Abstract submission 2022 Trination Edinburgh

Abstracts are organised in random manner

Number 1

Maintaining clearance of PD caused by SAV2 in Mid-Norway

Aoife Maloney Westgård, Paul J. Midtlyng, Erika Kunickiene, Arnfinn Aunsmo

In the most recent Tri-Nations meeting (Dublin, 2019) we reported on the preliminary success in territorial clearance of SAV-2 infection in the Nord-Trøndelag farming region, Mid-Norway. Based on continued vaccination of nearly all smolts transferred to the region´s marine sites since 2018, and continued monthly monitoring of infection status of sites using RT-qPCR, we can now report that so far, clinically apparent PD or SAV-2 infection are remaining absent from our farming region. Experiences made during this period with a novel type of side-effects of PD vaccination will be presented. Based on the results obtained regionally and recent published information, examples of the fish health econoimics of PD control in this region will be presented.

- Describe how this is relevant to the industry

PD is a viral disease of great importance in all the Trination countries that has shown to be difficult to combat in the field. The results from Mid-Norway, where a combination of vaccination in addition to biosecurity measures and unique production strategies, has resulted in clearance of SAV2, is of great interest to the indutry. We aim to convey these measures and experiences in our presentation and inspire other areas to achieve similar results.

- To which topic does the presentation belong? (chose as many as appropriate)
    - Situation update
    - Experience from the field
    - Management of disease
    - Welfare
    - Effect of vaccination
    - Economic consequences

Number 2

Characterisation of early phases of cardiomyopathy syndrome (CMS) pathogenesis in Atlantic salmon through various diagnostic methods

Camilla Fritsvold, Hilde Sindre, Aase B. Mikalsen, Øyvind Haugland, Haitham Tartor

In this study, CMS was induced in pre-smolt Atlantic salmon by injection (i.p., intra peritoneal, into the abdominal cavity) of a homogenate made of spleen samples originating from CMS diagnosed salmon. The resulting heart lesions of the experimental fish were graded by light microscopy (histopathology) and compared to the viral levels detected by real-time

RT-PCR in the hearts, in blood and mucus. In addition, detection of virus by positive staining in tissue slides by two different methods (immunohistochemistry (IHC) and a new in situ hybridisation (ISH) method, RNA scope) was compared. The results confirmed previous findings of the heart as the organ of choice for virus detection at later time points of infection. Detection of PMCV specific RNA in blood plasma at early time points is indicating a viremic phase, and mid-kidney also showed a relatively high load at these time points. The RNAscope ISH was shown to be both a more sensitive and robust method for in situ detection of PMCV compared to the established IHC, and can be a valuable support to histopathology and PCR results in CMS diagnostics, especially in early screening and in cases of untypical microscopical lesions or mixed viral infections.

- Describe how this is relevant to the industry

Together with salmon lice infestation, CMS is now considered one of the most important problems for Norwegian aquaculture, reflected both in the annual number of diagnoses and the economic losses due to mortality, and is also an increasing problem in other fish farming countries on the northern hemisphere, like Scotland, UK, Ireland and the Faroe Islands. Increasing focus on fish welfare and stronger demands for implementation of the 3Rs (replacement, reduction and refinement) both in farms and in research, makes research on non-lethal sampling methods like bloodsampling and mucosal swabs for CMS diagnostics very relevant. The fact that PMCV, the virus causing CMS, in non-culturable, makes new PMCV specific diagnostic tools valuable: Higher specificity than the established method, combined with a quite fast procedure that is easy to perform, makes the new in situ-method described a welcome and useful tool for both routine diagnostics involving CMS, and for further CMS research.

- To which topic does the presentation belong? (chose as many as appropriate)
    - Diagnostics

Number 3

A time-course study on the host responses induced at different time points post PMCV infection

Amr Ahmed Abdelrahim Gamil, Aase B. Mikalsen, Sunil K. Mor, Vikash Singh, Øysein Evensen

Piscine myocarditis virus is a toti-like virus that cause cardiomyopathy syndrome (CMS) in Atlantic salmon. Currently, CMS is one of the main disease challenges facing the salmon industry and continues to cause significant economic losses. Many aspects of the virus-host interaction are still not known including pathogenesis and the host responses generated at different phases of infection. In the present study, Atlantic salmon post smolts were infected with a heart homogenate prepared from PMCV infected fish and the hearts were collected at 6, 8 and 10 weeks post infection (p.i.). RNA was subsequently extracted from the heart tissues collected from infected as well as from control fish. Next generation sequencing was then used to evaluate the changes observed in the transcriptome at the different times p.i. In addition, a histopathological evaluation was also performed. Different transcriptomic profiles were observed at the different times post infections with the late times points (i.e. 8 and 10 weeks) being more similar. The list of genes that were differentially expressed included amongst others immune response related genes such as the MHC I & II, STAT proteins, type I interferon related genes as well as genes involved in different biological processes, the proteolytic process and antigen processing and presentation. Some of these genes were differentially regulated during the whole experimental period, while others were differentially regulated at specific time point p.i. Autophagy, FoxO signaling pathway, carbon metabolism pathways as well as MTOR and TNF signaling pathways were among the top differentially regulated pathway. One of the most noteworthy observations was that several immune response related pathways were down regulated at 8 weeks p.i. Overall, the results showed that different profiles of responses are generated in the heart of infected fish at different times post infections and that week 8 weeks of the infection may be critical for the development of infection as many immune related genes were differentially downregulated at this time point.

- Describe how this is relevant to the industry

The generated knowledge, in this study, contribute to the understanding of pathological mechanisms employed during PMCV infection and is a step forward towards understanding the infection biology of PMCV. This in turn can eventually aid in developing future control strategies.

- To which topic does the presentation belong? (chose as many as appropriate)
    - Molecular epidemiology

Number 4

Dynamics of Piscine Orthoreovirus-1 (PRV-1) Infection During Pre-smolt Stages of Atlantic Salmon (Salmo salar)

Dhamotharan Kannimuthu, Ma Michelle Demogina Penaranda, HyeongJin Roh, Ghebretnsae Dawit Berhe, Håkon Berg-Rolness, Stig Mæhle, Craig Morton, Bjørn Olav Kvamme, Søren Grove; Øystein Wessel

Piscine orthoreovirus (PRV) causes the disease heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon and it is predominantly observed after seawater transfer. In this study, we conducted a long-term PRV-1 challenge experiment to examine the temporal profile of viral load, shedding and/or clearance in Atlantic salmon over the course of its growth from fry to parr stage. Fish (mean weight: 1.1±0.19g) infected with PRV-1 (high virulent variant) via intraperitoneal (IP) injection reached peak viral RNA load at 2 weeks post-challenge (WPC) in heart and muscle tissues. The viral RNA load was maintained at relatively high levels in whole blood (25.0±2.4), spleen (26.0±1.4), and head kidney tissues (27.8±0.9) even after 54 WPC. Heart lesions typical of HSMI, including epicarditis and myocarditis, were observed at 6 and 8 WPC, but heart inflammation already began to resolve by 10 WPC. Despite achieving high viremia, PRV-1 infection failed to cause any mortality during the 54-week virus challenge period. However, significant growth differences between infected and control fish were observed during the experimental period. Cohabitation of persistently infected fish (10 and 31 WPC) with naïve Atlantic salmon fry did not result in successful infection, suggesting that fish during this phase of infection are not shedding significant amount of virus to initiate horizontal transmission. Moreover, stress exposures did not affect the viral RNA load or shedding of PRV-1 at 12 and 31 WPC. Recurrent blood sampling of PIT tagged individual fish from 44-56 weeks revealed maintenance of viral RNA in blood in a steady state. Superinfection of PRV-1 persistently infected fish with salmonid alphavirus 3 (SAV3) did not show any differences in SAV or PRV viral load compared to control fish. These findings confirm the persistence of PRV-1 for a long period in blood and lymphoid organs and limited shedding of Atlantic salmon pre-smolts.

- Describe how this is relevant to the industry
  - In Norway, Heart and skeletal muscle inflammation (HSMI), caused by Piscine orthoreovirus (PRV), is one of the most common viral diseases of farmed salmon. In recent years, the number of cases detected has been slowly increasing, and HSMI has been flagged as an escalating problem for hatcheries and ongrowing facilities. We have conducted a long-term experiment, by infecting salmon fry with PRV and following the course of infection for more than 1 year. Our results show that viral RNA could be detected in the fish throughout the experiment. However, co-habitation experiments suggested that infected fish did not shed significant amounts of live infectious virus after week 10. The results of this experiment will contribute to a greater understanding of persistant long-term PRV infection in salmon aquaculture.
- To which topic does the presentation belong? (chose as many as appropriate)
    - Management of disease
    - Welfare

Number 5

Histopathologic cardiac scoring methods used in Cardiomyopathy syndrome (CMS), Pancreas disease (PD) and Heart and skeletal muscle inflammation (HSMI)

Marta Alarcón, Liv Østevik, Helene Wisløff, William Reed, Anne Katrine Reed, Mette Hofossæter, Marianne Kraugerud, Hege Hellberg, Kai-Inge Lie

Viral induced myocarditis-related diseases (CMS, PD and HSMI) have been a health and welfare problem in farmed Atlantic salmon for several decades and have caused significant economic losses to the aquaculture industry. To diagnose and differentiate between these three conditions, histopathological examination of the heart is essential. There is ongoing research on how nutrition, genetics and vaccines can be used to reduce the impact of these diseases and improve the cardiac health of farmed salmonids. In most of these research projects, cardiac histopathology is included, and a histopathologic score is used. Scoring (grading) of the microscopical lesions can be performed in many ways, but three main characteristics are important: The scoring method should be definable, reproducible and it should produce meaningful results. There are several published cardiac histopathological scoring methods used for CMS, PD and HSMI, but it is important to remember that there is no universal cardiac score protocol that can be used for all types of projects. To be able to detect biological differences between groups, a proper scoring system tailored for the purpose of the specific research should be designed. There are important considerations to apply before cardiac histopathological examination. Sampling should be done to avoid autolysis and in a consistent way. If part of the heart is required for other analyses like PCR, all atrium and most of the ventricle should be included for histopathology and removing the apex of the heart must be done carefully to avoid accidentally removing or damaging the epicardium. The group level identity of the samples should be blinded (masked) for the pathologist. However, a proper understanding of the study objective and the experimental design is crucial and fluent communication with the client, or the remaining research group is essential. It is advisable to read the samples in a random order (not per group) and within a limited time period. In projects with high number of samples where several pathologists need to be involved to meet the deadlines, it is important to start with a calibration of the different pathologist with a subset of samples and ideally to do some interobserver analyses to provide consistency to the results. During the last eight years, we have histologically evaluated and scored many thousand hearts from numerous projects of different kind. We will show our own experiences from lab trials and field material and discuss the different scoring approaches, weakness, and strengths, from qualitative or semiquantitative to quantitative data. We will present data from the development of an adapted CMS histopathologic cardiac score for a project where the hearts were evaluated by two blinded pathologists and the interobserver agreement have been calculated. Lastly, we will briefly discuss how the methods used for statistical analyses should respect the properties of the score used, ordinal or numerical (continuous variables).

- Describe how this is relevant to the industry
  - Cardiac histopathology scoring methods are widely used in the salmon farming industry, and histopathology remains the gold standard method to evaluate the effect of diets, vaccines and breeding programs in fish affected with CMS, HSMI and PD. There are multiple factors that can affect the histopathological score that might impact the results of the research, from bad experimental design, incorrect sampling, inadequate scoring system, lack of experience to interpret histopathological sections to improper statistical analysis of the resulting data. Experiences from lab trials and field material will be presented and discussed.
- To which topic does the presentation belong? (chose as many as appropriate)
  - Diagnostics

Number 6

Effect of a vaccine against CMS

Marius Karlsen

Cardiomyopathy syndrome (CMS) in Atlantic salmon is a serious cardiac disease affecting Atlantic salmon during sea water stages. PHARMAQ will present an update from the work with developing a vaccine against CMS, including efficacy data of vaccine candidates tested in a laboratory challenge model.

- Describe how this is relevant to the industry

- There is a great need for a CMS vaccine in salmon aquaculture
- To which topic does the presentation belong? (chose as many as appropriate)
  - Effect of vaccination

Number 7

PMCV infection results in presence of defective genomes including deletions
RACHEAL AMONO, Turhan Markussen, Aase B. Mikalsen, Øystein Evensen

Piscine myocarditis virus (PMCV) is a double stranded RNA virus that has been known as the infectious agent causing cardiomyopathy syndrome for more than a decade, but the infection mechanisms and virulence factors associated with infection progress and disease severity still remain elusive. Most RNA viruses evolve rapidly due to the lack of a proofreading function in their polymerases when their genome is replicated, and this results in flexibility of their genomes. The genetic changes that accumulate are believed to play a role in the interactions between the pathogen and the host, with a favoring towards mutant genotypes that display improved fitness in the given pathogen-host environment. Single nucleotide mutations that occur in the viral genomes may result in amino acid changes in encoded proteins. In the event of a changed amino acid, structural and/or functional changes in the viral protein including a change in virulence may occur. Viral RNAs harboring single nucleotide substitutions, deletions of various sizes or other changes that render the viral RNA non-replicative are well-known phenomenon in nearly every virus family. These are often referred to as defective viral genomes (DVG) and may have confirmed or expected characteristics causing interference with the viral replication as it may be packed as virus particles and propagates and accumulates when it infects the cells at high concentrations and attenuates the virus (and/or its replication) or it may interfere with the immune response of the host. DVGs described as genomes having deletions have mainly been characterized for the mammalian viruses, e.g. Chikungunya, Zika and SARS-CoV-2, but has also been identified in fish viruses as described for salmonid alphavirus (SAV), by our group previously and also in an ongoing project. Epidemiological studies on PMCV have so far shown limited genetic variability for this virus, still the genetic variation seen reflects variation both between hosts in a single infection case and also within single hosts. Most often there is an apparent lack of any clear epidemiological or evolutionary linkage between the genotypes sequenced. We have, from our recent studies of sequencing PMCVs third open reading frame (ORF3), identified genomic deletions also in this virus. These were first indicated by the unexpected presence of products of smaller size following PCR and electrophoretic separation. In addition, for apparently full-length amplicons, we experienced regions of reduced quality in the chromatograms from Sanger sequencing. As a follow-up of these findings, we initiated a small pilot study in order to characterize in more detail these smaller-sized amplicons by sequencing and also attempted to characterize deletions originating in other parts of the genome. Our preliminary results describing deletions originating from all parts of the PMCV genome will be presented. Putative underlying mechanisms behind their generation and how the presence of DVGs may affect the infection outcome and diagnostics will be discussed.

- Describe how this is relevant to the industry

    - There is still a lot of unknown regarding CMS field outbreaks and how the virus and its infection mechanisms affect disease outcome. Different factors contribute, including both host and virus pathogen factors and also environmental factors. PMCV has been observed to have varying infection progression between different CMS outbreaks but factors behind the variations are not known. Pathogen factors may include differences in virulence characterized by genetic variations causing changes in the characteristics of virus proteins, but in the latest years defective viral genomes have been noted to play an essential role in the host-pathogen relationship for several mammalian and arboviruses. Defective virus genome variants may contribute to means for adaptation, immune escape and/or virus perpetuation and may in general play a major role in interfering with replication of the infectious/replicative virus and be important in virus persistence. Knowledge of how these defect genomes interfere with PMCV infection mechanisms may aid in development of more efficient vaccines and also therapeutical applications could be included. Also, high concentrations of defect verses full-length genomes can create biases and influence results obtained in PCR-based diagnostics.
- To which topic does the presentation belong? (chose as many as appropriate)
    - Diagnostics
    - New technology

Number 8

The development of nucleic acid vaccines for the protection of infection from Pancreas Disease in Ireland.

Dr Anita Talbot, Hamish Rodger

The overall purpose of this research is to develop and test nucleic acid vaccines for their ability to protect salmon against the salmon alphavirus subtype 1 (SAV1), the dominant virus subtype causing salmon Pancreas Disease (PD) in farmed salmon in Ireland. Nucleic acid vaccines are made the newest form of vaccines, known as third-generation vaccines, and are made using deoxyribonucleic acid (DNA) and messenger ribonucleic acid (mRNA). Traditional vaccines, or first-generation vaccines, have been used for the past 100 years in human and veterinary medicine include whole inactivated pathogens, and live-attenuated pathogens. Traditional vaccines have drawbacks including less than optimal efficacy with certain pathogens, safety issues with regard to live-attenuated vaccines and time from development to getting to market. Second-generation vaccines, instead of using the whole organism, uses pieces of the organism and includes subunit elements, conjugated/recombinant antigens, or synthetic proteins. The effectiveness of the plasmid DNA vaccine (pDNASAV1ATU) and its ability to induce an active immunisation in Atlantic salmon will be tested in salmon parr. One measure of effectiveness will be the vaccines' ability to reduce the number of cardiac and skeletal muscle lesions that result from infection with SAV. Although mRNA vaccine development is in its infancy, huge strides have been made since 2020 with the development and administration of mRNA vaccines for Coronavirus. Developing mRNA vaccines for food-producing species, including salmon, raises many questions for which there are, as yet, no answers. The mRNA vaccine for protection against SAV1 (mRNASAV1ATU) will be tested first using in-vitro cell culture methods before being tested in-vivo, in fish

- Describe how this is relevant to the industry
  - The relevance of the current research to the salmon industry will include: -The development of more effective vaccines to prevent PD outbreaks in Ireland and the subsequent generation of internal organ lesions, which leads to compromised fish welfare, poor condition, and the downgrading of fish quality at harvest. -The capacity to produce and test nucleic acid (pDNA/mRNA) vaccines in Ireland for fish, and other aquaculture species -The potential for the rapid deployment of effective mRNA autogenous vaccines to the Irish Aquaculture industry to prevent losses from new and emerging viral diseases.
- To which topic does the presentation belong? (chose as many as appropriate)
  - Management of disease
  - Welfare
  - Effect of vaccination

Number 9

Salmonid alphavirus accumulates deletion variants localized to specific regions of the viral genome

Turhan Markussen, Thomas Vallet, Øystein Evensen, Marco Vignuzzi, Aase B. Mikalsen

We have in previous studies shown that the SAV3 genome can recombine during infection and generate so-called defective viral genome variants (DVGs). These DVGs are mainly characterized by deletions of varying in size, occurring in different parts of the genome. Still, some deletions have been found to occur at certain positions with higher frequency than in others. It has been shown for other RNA viruses that certain DVG types can be packaged, exit the cell, and re-infect new cells. Recent studies on mammalian viruses such as Chikungunya and Zika virus have shown that both the composition and number of DVGs produced during infection can influence the host immune response and disease severity. From our new project, we present early results obtained from serial passaging of SAV3 in Chinook salmon heart (CHH-1) cells with subsequent sequence analyses combining next generation sequencing (NGS) and a computational approach previously used to detect DVGs in Chikungunya and Zika virus infections. This approach enabled us not only to achieve a massive increase in the number of deletion types identified but also to detect deletions covering larger portions of the SAV3 genome than previously possible from the PCR-based studies. The results show that the number of different deletion types increase during passaging and that larger deletion types, encompassing parts of non-structural nsp3/4 coding regions and/or all portions of the structural genes, accumulate during later passages. The putative mechanisms involved in generating these deletions and the potential future significance and applicability of DVGs in controlling PD, will be discussed.

- Describe how this is relevant to the industry
  - Should genome deletion variants (defective viral genomes, DVGs) identified during SAV infection be linked to differences in PD severity, these could represent tempting targets for molecular manipulation. Together with more traditional strategies for virus attenuation, DVGs could aid in the development of more efficient and safer attenuated DNA-based vaccines,

with lesser risk of reversion to virulent wild type. Potential therapeutical applications of DVGs should also not be excluded. In addition, high concentrations of DVGs compared to full-length genomes can influence results obtained in PCR-based diagnostics. Hence, knowledge of DVG types and their abundance during infection is of importance.

- To which topic does the presentation belong? (chose as many as appropriate)
  - Diagnostics
  - New technology

Number 10

Novel myocardial pathology in farmed salmonids in Norway

Helene Wisløff, Anne Katrine Reed, Mette Hofossæter, Marta Alarcón, Liv Østevik, William Reed, Marianne Kraugerud, Hege Hellberg, Kai-Inge Lie, Trygve Poppe

Over the last few years, a novel histopathological finding has been observed in the compact myocardium of Atlantic salmon (Salmo salar L) and rainbow trout (Oncorhynchus mykiss Walbaum). This finding has been observed in salmon which have died during or after non-medical treatment against sea lice (Lepeophtheirus salmonis Krøyer). Reported gross lesions have been hemopericardium, distended hearts, pale myocardium, ascites and congestion. The histopathological findings in typical cases are multifocal to coalescing areas in stratum compactum with paler cardiomyocytes with granular or vacuolated cytoplasm. The lesions are centered around terminal branches of the coronary arteries. No significant inflammatory response is observed, apart from mild to moderate epicarditis. Concurrent mild inflammatory lesions consistent with heart and skeletal muscle inflammation (HSMI) and cardiomyopathy syndrome (CMS) has been seen in some cases. In some of the fish with severe lesions in the myocardium, zonal ischaemic necrosis is observed in the liver. Organs from wild salmon have been examined for comparison, and no similar lesions have been found in the heart. Farmed salmon frequently experience severe stress during crowding, transportation, pumping and mechanical/thermal lice treatments. The report “The Health Situation in Norwegian Aquaculture 2021” points at thermal and mechanical de-lousing as one of the two most serious welfare and health issues in Norwegian salmon farming. Transportation may also be a serious stressful experience for the fish. Similar myocardial lesions have been observed in salmon transported for long distances to slaughter. The observed lesions in stratum compactum are interpreted as degeneration of cardiomyocytes. The cause(s) of the lesions is not clear. It is, however, reasonable to suggest that they are ischaemic lesions. The distribution of the lesions around terminal branches of the coronary arteries may indicate impaired vessel function resulting in reduced blood perfusion and ischaemia. One important cause of myocardial ischaemia is arteriosclerosis of the coronary artery. Other mechanisms may also reduce the blood supply to the compact myocardium. Increasing the water temperature from 13 to 25℃ has been shown to cause cardiac arrhytmias, acidosis and increased blood lactate levels, which compromises the oxygen supply to the myocardium. In human medicine, coronary artery spasm (CAS) is a well-established entity that severely reduces the arterial blood supply to the myocardium and play an important role in the pathogenesis of sudden cardiac death and ischaemic disease. Some predisposing factors for CAS are chronic inflammation, endothelial damage, temperature shock/changes and physical challenges. Several of these factors may apply for farmed salmonids. A common factor for most cases in our material is a history of acute severe physical or thermal stress, which may induce CAS and thereby reduce the blood supply to the compact layer of the myocardium. Arteriosclerosis may be an aggravating factor in many cases, but the high acute prevalence of heart failure associated with stressful situations may indicate that interaction(s) with other mechanisms is necessary, or that other mechanisms alone are responsible for the lesions. Ventricular lesions similar to those described in farmed salmon have also been diagnosed in rainbow trout recently transferred to sea water at low temperatures (2–4°C). The actual prevalence of the observed lesions is not known, but an increasing frequency of observations the last few years may indicate an increasing occurrence. The findings are interpreted as degenerative changes although they are not exactly alike the textbook definition of myocardial degeneration. The changes may represent either degeneration due to ischemia or primary degeneration as in cardiomyopathies. Further investigation of these findings is necessary to get more knowledge and to improve the welfar...

- Describe how this is relevant to the industry
    - These novel findings are relevant to the cardiac health of farmed salmonids and deserve more attention/further research/investigation to improve the welfare of the fish. To improve the fish welfare and reduce the mortality associated with stressful handling, both breeding programmes, rearing strategies and improvement of treatment-and handling methods need to be investigated. Histopathological examination is an important tool to reveal these cases, and will serve as a valuable contribution to gather more data and information about the possible causes and disease mechanisms.
- To which topic does the presentation belong? (chose as many as appropriate)
    - Diagnostics

Number 11

Serum Proteomics of CMS Field Outbreak Samples

Lewis Moore, P. Sourd, A. Bordeianu, C. Chadwick, K. Thomson, J. Del-Pozo

Cardiomyopathy syndrome (CMS) in Atlantic salmon (Salmo Salar L.) is caused by piscine myocarditis virus (PMCV). This viral cardiomyopathy has a major impact on farmed Atlantic salmon production in both Scotland and Norway through sudden death from heart failure and reduced growth. Prophylactic and treatment measures for CMS are currently unavailable, therefore, salmon farmers rely on functional feeds and early harvesting to manage the impact of the disease. Diagnosis of CMS is routinely performed using clinical signs, such as small haemorrhages spread diffusely across the ventrum, histopathology, and RT-qPCR. Although histopathology and RT-qPCR are accurate for diagnosis they require terminal sampling of valuable fish at the end of their production cycle. An early indication of the onset of CMS in subclinical salmon, preferably using non-lethal diagnostic methods, would improve the timings associated with mitigation steps to decrease the economic burden of CMS. The tools currently employed for diagnosis of CMS do not allow for predictive diagnostics or give insight into viral proliferation throughout a cohort and, therefore, the success of mitigation strategies is difficult to gauge. Identifying CMS in subclinical stages would allow for better assessment of viral proliferation at a population level allowing for the implementation of superior targeted mitigation steps to decrease the economic burden of CMS In other animal species, it is possible to diagnose cardiac disease using appropriate biomarkers. In both human and veterinarian medicine, serum biomarkers are routinely used to prognostically indicate disease status and identify individuals with increased risk of disease, allowing for early implementation of management strategies. Knowledge of biomarkers associated with cardiac disease in salmon is limited, although several candidates have been highlighted as potential cardiac biomarkers for fish, including creatine kinase (CK), lactate dehydrogenase (LDH), natriuretic peptides (salmon cardiac peptide (cSP)) and troponins. Here, we aimed to generate serum proteomic data to identify putative biomarkers for subclinical CMS. To do this we followed our previous qualitative proteomics study (LC-ESI-MS/MS) with a semi-quantitative proteomic study using isobaric tags for relative absolute quantitation of proteins (iTraQ). This used a field case and controls design using CMS negative controls, clinical moribund CMS fish and sub-clinical positive CMS fish which were identified as such using histopathology and RT-qPCR. We performed seven four-plex analyses, each experiment had a negative control (reference), a low, moderate, and high viral PMCV load. Common proteins across the seven experiments were collated, and summary statistics were conducted on the relative ratios of positive samples to their negative control. Overall, 108 protein proteins were relatively higher in subclinical CMS than in controls. None of these was exclusive to serum from fish with subclinical CMS, but it was possible to identify putative biomarker candidates based on biological meaningfulness, previous use as biomarkers, and relative concentrations. Relevant putative biomarkers noted included several acute phase proteins (e.g., haptoglobin, retinol-binding protein, A2-macroglobulin-like), and leakage biomarkers (e.g., creatine kinase), among others. Clinical evaluation of the suitability of these proteins as biomarkers for CMS, either individually or as a part of a panel, is now required to establish their potential as tools for the early diagnosis of CMS.

- Describe how this is relevant to the industry
- CMS is a viral-induced pathology that accrues a huge economic burden for the aquaculture industry each year due to sudden death before harvest in subclinical fish. Using specific protein biomarkers for the detection of viral pathologies in subclinical salmon would allow for an early diagnosis and could allow farms to introduce preventative measures. As PMCV is incurable, has no vaccine treatment, and can present without symptoms, identification of a disease-specific protein biomarker(s) in subclinical fish could allow for a comprehensive diagnostic tool for the identification and prognosis of the disease. Aquaculture farms could use biomarker tests to pre-empt disease outbreaks hence decreasing the economic burden of the viral pathology.
- To which topic does the presentation belong? (chose as many as appropriate)
- Management of disease
- Diagnostics

Number 12

Genome sequencing for epidemiological studies of SAV2 and SAV3 in Norway

Marius Karlsen, Svein Alexandersen, Dan MacQueen

Genomic epidemiology can support disease control by rapidly informing on pathogen spread. We have sequenced genomes of SAV2 and SAV3 collected from Norwegian fish farms, covering the major time-frames and geographical regions where these epidemics have been active. The SAV genomes were obtained by nanopore sequencing, allowing extensive data to be rapidly generated from many samples. The sequences were used in phylogenetic analyses that provide new insights into the spread of PD in the Norwegian salmon industry. Several cases of likely anthropogenic PD spread were strongly supported. The analyses confirmed that SAV genomes accumulate enough genetic changes to inform on epidemiology over relevant time-scales, such as the salmon lifecycle. The strategy presented offers a useful new tool to track viral disease transmission and viral genetic changes relevant to pathology or vaccine efficacy, and is being further pursued in a new collaborative project between the Roslin Institute in Edinburgh and PHARMAQ, which will include additional viral pathogens and samples from Norway and the UK.

- Describe how this is relevant to the industry
    - A new tool for studies of PD epidemiology is presented. The method allows for tracking virus spread through rapid full-genome sequencing. Examples of its use on field samples from Norway will be presented.
- To which topic does the presentation belong? (chose as many as appropriate)
    - Molecular epidemiology

Number 13

Effect of Vaccines Against PD on Viral Shedding and Disease Transmission from Atlantic Salmon in Seawater Challenged with SAV, Subtype 2

Ragnar Thorarinsson, Hilde Sindre, Jeffrey C. Wolf, Eystein Skjerve, Jose F. Rodriguez, Anne Ramstad

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 enteric redmouth 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, subtype 2 (SAV2) using a cohabitation challenge in seawater. At 19 days post challenge (DPC), 20 representative fish from each of the study groups were transferred and placed separately into 10 smaller tanks with identical flow-through levels. Water samples were taken two days later from each of these tanks followed by addition of non-infected fish, either as naïve or as PD vaccinated fish. Quantitative results will be presented including SAV RNA concentrations in water, RT-qPCR from hearts and pathological changes in the heart and pancreas.

- Describe how this is relevant to the industry

  - PD threatens the successful rearing of Atlantic salmon in Ireland, UK and Norway. PD is reported to spread effectively between farms and poses a risk to spread from the SAV2 zone further north into Norway's PD-free zone. The results to be presented provide insights into how vaccination against PD can help reduce the environmental spreading of SAV and thus helping to improve animal welfare and economic sustainability of the salmon industries in Ireland, UK and Norway.
- To which topic does the presentation belong? (chose as many as appropriate)
  - Management of disease
  - Welfare
  - Effect of vaccination

Number 14

Effect of Vaccines Against PD in Atlantic Salmon Challenged with SAV, Subtype 2, Using a Seawater Cohabitation Challenge Model

Ragnar Thorarinsson, Hilde Sindre, Jeffrey C. Wolf, Eystein Skjerve, Jose F. Rodriguez, Anne Ramstad

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 enteric redmouth 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, subtype 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 prior to challenge will be presented. Post challenge data including mortality, viremia, levels of neutralizing antibodies, levels of pathological changes in target organs and loss of growth caused by PD through the challenge period will, in addition, be presented.

- Describe how this is relevant to the industry
  - PD caused by SAV2 threatens the successful rearing of Atlantic salmon in Norway's SAV2 zone and in Scotland. Data on vaccine efficacy against SAV2 is to date very scarce. Vaccination against PD caused by SAV2 represents an important management tool to improve animal welfare and economic sustainability of the salmon industry in these two countries.
- To which topic does the presentation belong? (chose as many as appropriate)
  - Management of disease
  - Welfare
  - Effect of vaccination

Number 15

Metabolomics in aquaculture: metabolic changes in response to Piscine orthoreovirus (PRV) infection of Atlantic salmon

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Metabolomics is the holistic study of small molecules (<1500 Da) and commonly makes use of advanced analytical chemistry with the aim to study physiological responses of living organisms to external stimuli. Several studies have shown the great potential of metabolomics to provide detailed molecular information about disease progression in fish by determining pathogen-associated biomarkers. In the present study, we looked at the metabolic changes in plasma of Atlantic salmon (Salmo salar) challenged with Piscine orthoreovirus (PRV). The virus causes lethal heart and skeletal muscle inflammation in fish, and pose a major problem to the global salmon farming industry. Both targeted and untargeted metabolomic analyses were performed using liquid chromatography coupled to high-resolution mass spectrometry (LC−HRMS). For targeted metabolomics, the commercial Absolute IDQ p400 HR metabolite reference kit was applied to identify differences in the metabolic composition of plasma samples from infected and non-infected fish. The untargeted approach included the analysis of the same plasma samples by hydrophilic interaction liquid chromatography–HRMS, measuring the highest possible number of metabolites. The results showed that several metabolites involved in the glycerophospholipid, glycerolipid, sphingolipid and fatty acid metabolism were decreased in infected fish as compared to healthy controls, indicating the critical role of lipids during a progressing PRV infection. Furthermore, the metabolomic analysis revealed the alteration in several amino acids in the course of HSMI development. We found that multiple metabolic pathways such as aminoacyl-tRNA biosynthesis, arginine and proline metabolism, valine, leucine and isoleucine degradation/biosynthesis were disturbed. However, further exploration of these pathways will contribute to the better understanding of the viral interference with the host metabolism and, potentially, help us understand the processes involved in development of HSMI and suggest targets for therapeutics.

- Describe how this is relevant to the industry
  - Metabolomics is a new and innovative approach that has the potential to make a considerable contribution regarding to the Piscine orthoreovirus (PRV). Metabolomihcs offers new possibilities to identify the particular metabolic pathways and explore underlying mechanisms of PRV progression in Atlantic salmon. The data provided in the present study could be further used for the identification of suitable biomarkers facilitating development of novel antiviral therapeutics.
- To which topic does the presentation belong? (chose as many as appropriate)
  - Management of disease
  - Diagnostics
  - New technology

Number 16

Does PMCV primarily infect salmon?

Espen Rimstad, Ingvild B. Nyman, Øystein Wessel

Piscine myocarditis virus (PMCV) has a physical and chemical construction common to members of the virus family Totiviridae. The totiviruses infect unicellular animals and fungi. There is an evolutionary similarity between PMCV and viruses that infect unicellular organisms, but there is an evolutionary distance of at least several hundred million years between unicellular parasites and salmon. In general, viruses and host cells have over millions of years evolved together and are carefully adapted to each other. All viruses multiply intracellularly and are selected for interactions with the proteins and intracellular responses that the virus encounters. Viral genes and proteins counter-react to reduce the effect of the cell's antiviral response. The antiviral responses of unicellular organisms are very different from those of vertebrates and viruses that infect vertebrates have therefore genes that are different from those of viruses that infect unicellular animals. Based on these basic viral properties, we have investigated the hypothesis that PMCV primarily infects single-celled organisms within salmon, and not salmonid cells. We have established RNAscope in situ hybridization assay for the detection of RNA from PMCV. The results from heart tissue show staining of the spongiosum layer, but no staining of the compactum layer. The data indicate that there is no detectable viral RNA in compactum. The PMCV particle has a genome of double-stranded RNA (dsRNA), while virus proteins are encoded by mRNA, which is single-stranded (ssRNA) produced by intracellular viral sub-particles. We have established an RT-qPCR as well as an enzymatic assay that both distinguishes between dsRNA and ssRNA. We have used these methods for investigation of the forms of viral RNA found in various organs. The results show that in heart tissue, the dominating form of viral RNA is viral mRNA. The results indicate that there are no assembly of new infectious PMCV in the heart. The spongiosum layer, that is the only compartment of the heart where viral RNA can be found, is a dead end for virus replication.

- Describe how this is relevant to the industry
  - Effective specific measures against a disease require that the etiology and pathogenesis of the disease are mapped. If PMCV is primarily an infection of a unicellular organism, the current strategy to reduce CMS in salmon will not be successful. This current project is an attempt to uncover the hiding places of PMCV and how this virus contributes to the pathological changes found in CMS.
- To which topic does the presentation belong? (chose as many as appropriate)
  - Diagnostics
  - New technology
  - Emerging diseases (ie PRV3)

Number 17

Promoting cardiac robustness to withstand infections: Is it important?
Ida Beitnes Johansen, Marco A. Vindas, Ole Folkedal, Simona Kavaliauskiene, Erik Höglund, Tormod Haraldstad, Michael Frisk, Vilde Arntzen Engdal, Øyvind Øverli
Reported mortality in farming of Atlantic salmon over the past years show a clear transition in mortality trajectory, with a marked increase during the latter months of the production cycle.

Of particular concern, clear signs of compromised welfare and huge production losses are reported in association with stressful interventions. Heart disease of both non-infectious and infectious nature is an increasing problem in farmed salmonids and increasing evidence suggest cardiac failure as a primary contributor to mortality. Curiously, farmed salmonids tend to have more rounded ventricles with crooked (i.e. misaligned) outflow tracts (bulbus arteriosus). Both morphological traits are clearly associated with increased work load on the heart (i .e. cardiac stress), impaired cardiac performance and likely impact on general cardiac robustness. Adding to such non-infectious cardiac conditions are viral diseases affecting the heart. Cardiomyopathy syndrome triggered by the piscine myocarditis virus (PMCV) is, for example, a severe cardiac disease in Atlantic salmon. Over the last decade it has become one of the leading causes of morbidity and mortality in the Norwegian salmon aquaculture industry. Of note, PMCV infection does not necessarily lead to CMS and in locations with confirmed PMCV virus outbreaks only a small fraction of fish develop CMS. These observations indicate large inter-individual variation in disease susceptibility. Factors contributing to both deviating cardiac morphology and variation in susceptibility to viral diseases are largely unknown. Here, we ask whether these traits may be linked and specifically, whether general cardiac robustness may be an important factor in disease resilience. Indeed, our data indicate a link between cardiac morphology and CMS susceptibility highlighting the possibility that general cardiac robustness may affect the ability to withstand cardiac infections. If so, implementing production practices aimed at improving cardiac morphology may alleviate cardiac morbidity and mortality in the production of Atlantic salmon. Finally, we have recently identified production practices associated with improved cardiac morphology and robustness, perhaps providing a means to improve disease resilience.

Relevance for the Industry:

Both cardiac deformities and CMS are severe cardiac conditions contributing to poor welfare and mortality of Atlantic salmon. Indeed, disease-related production losses remain a serious risk to the reputation and profitability of the aquaculture industry worldwide. Thus, identifying practices that improve cardiac health and robustness is a prerequisite for sustainable aquaculture.

Topic: Production strategies on disease management

Number 18

Decreased Water Temperature Enhance Efficient Piscine orthoreovirus Genotype 3 Replication and Severe Heart Pathology in Experimentally Infected Rainbow Trout

Juliane Sørensen, Argelia Cuenca, Anne Berit Olsen, Kerstin Skovgaard, Niels Jørgen Olesen and Niccolò Vendramin

Piscine orthoreovirus genotype 3 (PRV-3) was first discovered in Denmark in 2017 in relation to disease outbreaks in rainbow trout (Oncorhynchus mykiss). While the virus appears to be widespread in farmed rainbow trout, disease outbreaks associated with detection of PRV-3 has only occurred in recirculating aquaculture system, and has predominantly been observed during the winter months.

To explore possible effects of water temperature, an in vivo cohabitation trial was conducted in rainbow trout 5°C, 12°C, and 18°C. For each water temperature, a control tank containing mock-injected shedder fish and a tank with PRV-3 exposed fish were included. Samples were collected from all experimental groups every second week post challenge (WPC) up until trial termination at 12 WPC.

PRV-3 RNA load measured in heart tissue of cohabitants topped at 6 WPC maintained at 12°C and 18°C, while reached its peak at 12 WPC in fish maintained at 5°C. In addition to the time shift, significantly more virus was detected at the peak in fish maintained at 5°C compared to 12°C and 18°C.
Furthermore, a significant reduction in the haematocrit levels was observed in the cohabitants at 12°C in correlation with the peak in viraemia at 6 WPC; no changes in haematocrit was observed at 18°C, while a non-significant reduction (due to large individual variation) trend was observed at cohabitants held at 5°C.

In shedders, fish at 12°C and 18°C cleared the infection considerably faster than the fish at 5°C: while shedders at 18°C and 12°C had cleared most of the virus at 4 and 6 WPC (10 dCt in difference from week 4 to week 12), respectively, high virus load persisted in the shedders at 5°C until 12 WPC.

Immune gene expression analysis showed a distinct gene profile in PRV-3 exposed fish maintained at 5°C compared to 12°C and 18°C. The immune markers mostly differentially expressed in the group at 5°C were RIGI, IFIT5 and RSAD2 (viperin).

In conclusion, these data show that low water temperature allow for significantly higher PRV-3 replication in rainbow trout, and a tendency in more severe heart pathology development in PRV-3 injected fish. Increased viral replication was mirrored by increased expression of important antiviral genes. Despite no mortality being observed in the experimental trial, the data comply with field observations of clinical disease outbreaks during winter and cold months.

Number 19

Infection challenges for rainbow trout farmed in RAS – from a diagnostic challenge to an opportunity for innovative diagnostic approach

Niccolò Vendramin, Juliane Sørensen, Jacob G. Schmidt, Tine Iburg, Lone Madsen, Kerstin Skovgaard and Argelia Cuenca

During the last decades recirculating aquaculture (RAS) has gained in numbers, and an increasing percentage of cultured fish comes from some type of RAS. The transition from traditional flow through farms to RAS, however, has brought along some challenges for health and welfare of farmed fish.

The large investments and running cost to produce in RAS imply an intensification of production, and this can hesitate, as a consequence, in a reduced attention to biosecurity level. The frequent introduction of new batches of fish, compared to flow through system, is a risk factor in itself. Recirculating the water to a higher degree intrinsically changes the farming environment, and this can be correlated with a favorable environment for the emergence of opportunistic pathogens. This is what we have observed with Piscine orthoreovirus 3 (PRV-3), which to our knowledge cause disease problems predominantly in RAS. Besides PRV-3, commonly detected pathogens are other virus as Infectious Pancreatic Necrosis Virus (IPNV) as well as bacterial pathogens as Flavobacterium psychophilum, Yersinia ruckeri, Aeromonas salmonicida as well as intracellular bacterium Renibacterium salmoninarum the causative agent of Bacterial Kidney Disease (BKD).

By collating new knowledge from applied research programs conducted on different RAS types in Denmark and diagnostic investigation at our laboratory we observe frequent co-infections in RAS producing rainbow trout in freshwater.

The current traditional and gold standard diagnostic methods do not allow to assess the health challenges in a quantitative manner and thereby to rank them and formulate tailor made prevention strategies. We have designed a new diagnostic tool, based on microfluidic qPCR assays (FLUIDIGM), to assess known and putative emerging pathogens in farmed salmonids. The current level of its implementation and future perspectives will be presented and discussed.

Number 20

Clinical Blood Biochemistry as a Predictive Tool for Managing infection with SAV

Josip Barisic, Chris Mitchell, Brian Quinn

The use of blood borne biomarkers as health indicators is well established in both human and terrestrial veterinary medicine. Whilst less advanced in aquatic animal health, research in this area over the past three years is now closing the gap. By using AI techniques applied to a large and expanding database of biomarker measurements, WellFish Diagnostics are now able to predict health outcomes in salmonid fish based upon their biomarker profiles. This ability is described here the context of SAV infection, showing how reported biomarker profiles have led to intervention by health managers with a view to mitigating disease outcomes.