNAlater, 2) gills in RNAlater, 3) blood, 4) kidney swab, 5) heart, spleen and kidney in medium and 6) water. Swabs and tissue in medium were tested with standard diagnostic methods while tissue in RNAlater and the filters from water were tested with qPCR and HT-qPCR. At each time- point, five clinically healthy and five clinically affected fish were sampled.

Additionally, production data was recorded during the sampling period including weights, feeding, disease outbreaks, treatments, and water quality parameters.

Virological examination found no presence of IHNV, VHSV, or IPNV, while Yersinia ruckeri and Flavobacterium psychrophilum were found occasionally, primarily in clinically affected fish. PRV-3 was detected by RT- qPCR across the study and in connection with a disease outbreak.

Using HT-qPCR, PRV-3 and the putative gill pathogen Candidatus Branchiomonas cysticola were found at all time-points, but at high load around the time of the disease outbreak. The disease outbreak lasted for 5 weeks, resulting in overall mortality of 2.2 tonnes. The examined batch had been moved to a new unit a few days prior to the disease outbreak, suggesting that stress from handling may have been a contributing factor. Filters from water samples were tested by HT-qPCR as well. In this study, we were unable to reliably detect PRV-3 in the water, but found several bacterial pathogens which were not observed in fish samples, including Yersinia ruckeri and Flavobacterium psychrophilum which were found at all time-points in the water but only occasionally in fish.

In conclusion, PRV-3 associated disease outbreaks in RAS farms are complex, and often other pathogens are found in addition to PRV-3. Here we show that stress induced by farm practices may be a contributing factor to disease outbreaks. Additionally, several pathogens that were not found in fish samples were found in water samples, showing that in RAS systems, the use of environmental sampling for pathogen monitoring should be accompanied with fish samples.

Describe how this is relevant to the industry

This project was developed in collaboration with the industry in order to gain more knowledge on the pathogen situation in a RAS farm, and to better understand PRV-3 associated disease outbreaks.

HT-qPCR has previously been used for pathogen monitoring in wild populations, but this study reports the first use of the method for diagnostic purposes in aquaculture.

This study shows that disease in RAS systems is often complex with more than one pathogen being involved. Additionally, it shows that environmental samples such as water may not be a good indicator of the pathogen situation in a given unit or farm, as bacterial pathogens were detected in the water but were not necessarily found in the fish samples and vice versa for the viral pathogens.

Furthermore, this study shows that stressors from handling and farm practices may be contributing to PRV-3 associated disease outbreaks. Frequently monitoring the pathogen level in a batch of fish may provide better insight into when farm practices should be implemented, and can help determine and monitor the effect of treatments used.

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Salmonid alphavirus accumulates specific genome deletion variants to high frequencies during replication in cell culture

Turhan Markussen, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway
Co-authors (if relevant) Thomas Vallet, Fabian Kropp, Øystein Evensen, Marco Vignuzzi, and Aase B. Mikalsen

Summary of presentation

Most, if not all, RNA viruses produce so-called defective viral genomes (DVGs) during infection. These DVGs are characterized by deletions of varying size and they occur in different parts of the genome. We have shown in previous studies that SAV3 also produces DVGs and that some of these occur at positions in the virus genome at higher frequency than in others. For other viruses it has been shown that DVGs can be packaged, exit- and re-infect new cells, and that both the composition, number and specific types of DVGs produced can influence the host immune response and disease severity. Combining next generation sequencing (NGS) and a computational approach previously used to detect DVGs in other RNA viruses, we here present results obtained from serial passaging of SAV3 in Chinook salmon heart (CHH-1) cells, the infection initiated by the transfection of a plasmid-based SAV3 infectious clone. Analyses of the sequence data show that the overall total number of DVGs, as well as the type and frequency of different deletions produced, all increase during passaging. Those DVGs produced to very high frequencies include both large deletion types covering roughly half of the virus genome and smaller deletion types, tens to hundreds of nucleotides in size, the latter mainly located to the structural genes. Detailed analyses of the sequence data and preliminary results from cell culture work involving DVGs will be presented. The potential future significance and applicability of DVGs in controlling PD, will also be discussed.

Describe how this is relevant to the industry

DVGs influence viral fitness and virulence. Identifying those DVGs that are produced to high frequencies during SAV infection can provide new information that may aid in the development of better vaccine formulations and/or have therapeutic applications. In other RNA viruses, the presence of artificially high amounts of specific DVG types has been shown to lead to attenuation and reduce virus spread. Studies of interactions between DVGs and the host immune system may also provide more basic information on the molecular basis behind SAV pathogenicity.

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PMCV – characteristics of the virus that may affect the outcome of CMS

Aase B. Mikalsen, Norwegian University of Life Sciences (NMBU)

Summary of presentation

Piscine myocarditis virus (PMCV) is well known for its ability to cause necrotizing myocarditis in Atlantic salmon aquaculture, a disease known as cardiomyopathy syndrome (CMS). The disease relates to health and welfare issues for the fish and large economic losses to the industry as it mainly affects marked sized fish. In 2011, we described PMCV as the first vertebrate virus with similarities to Totiviridae, a family of viruses infecting single-celled organisms and spread from cell to cell following cell division or fusion, without leaving the cells.

In the last decade, many toti-like viruses found in multicellular organisms. These are all described with additional protein-encoding sequences in either ends of their genome, when compared to Totiviridae. Increasing evidence suggests that these additional proteins or peptides have functional properties related to infection and their spread in a more advanced host. PMCV was the first toti-like virus described with additional sequences included as a separate third gene. In recent years, toti-like viruses with similar protein coding additions have also been found in lumpfish, golden shiner, common carp and sea bass. Like totiviruses, these viruses seem to code for a capsid protein constituting the viral particle and a polymerase responsible for copying the virus genome in infected cells.

The unique third gene of PMCV, codes for a 33.4 kDa protein, not previously known in any viruses or elsewhere in biology.

Through various projects over many years, we have attempted to characterize the protein products of PMCV and understand their role in infection, replication and spread of the virus. In addition to genetic characterization of the capsid and polymerase proteins, we have focused on the protein resulting from the third gene, named p33 after its expected full-length size. This protein has been expressed recombinantly in cultured fish cells and characteristics of expression of the protein and resulting sub products and their effect on the cells have been described. In addition to understanding the functional role we have also attempted through sequencing studies, to understand if gene sequence variants for the proteins may affect their function, if genetic variants of the full PMCV genome may explain differences in severity of CMS on the basis of individuals or a salmon population or if genetic information can describe evolutionary selection of variants more fit to persist in the salmon population or cause disease.

Main results and relevance for the industry will be presented and discussed.

Describe how this is relevant to the industry

CMS is today the costliest viral disease in Norwegian salmon farming and improved control measures through better management of infected farms, prevention of spread and protocols for prediction of disease outcome would be of great benefit to the salmon industry. One of the key challenges when PMCV is detected in a farm, is the unpredictable nature of the infection, spread of the disease in the farm (and between farms) and variability between year classes. Knowledge on how the virus infects, replicates and spread from cell to cell and fish to fish and how the full- length genome and genes may vary is needed to understand if or how this may be related to the infection progress and hence subsequent handling.

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In vivo replication and pathogenicity of PMCV after infection with different doses of heart homogenate

Amr Ahmed Abdelrahim Gamil, Norwegian University of Life Sciences, Faculty of Veterinary Medicine

Co-authors (if relevant) Cheng Xu, Øystein Evensen

Summary of presentation

Cardiomyopathy syndrome (CMS), caused by piscine myocarditis virus (PMCV), is one of the main infectious challenges facing the salmonid industry. The disease is widespread in different production areas in Norway and the numbers of registered cases has been increasing in recent years.

The pathogenicity and infection dynamic of PMCV is yet to be well understood and only few experimental challenge experiments have been conducted thus far. Here we have infected Atlantic

salmon post-smolt with three different concentrations of heart homogenates obtained from infected fish. Fish were then sampled after 4 and 5 weeks of infection and histopathological evaluation was used to monitor the degree of heart inflammation induced in the heart. In addition, real time PCR was used to measure virus replication in the heart, head kidney, spleen, and blood. Our result show differences in virus induced pathogenicity in the heart of fish exposed to different infection dose. Moreover, the level of virus replication differed between the different doses as well as between the different tissues. The detailed findings of this study will be presented and discussed.

Describe how this is relevant to the industry

PMCV infections is one of the main challenges facing the Atlantic salmon industry. Control strategies are needed but is yet to be developed.

Understanding pathogenecity and disease development will help in developing such strategies. The current study is addresses virus pathogenecity after infection with different doses and can constitute the basis of developing a more controlled infection challenge model.

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The effect of synchronized fallowing in open sea cage farming of Atlantic salmon

Marit Stormoen, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway

Co-authors (if relevant) N. A. Vatne, Ø. Wessel, H. Trengereid, S. Haugsland, E. Rimstad

Summary of presentation

Mariculture of Atlantic salmon harbours intrinsic disease control challenges. Norwegian salmon farming struggles with viral diseases transmitted between farms. The Norwegian regulations demand unspecific control measures such as two-months fallow period between stocking cycles at a farm. Previous studies found that fallowing prevents transmission of Salmonid alphavirus between stocking cycles at the same farm, while this has been harder to prove for widespread viruses such as piscine orthoreovirus-1 (PRV-1). In this study we aimed to demonstrate the effect of synchronized fallowing of five sea sites in a geographically isolated area on the general disease control.

Five salmon sea sites underwent a synchronized fallowing period for one month. The following production cycle, monthly samples were collected from 20 moribund or recently dead fish from each site. The samples were screened for viruses using a commercial lab PCR. In total, 32 samples positive for PRV-1 were sequenced to trace connectivity between sites and timepoints.

We could trace the introduction of PRV-1 for four of the five sites and the connectivity between the sites based on the viral genetic variation. The variants, but one, differed from those isolated during the previous production cycle indicating that the fallowing had prevented transmission between the generations. Further, the dissemination of the variants between the farms in the area suggested that the main route of spread was anthropogenic and not via water currents.

The results suggests that the disease status of the Norwegian salmon farming industry could benefit from strategic fallowing of larger areas, and even viruses as widely distributed as PRV-1 are manageable by fallowing.

The anthropogenic nature of the transmission suggests that many diseases can be managed by proper biosecurity.

Describe how this is relevant to the industry

Synchronized fallowing of hydrodynamically connected sites could be more exploited to enable better diseases management in the salmon aquaculture industry.

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Blood based biochemistry in Atlantic salmon- prognostic tool for myopathies?

Martin Huun-Røed, Patogen, Norway

Co-authors (if relevant) Johanna Baily, Christian Wallace, Mads Sæther, Meritxell Diez-Padrisa,

Kjersti Gravningen, Hamish Rodger

Summary of presentation

Blood based biochemistry is used to learn and understand the biological processes and physiological responses which take place in cells and organisms. In this talk we will give an introduction to the use of blood- based biochemistry and the potential it brings in order to give a prognosis on myopathies relevant for farmed Atlantic salmon. We will provide preliminary results from an ongoing SAV2 (PD) trial where we have looked at 7 different biomarkers in serum that have relevance to organs effected during an outbreak of PD.

Describe how this is relevant to the industry

We need more and better tools in the diagnostic tool box to help our veterinarians and fish health biologist working hands on in the industry. Blood based biochemistry could help Increase our knowledge to see the bigger picture for improved decision making

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Scottish experience - Pancreas Disease & Hydrozoan Blooms

Kimberley McKinnell, Bakkafrost Scotland

Summary of presentation

Sharing Bakkafrost experience with a Pancreas Disease infection and

concurrent micro jellyfish blooms. Comparison of data between two farms in same management area.

Describe how this is relevant to the industry

Pancreas Disease is still a major disease impact in Scottish Industry causing losses through mortality and growth. Jellyfish have been an increasing issue in Scotland over the last 2 years, and their impact/risk are still being evaluated. This data gives a site by site comparison of the impact that viral myopathies have when concurrent challenges affect the fish.

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Situation update from the Faroe Islands on PD, CMS and HSMI

Debes Hammershaimb Christianssen, Senior Researcher and Head of Department

Summary of presentation

CMS, HSMI and PD are important diseases in the three North Atlantic salmon farming countries Ireland, Norway, and Scotland.

CMS was a major challenge in farmed Atlantic salmon in the Faroe Islands in the 90'ies. However, Following the ISA epidemic from 2000 – 2005, the complete reorganization of the Faroese aquaculture industry and the new legislations implementing significant improvements in biosecurity measurements CMS (and other diseases) disappeared and seemed to be eliminated for several years. The re-emergence of CMS in 2013 and subsequent fast increase in prevalence happened simultaneously with the introduction of large scale non-medical treatments for sea lice.

The causative agent for HSMI, PRV-1, has been detected in all three production stages of Atlantic salmon i.e. brood fish, freshwater smolts and marine fish since we started screening in 2010. Although very high viral loads were observed in several farms these cases were not associated with clinical signs of HSMI or increased mortality. One likely explanation was that only the low-virulent subtype of PRV-1 was circulating in Faroese aquaculture (Dhamotharan et al 2019). However, within the last couple of years this picture has changed as several cases of HSMI with increased mortality have been observed.

Whereas CMS and now also HSMI are important diseases in Faroese aquaculture PD or the causative agent SPDV has never been detected in the Faroe Islands.

Here I will give an update on the status of CMS and HSMI in the Faroe Islands including some preliminary genetic data on new PRV-1 variants causing HSMI have emerged in the Faroe Islands.

Describe how this is relevant to the industry

The knowledge from the Faroe Islands demonstrates that one of likely driver for the spread of PMCV and subsequently CMS (and most likely other pathogens) the last six to eight years seems to be the introduction of mechanical treatments for sea lice. These large treatment Wessels are mowed between farming sites most likely without appropriate cleaning and disinfection.

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Genomic Analysis Reveals Low Genetic Diversity and No Continuous Reintroduction of Piscine Myocarditis Virus (PMCV) in Faroese Farmed Salmon

Maria Marjunardóttir Dahl, Faroese National Reference Laboratory
Co-authors (if relevant) Debes Hammershaimb Christiansen, Petra Elisabeth Petersen

Summary of presentation

Piscine Myocarditis Virus (PMCV) is a dsRNA virus with 3 open reading frames and responsible for Cardiomyopathy syndrome (CMS) in Atlantic salmon which has proven to be of great concern to the farming industry. This study is based on a broad spatio-temporal representation of samples from Faroese salmon farming. Samples originating from 23 salmon farming production sites in the Faroe Islands and from a returning wild salmon were collected for disease surveillance purposes by the authorities and the farming companies over a period of 12 years. Whole genome sequences were obtained and phylogenetic analyses found PMCV to be highly homogeneous and revealed a monophyletic Faroese cluster comprising samples originating from farmed salmon. ORF1 and ORF2 both showed highly conserved regions, whilst the smaller ORF3 had no such region. The genome obtained from a returning wild salmon differed significantly from samples from the Faroe Islands, Norway, and Ireland. To set the phylogenetic data in context, information on roe and smolt origin, sampling site and date, Ct values and CMS clinical signs was collected for all samples in this study. Combined, the phylogeny and metadata show no continuous reintroduction of PMCV to Faroese farmed salmon and no evidence of vertical transmission being the main transmission mode.

Furthermore, the results show no apparent correlation between assigned CMS cases and potential virulence markers.

Describe how this is relevant to the industry

Although increasing number of CMS outbreaks have been recorded in the last decade, there is only one publicly available whole genome and currently no way of cultivating the virus. Hence, understanding PMCV transmission mode and route is fundamental for proper management and mitigation strategies in salmon farming. Here we present a fast amplicon- based whole genome sequencing method of PMCV directly from field samples.

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The successful story of combatting PD in Production Area 7 (Mid-Norway)

Anne Lene Dale, coordinator PO 7, Aqua Kompetanse AS, Norway

Coordinated preventive efforts among salmon producers, is key to ensure biosecurity at sea. It is evident that one producer alone only to a limited extent can avoid proliferation of disease once there is an outbreak. One effort that has proven successful is organizing marine production areas into smaller zones with coordinated production cycles and joint fallowing. Other important joint efforts would be the elaboration of common biosecurity plans with strong and equal demands on hygiene and trafficking for well boats, service boats and various vessels for delousing. Furthermore, a joint vaccine strategy, in combination with the above, could ensure the elimination of PD entirely.

Separate zones should be established based on known currents and average weather conditions, as well as on hydrodynamic modelling. Whereas it would be close to impossible to establish zones with

no hydrodynamic interaction, a well-prepared zone structure could prevent proliferation substantially. Knowing where a infectious agent would likely spread through the waters, can furthermore open for other preventive measures. Modelling is an excellent tool in this regard.

Norway's main policy on PD is to differentiate between endemic production areas (slaughtering out not required) and prevention areas (where slaughtering out is required).

This intervention will elaborate on efforts of coordination, drawing on examples from the ongoing excellent cooperation among companies in Production Area 7 (PO 7). The PD story of PO 7 is a very successful one – despite several outbreaks – companies have succeeded in keeping the area free from PD for five successive years. Examples will also be presented from efforts following the latest outbreaks in PO 8 in 2023.

Modelling spread of PD using water contact integrated in a three-dimensional hydrodynamic model

Erlend A. Mundal, Aqua Kompetanse, Norway
Co-authors Frank Gaardsted, Oceanbox

PD virus can be contagious from one site to another through water flow. Along the Norwegian coast the salmon farming sites are located at a variety of water bodies, with altering forces driving the water current. This makes the spread of contagious diseases to vary from site to site, and thus requires advanced modelling of a potential outbreak. By using a three-dimensional hydrodynamic model, the spread of PD from an infected site can be predicted using the method of water contact. From some cases of disease outbreaks along the coast, the method of water contact has been applied, and presents a new tool to obtain the possible spread of PD.

Describe how this is relevant to the industry

By applying the model FVCOM through Oceanbox the time required to obtain a likely spread of PD can be performed within hours.

An atypical course of Cardiomyopathy syndrome in Farmed Atlantic Salmon fed a clinical nutrition diet

Julia Mullins, Skretting Norway
Co authors: Johan Rennemo, Kjetil Berge (Skretting AS, Norway); Muhammad Naveed Yousaf, Tommy Berger Eriksen, Eirik Welde (Skretting Aquaculture Innovation, Norway); Camilla Robertsen, Bjarne Johansen (Nordlaks Havbruk AS); Charles McGurk, Espen Rimstad, Erling Olaf Koppang and Håvard Bjørgen (Norwegian University of Life Sciences, Norway)

Summary of presentation
Cardiomyopathy syndrome (CMS) poses a significant threat to farmed Atlantic salmon (Salmo salar), leading to high mortality rates during the seawater phase. Given that controlled experimental challenge trials with PMCV do not reproduce the mortality observed in severe field outbreaks of CMS, field trials on natural CMS outbreaks are warranted. This field study explored the impact of a clinical nutrition intervention, specifically a diet enriched with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), on a severe CMS outbreak in a commercial sea farm. CMS was diagnosed in a single sea cage with high mortality rates. Histopathological analysis, RT-qPCR in situ hybridization for virus detection, and fatty acid composition analysis were used to monitor the impact of disease and the inclusion of EPA and DHA in heart tissue. Following the implementation of

clinical nutrition, a decline in mortality rates, regression of CMS-associated changes, and a significant reduction in piscine myocarditis virus (PMCV) RNA load were observed within the salmon population. Fatty acid composition analysis of heart samples demonstrated increased levels of EPA and DHA, reinforcing the association between dietary factors, viral load dynamics, and overall fish health. Although further validation is needed in future studies, as field trials may not be sufficient to establish causation, our results indicate that optimizing the EPA + DHA levels may prove beneficial in severe CMS outbreaks.