March 29, 2018

Re: Exploring PRV and HSMI in Europe and British Columbia – Workshop Report

Over November 27 and 28, 2017, our Association, in collaboration with the BC Centre for Aquatic Health Sciences, hosted a workshop entitled 'Exploring PRV & HSMI in Europe & BC' in Campbell River, BC. The objective of the workshop was to better understand Piscine orthoreovirus (PRV) and its association with Heart and Skeletal Muscle Inflammation (HSMI) in Norway and British Columbia.

This workshop brought together 13 leading, international experts in PRV and HSMI research and was moderated by Dr. George Iwama, President and Vice Chancellor of Quest University, BC.

The BC Salmon Farmers Association has worked collaboratively with all experts involved in the workshop over the past number of weeks to develop this report as an accurate representation of the research presented at this workshop. Much of what was presented and discussed at the workshop is new and on-going research, and has yet to be published.

Our members recognize that rigourous, peer-reviewed science is at the core of understanding complex issues such as the relationship between PRV and HSMI and the variance in how this pathogen and disease are recognized in Norway, British Columbia and elsewhere, globally. Therefore, although there are some areas where consensus of all speakers could not be found on the points recorded in this report, the Association supports the communication of all perspectives and research discussed.

Key Findings
We would like to take this opportunity to highlight some of the key findings discussed in this report.

PRV First Evidence: Archived samples indicate the presence of PRV in the environment over twenty years before its initial identification in Norway, in 2010 (1988, Norway, and 1977, BC).

PRV Range, Globally: Several genotypes of PRV have evolved regionally and have been identified globally in the marine environment, to date, in Norway, Chile, the North Pacific (BC and Washington), Atlantic Canada, and Japan.

PRV Hosts: The known host range of PRV globally includes: Cutthroat Trout, wild and farmed Chinook Salmon, Sockeye Salmon, Steelhead Trout, Coho Salmon, Chum Salmon, Pink Salmon and wild and farmed Atlantic Salmon, and a range of non-salmonid species.

PRV Prevalence (North Pacific Coast): On the west coast of North America, reports in 2014 and 2015 indicated that the prevalence of PRV in wild Pacific salmon was less than 20% (from BC studies, Miller et al. 2014 and Marty et al. 2015) but more recently, new US research (Purcell et al. 2017), revealed that PRV prevalence within adult Pacific Salmon and trout stocks was, on average, very low at 3.4%.

Effects of laboratory-based studies of PRV infections on pathology, immune function and respiratory physiology performed in BC: Several studies on Atlantic Salmon and Sockeye Salmon infected with PRV have shown no pathological evidence of disease. Moreover, in Atlantic Salmon, a comprehensive examination of the fish's respiratory physiology revealed no effect of PRV infection on overall fitness. This research is currently underway on Sockeye Salmon.

HSMI First Evidence: HSMI was first characterized in 1999 in Norway.

Correlation between PRV and HSMI: In 2017, PRV was correlated with the development of HSMI, in Norway. In contrast, in BC, HSMI has not been induced to date using BC Atlantic and Sockeye Salmon exposed to a BC strain of PRV.

To date, HSMI has only been described in farmed fish, globally (never diagnosed in wild fish). Like any other disease, the development and severity of HSMI is likely dependent upon the interactions of the host, pathogen, and environment.

Salmon Farms and HSMI: In Norway, HSMI is a production concern for farmed salmon but only accounts for approximately 2% of industry mortality. In contrast to the 600+

farms that develop HSMI each year in Norway, HSMI has rarely been diagnosed on salmon farms in B.C. and it has never been associated with an elevation in mortality.

Should you have any questions on the attached report, or require further information, please do not hesitate to get in touch.

Jeremy Dunn
Executive Director

Exploring PRV and HSMI in Europe & British Columbia (2017)

Final Workshop Report

March 20, 2018

BC Salmon Farmers Association

Acknowledgements

The BC Salmon Farmers Association (BCSFA) gratefully acknowledges the support of Fisheries and Oceans Canada's Aquaculture Collaborative Research and Development Program in contributing funding for this workshop. The BCSFA is appreciative of the efforts of the speakers and participants who have contributed their time to make the workshop a success and have helped in the generation of this report.

Introduction

The BC Salmon Farmers Association identified an opportunity for international researchers to share current information on Piscine orthoreovirus (PRV) and Heart and Skeletal Muscle Inflammation (HSMI) and advance the state of knowledge.

Therefore, in collaboration with the BC Centre for Aquatic Health Sciences, they hosted a workshop entitled 'Exploring PRV & HSMI in Europe & BC', over November 27th and 28th, 2017, in Campbell River, BC. The objective of the workshop was to better understand PRV and its association with HSMI in Norway and British Columbia.

Workshop presentations and discussions highlighted the regional similarities and differences in the way PRV and HSMI are characterized and presented. This discussion also highlighted the need for further research to better understand why these regional differences exist, and to better understand PRV and HSMI in farm-raised and particularly wild fish. Future needs for research and collaborations were identified to eliminate knowledge gaps.

Speaker Representation
Workshop participants represented a wide range of experiences and perspectives in this field of fish health research. This report incorporates all perspectives and research presented.

Workshop Agenda

Day 1 – November 27, 2017		
Time	**Topic**	**Speaker**
1:00-1:10p	**Welcome**	Jeremy Dunn, BCSFA and Vincent Erenst, Marine Harvest Canada
1:10-1:25p	**Introductions + Outline for the day**	Moderator - George Iwama
1:25-2:05p	**Overview HSMI and PRV**	Stewart Johnson – DFO
2:05 – 2:50p	**HSMI in Norwegian Aquaculture**	Kathleen Frisch – Cermaq
2:50-3:05p	*Break*	
3:05 – 3:50p	**Diagnosing HSMI in Europe + Sampling/Auditing**	Renate Johansen – Pharmaq Analytiq
3:50-4:35p	**Laboratory studies into HSMI and the link with PRV**	Espen Rimstad – Norwegian University of Life Sciences
4:35 – 5:30p	***Afternoon Speaker Panel Discussion and Questions***	
Day 2 – November 28, 2017		
Time	**Topic**	**Speaker**
8:30-9:15a	**Diagnosing HSMI in BC and new in situ analyses providing insight into pathogenesis of PRV**	Emiliano DiCicco - DFO
9:15-10:00a	**Transcriptome interactions between PRV and the salmon host through HSMI disease progression**	Kristi Miller - DFO
10:00-10:45a	**Laboratory studies into the pathogenicity of western North American PRV**	Mark Polinski - DFO
10:45-11:00a	*Break*	
11:00-11:45a	**PRV effect on salmon physiology: do PRV infected salmon have compromised fitness?**	Anthony Farrell – UBC
11:45-12:15p	***Morning Speaker Panel Discussion and Questions***	
12:15-1:15p	*Lunch*	
1:15-2:00p	**Surveillance and laboratory studies on western North American PRV in Pacific Salmon**	Maureen Purcell – USGS
2:00-2:45p	**PRV research from the east coast of Canada**	Nellie Gagne - DFO

Time	Topic	Speaker
2:45-3:15p	**The genetics of PRV, are there regional strain differences?**	Ahmed Siah - BCCAHS
3:15-3:45p	**Research in the works: Building on current knowledge**	Kyle Garver - DFO
3:45-4:00p	*Break*	
4:00-5:00p	**Regional comparisons and Next Steps: Are we on the same page and where do we go from here – Structured Speaker Panel Discussion**	George Iwama - Moderator and Gary Marty – BC MAL

Speaker Biographies

Moderator: Dr. George Iwama, University of British Columbia and Quest University

Dr. Iwama is the President and Vice-Chancellor of Quest University. He is the past President and Vice-Chancellor of the University of Northern British Columbia. He recently was the Executive Vice President, and the Provost and Vice-CEO of the Okinawa Institute of Science and Technology Graduate University in Japan. He is a graduate of UBC, where he later served as Full Professor. As a comparative physiologist, he applied his science to aquaculture and environmental issues. He has served as Director General of two NRC Institutes. He has been the Dean of Science at Acadia and Carleton Universities. He also is an Editor for the journal Aquaculture, Elsevier Press.

Dr. Stewart Johnson, Fisheries and Oceans Canada

Dr. Johnson leads the Aquaculture Ecosystems Interactions Program at the Pacific Biological Station and is an adjunct professor in the Health Management Faculty at UPEI. His current disease research focuses on investigations into the nature of interactions between aquatic animals and their pathogens and the role that the environment plays in determining the outcomes of these interactions. Much of this research is directed to improving our understanding of the nature and importance of disease interactions between farmed and wild salmonids. This work is conducted in collaboration with national and international researchers from a variety of universities, government institutes and industry partners.

Kathleen Frisch, Cermaq

Dr. Frisch is currently involved in an industrial PhD project in the field of infectious aquatic diseases at the University of Bergen in collaboration with Pharmaq. Kathleen received her veterinarian certification at the University of Melbourne in Australia and then specialized in aquatic animals by earning a Masters in Aquatic Veterinary Studies from the University of Stirling in Scotland. As the fish health technical manager and interim fish health manager of Cermaq Canada, she led and coordinated projects and screening programs. As a veterinarian, she investigated fish health and product quality concerns, with a focus on prevention.

Dr. Renate Johansen, Pharmaq Analytiq

Dr. Johansen started her carrier as a fish vet in Kristiansund 1995. She moved back to Oslo in 1998 and began a PhD on nodavirus infection in halibut. After 12 years of fish diagnostics and several research projects at the institute, she started her own company in 2014. Now, she is the Histology manager at Pharmaq Analytiq leading a group of 4 fish-vet-histo-pathologists. Pharmaq Analytiq focuses on diagnostic services along with consultancy to all fish farming markets that Pharmaq AS is selling vaccines including Canada, Chile, UK, Asia and the Nordic countries. Johansen has a special interest in finding new fish-viruses and was involved in the groups detecting both PRV in rainbow trout and flavivirus in Lumpfish.

Dr. Espen Rimstad, University Norwegian University of Life Sciences

Dr. Rimstad, Norwegian University of Life Sciences (NMBU) in Oslo, Norway has extensive experience (25 years) in fish virus research and leads the research group in fish virology at NMBU. The group was pivotal in the original detection of PRV. The PRV related research focus has been on the pathogenesis of infection, especially the infection of erythrocytes, and the study of viral genomes and encoded gene products. He is also currently heading the project ViVaFish, a platform for fish virus vaccines in Norway.

Dr. Emiliano DiCicco, Fisheries and Oceans Canada

Dr. DiCicco obtained both his Veterinary Medicine Doctorate and his PhD at the University of Camerino (Italy), where he spent several years dealing with Fish Health and Fish Histopathology on rainbow trout and aquarium/ornamental fish. He also spent the first year of his PhD studies at the Centre of Coastal Health at Vancouver Island University focusing on the assessment of sustainability indicators for salmon farming in British Columbia. He then worked as a Fish Histopathology Consultant for the Department of Veterinary Sciences at the University of Camerino. Dr. DiCicco is currently the Project Manager of the Sustainable Salmon Health Initiative, but he also acts as a Fish Histopathologist for the project, providing new insights on the pathogenesis of fish diseases utilizing novel molecular approaches.

Dr. Kristi Miller, Fisheries and Oceans Canada

Dr. Miller received her PhD from Stanford University, and is head of the Genetics and Genomics section at the Pacific Biological Station, where she has conducted research in the fields of population genetics, functional genomics, immunogenetics and salmon and shellfish health over her 25 years at DFO. She is currently the scientific lead of the "Strategic Salmon Health Initiative", a project that is providing a comprehensive evaluation of the infectious agents and diseases potentially impacting wild, hatchery and aquaculture salmon in BC. In the area of fish health, Dr. Miller has published dozens of manuscripts focussed on host response to disease, genetic susceptibility to disease, molecular disease diagnostics, and quantitative high throughput agent monitoring.

Dr. Mark Polinski, Fisheries and Oceans Canada

Dr. Polinski is an aquatic animal health research scientist at the DFO Pacific Biological Station. He has acquired a broad range of experience centering on host-pathogen interactions. This has ranged from investigating the immunological responsiveness of Bluefin tuna (PhD, University of Tasmania) to pathogen transmission potential of oyster microcell parasites in Canada (Post-doc, NSERC/DFO). Currently, Dr. Polinski's research focuses on the host responses and disease causing potential of PRv in salmon. This work utilizes both in vitro and in vivo challenge models to evaluate cellular responses at the single gene, transcriptome, and organism level.

Dr. Anthony Farrell, Fisheries and Oceans Canada

Dr. Farrell is a professor of Zoology at the University of British Columbia and a Fellow of the Royal Society of Canada. With over 400 research publications in scientific journals, Tony's research emphasis is to understand fish cardiorespiratory systems and apply this knowledge to salmon migratory passage, handling stress and recovery, sustainable aquaculture and aquatic toxicology. He has received multiple awards, including the Fry Medal, which is the Canadian Society of Zoologists highest honour to a scientist, the Beverton Medal, which is the Fisheries Society of the British Isles highest honour to a scientist, and the Medal of Excellence, which is the highest honour of the American Fisheries Society. He is a former President of the Society of Experimental Biologists and is the incoming Editor-in-Chief for the *Journal of Fish Biology*.

Dr. Maureen Purcell, US Geological Society Western Fisheries Research Centre

Dr. Purcell is the Chief of the Fish Health Section at the Western Fisheries Research Center (WFRC) in Seattle, WA. She earned her Ph.D. from the University of Washington and took a position at the WFRC after graduation. Her research focuses on infectious diseases of fish, molecular taxonomy and epidemiology of fish pathogens, ecology of fish diseases in wild populations and fish host defenses. A portion of her time is spent providing technical assistance to federal, state and tribal fisheries agencies, including activities such as training, technology transfer and response to emerging fish pathogens.

Dr. Nellie Gagne, Fisheries and Oceans Canada

Dr. Gagné is a scientist and head of the Molecular Biology group at DFO-Gulf Fisheries Center since 2001. The expertise of her group is in the development and validation of molecular diagnostics of fish and shellfish diseases and the development of improved in situ assays. As well, her research focuses on aquatic animal disease in general, immune response, pathogen characterisation, and host susceptibility.

Dr. Ahmed Siah, BC Centre for Aquatic Health Sciences

Dr. Siah is involved with numerous research projects developing new technologies in the field of aquatic health diagnostics and implementing and validating molecular biology technologies for diagnostics. Dr. Siah pursued his Postdoctoral studies in Molecular Ecotoxicology at the University of Le Havre in France after earning his PhD in Oceanography at the Institute of Marine Sciences in Rimouski, Quebec. As a Research Associate at PEI's Atlantic Veterinary College, he led and managed several projects on mollusk health management and the development of molecular diagnostic tools. He has extensive experience in developing and implementing diagnostic methods for emerging pathogens of interest.

Dr. Kyle Garver, Fisheries and Oceans Canada

Dr. Garver (BSc, PhD), Research Scientist (DFO), Pacific Biological Station, leads the Virology Research Program in the Aquatic Animal Health Section and holds adjunct status at Vancouver Island University. He has authored over 40 peer reviewed publications on various aspects of fish virology ranging from molecular epidemiology, diagnostics, transmission potential, and virus-host interactions. Dr. Garver has active

research investigations into piscine reovirus and has published several papers on the pathogenic potential of this virus in Pacific and Atlantic Salmon.

Dr. Gary Marty, BC Ministry of Agriculture
Dr. Marty is the senior member of a group of four fish pathologists at BC's independently accredited veterinary diagnostic laboratory in Abbotsford. The group provides diagnostic fish pathology services for clients that include federal agencies, BC regional districts, major research universities, and the licenced veterinarians that work within aquaculture. Dr. Marty has an MSc in fisheries biology, a PhD in comparative pathology, a veterinary degree (DVM), and he is board certified in anatomic veterinary pathology.

Workshop Discussion Summary

The following sections provide a synopsis of workshop discussions, and key outcomes. *Where specific speakers contributed significant information on a particular topic area, and those details are reported here, the section has been attributed to the speaker.*

Piscine orthoreovirus (PRV)

First Evidence: PRV was first reported in 2010 in Norway; however, data from archived samples indicates the presence of PRV in the environment over thirty years before this report (1988, Norway, and 1977, BC (Marty et al 2014)).

Virus Structure: PRV is a segmented, non-enveloped, double stranded RNA virus, and a member of the family Reoviridae. Its genome contains 10 segments, which encode 11+ proteins.

Several genotypes, globally: Several genotypes have been identified globally to date. These genotypes are based on analysis of genomic segment S1 sequences. BC's genotype is PRV-1a (98% genetically similar for segment S1 to the Norwegian genotype and 97% similar to PRV-1b shared by Norway and Chile).

Farm Diagnoses: In Norway, all marine salmon farms become PRV positive by RT-qPCR[1] testing 4 – 12 months after transfer to sea. Similarly, in BC, the majority of farms are also RT-qPCR positive after 4 – 12 months after transfer to sea. Transmission occurs in fresh and saltwater environments. In the marine farming environment, PRV loads

[1] Reverse Transcriptase quantitative Polymerase Chain Reaction (RT-qPCR) is a diagnostic tool used to detect the presence of a specific RNA partial sequence (e.g. part of a virus) in a sample. It does not indicate whether the detected virus is viable or is causing disease.

appear to peak several weeks (in Norway) to months (in British Columbia) after transfer to saltwater sites, reaching up to 100% prevalence. PRV infections persist for long periods at a relatively high loads in both BC and Norway.

Infection and Tissue Tropism[2]

Atlantic and Pacific Salmon have been infected by injection with PRV positive tissue homogenates and by co-habitation with PRV-infected individuals. Infections have been established in fresh and saltwater. There is evidence that PRV is shed from infected fish via the intestine (Hauge et al. 2016). The route/s by which PRV enters the body are not known.

In Atlantic and some species of Pacific Salmon PRV can be detected in most tissues by RT-qPCR. Highest virus loads are usually reported in the blood, which appears to be the primary tissue in which PRV actively replicates. Infection with PRV does not appear to kill red blood cells of Atlantic Salmon, to large extent, but rather, creates transient inclusion bodies. PRV has a consistent replication pattern during blood infection, in three distinct phases (amplification, peak and persistent). Immunohistochemical staining and in-situ hybridization has localized PRV in red blood cells, cells of the heart muscle and in some instances, myocytes of the skeletal muscle.

There are some examples where PRV infected Chinook Salmon and Coho Salmon have displayed lower hematocrits indicating red blood cells lysis which releases virus and haemoglobin into tissues, especially kidney, liver and spleen and this is thereby suspected to be linked to anemia and jaundice-related disease (presented by Miller). However, there has been no research to date to determine the nature of the relationship between PRV and these outbreaks (Miller et al. 2017).

PRV cannot be cultured: The virus can be purified from red blood cells using ultracentrifugation but, to date, the virus has not been cultured in established cell lines.

Hosts:
The known host range of PRV in the North Pacific Ocean includes: Cutthroat Trout, wild and farmed Chinook Salmon, Sockeye Salmon, Steelhead Trout, Coho Salmon, Chum Salmon, Pink Salmon and wild and farmed Atlantic Salmon.
PRV has most commonly been reported in salmonids but has been found in some non-salmonid hosts in Norway. New US research (Purcell et al. 2017), revealed that PRV RNA prevalence within adult Pacific Salmon and trout stocks varied and ranged from 2-73%.

[2] Tissue tropism is the cells and tissues of a host that support growth of a particular virus or bacterium. Some pathogens infect many types of cells and tissues and some may infect primarily a single tissue.

Overall prevalence across all stocks was 3.4% (returning results sampled in Washington and Alaska) with PRV found most commonly in Coho and Chinook Salmon.
In BC, prevalence of PRV in analyzed wild salmonid smolts ranged from 0 – 20% (Miller et al. 2014 and Marty et al. 2015). Additional work needs to be done to evaluate whether non-salmonids are the prime reservoir for this virus.

In Norway, in studies of wild and hatchery-raised Atlantic Salmon, PRV prevalence has been noted to be twice as high in returning hatchery-raised (enhancement) salmonids than in wild returns.

Effects of laboratory-based studies of PRV infections on pathology, immune function and respiratory physiology performed in BC (presented by Farrell): Atlantic Salmon and Sockeye Salmon infected with PRV (via a PRV injection) showed no elevated immune response or pathological evidence of disease. Moreover, in Atlantic Salmon, a comprehensive examination of the fish's respiratory physiology revealed no effect of PRV infection on measures of maximum oxygen carrying capacity, oxygen binding ability, whole animal maximum oxygen uptake and whole animal hypoxia tolerance.

Heart and Skeletal Muscle Inflammation (HSMI)

HSMI was first characterized in 1999 in Norway. PRV was regarded as associated with HSMI after the first identification of PRV in 2010. In 2017, PRV was correlated with the development of histopathological lesions characteristic of HSMI in naïve fish (presented by Rimstad).

To date, HSMI has only been described in farmed fish, globally (never diagnosed in wild fish). Like any other disease, the development and severity of HSMI is likely dependent upon the interactions of the host, pathogen, and environment.

HSMI is an inflammatory disease that affects heart and skeletal tissues. Because skeletal muscle inflammation is more fleeting, or less common than heart inflammation associated with HSMI, not all individuals with HSMI necessarily show skeletal inflammation.

Salmon Farms and HSMI

HSMI is a production concern for farmed salmon in Norway, where it causes variable mortality levels (accounts for approximately 2% of industry mortality; range 0-20%). Also, the high prevalence of HSMI (10% of a farm affected by the disease over months) leads to impacts on feeding and growth and susceptibility to stressors. In contrast to the 600+ farms that develop HSMI each year in Norway, HSMI is not defined as a concern in British Columbia either by producers, or by Fisheries and Oceans Canada (Appendix III, Marine Finfish Aquaculture Licence).

In Norway HSMI has been occasionally reported in freshwater but is more common in salt water. Research has repeatedly shown that on Norwegian farms, HSMI appears a few weeks after the peak of PRV load in blood. Mortality is generally higher in freshwater outbreaks or outbreaks that occur soon after transfer of PRV-infected fish into ocean net pens (upwards of 50%).

Histopathological lesions diagnostic of HSMI have been observed in a few instances on farms in BC. In these cases, industry has reported no associated elevation in farm salmon mortalities at the farm level, unlike in Norway. In the BC-HSMI diagnosis farm case (DiCicco et al. 2017) – HSMI was present farm-wide (peaking at >40% of moribund fish sampled) for about 9 months with additional minor heart inflammation being noted in up to 90% of moribund fish sampled. 10% of samples gathered from fish health audit (carcass) populations showed muscle inflammation as an incidental observation. Skeletal muscle lesions closely tracked the development of moderate to severe lesions in the heart, and as such, were not evident in all fish at early and late stages of disease development. Hence, while at the farm level, both heart and skeletal muscle lesions occurred, not all individual fish carried both lesions.

Idiopathic cardiomyopathy (a heart disease of unknown cause) is common in fish and the BC industry and provincial vets have identified heart lesions by histology using samples of farmed salmon in BC since 1990. The prevalence for idiopathic cardiomyopathy in samples submitted for histological examination ranging from 0.9 – 3% (~2%/year) of audited samples *(recent mortalities)* has been reported over the period of 1990-2017 (G Marty, unpublished). In the absence of clinical signs of disease on farms, and an unknown etiology of the lesions, the diagnosis of these cases is limited to a morphologic diagnosis rather than a specific disease or cause. However, heart inflammation is considered "sufficient to cause of death" in 2% of farmed fish audit mortality samples collected over 8 years (# of fish examined = 470 – 771 per year). [3]

Histopathology has been used as a key investigative tool in fish health audits for many years. However, some speakers identified that it would be of importance to use histology to follow a potential HSMI outbreak through a mortality event to more fully evaluate the disease's effects on mortality in farm-raised salmon in BC.

Current BC diagnostic standard for diagnosing HSMI requires the presence of histopathological lesions in heart and skeletal muscle, clinical signs and mortality. There was discussion among workshop participants on the differences in how HSMI is

[3] *Note:* Dr. Gary Marty estimated in 2015 that less than 1% of the total farm-raised salmon population in BC die of diseases that might be infectious to wild salmon (Marty, 2015). Fish health audited samples are a sub set of this overall population mortality level.

diagnosed, globally. For example, in Norway, Scotland, and Chile, the presence of histopathological lesions alone is diagnostic of the disease.

In Eastern Canada, HSMI has not been diagnosed but PRV has been detected in farmed and wild Atlantic Salmon (presented by Gagne).

Studies on HSMI in a Laboratory Environment (Presented by Polinski)

In Norwegian studies, injection of purified PRV infection induced HSMI but laboratory challenges generally do not result in mortality, other clinical signs or lesions as severe as seen in the field.

In BC studies, HSMI has not been induced to date using BC Atlantic and Sockeye Salmon exposed to a BC strain of PRV. Lab injection challenges with PRV in BC have resulted in PRV loads equivalent to those obtained Norwegian studies yet only minimal host antiviral response was observed 4-8 weeks post-challenge and minimal to no inflammatory transcriptional response. Moreover, despite inducing a stressful event (i.e. transition to seawater), and/or co-infection with another pathogen (IHNV), PRV infections did not lead to HSMI. This could be due to differences in virus strain, environmental effects, host genetic differences, or other factors impacting the disease development pathway.

Disease Development Pathway (Presented by DiCicco)

Viral Disease Development Biomarker Evaluation

One study on an incidence of HSMI in BC farmed Atlantic Salmon utilized a combination of a novel molecular disease diagnostic tool and in situ hybridization (ISH) for fine-scale localization of PRV within tissues (DiCicco et al. 2017). In some instances in this study, in Atlantic Salmon, ISH localized PRV in plasma and tissues other than red blood cells, including cardiomyocytes, where inflammation appeared (panmyocarditis), and a powerful host transcriptome response predictive of the presence of viral disease was detected. 50% of fish that were carrying loads of PRV in excess of 500 copies per microliter of blood were in a viral disease state. However, this research also further corroborated that PRV from BC can reach high viral loads in the blood without triggering an immune reaction or showing evidence of pathology (as previously shown by Garver et al. 2016 and Polinski et al. 2016).

Observations on Co-Infections

In Norwegian studies, well-developed host antiviral and inflammatory responses have been identified in infected blood cells, and protection to Pancreas disease (PD) caused by Salmonid alphavirus (SAV) has been found.

In Norway, since all farmed fish in seawater are PRV positive, it is difficult to determine if heart lesions also could have other causes. Mixed infections (in particular with other viral diseases like PD and Cardiomyopathy Syndrome (CMS)) have been indicated as a source of “undiagnosed” HSMI cases.

In cases where inflammatory reactions are present and PRV is free in plasma (in Norwegian studies), well-developed host antiviral and inflammatory responses have been identified, as observed in the BC research by DiCicco and Miller. Studies in Norway and Denmark have found that a strong innate response to PRV protects/interferes with other infections (e.g. PD and SAV). Additionally, a PRV vaccine was observed to protect against HSMI but did not completely block the viral infection or eliminate the virus in the environment.

In BC lab studies, during co-infection with IHNV, PRV loads in erythrocytes were not affected by well-developed antiviral response to IHNV, nor did pre-infection with PRV have a protective or aggravating effect on subsequent IHNV exposure.

In Eastern Canada, PRV has often been a previously unknown co-infection in ISAV challenge studies. Whether PRV contributed to the disease development in these trials is not known.

Management Measures in Norway (Presented by Frisch / Johansen)

Stressors seem to cause a higher likelihood of clinical manifestation of HSMI in Norway. These include fish transport, crowding for treatments, and predators. A key mitigation measure is to reduce stressors and avoid management routines that stress fish. If a farm has HSMI, using leaner diets, with higher EPAs from marine oils seems to help in lowering mortality.

PRV infection in freshwater is also a risk factor towards the development of HSMI, and the severity of the disease if it occurs. The Norwegian industry is trying to reduce PRV load in smolts by testing broodstock and evaluating the use of land-based broodstock.

Comparing Norwegian and BC Laboratory Studies (Presented by Garver)

The most significant different between Norway and BC studies regarding PRV & HSMI is the inability of BC laboratory challenges to reproduce the well-developed heart lesions reported in Norwegian laboratory challenges.

While the BC and Norwegian exposure studies result in equally high PRV loads in the infected fish, the outcome as it relates to lesion development is very different. This begs the question as to what factors are responsible for the altered disease scenarios, and what are the conditional requirements that exacerbate non-virulent PRV infections into the HSMI disease state. Currently the mechanism(s) responsible for this difference are unknown however recent studies by Garver et al. and Polinski et al. have highlighted some key differences that may provide clues to this mystery. These researchers have noted through their studies in BC, that PRV is often absent from the plasma while in Norway PRV is present in the plasma especially at peak loads. It is hypothesized that the absence of PRV in the plasma may account for the limited immune response that has been so far observed in BC. In addition, the timing and severity of the host responses

seen in BC lab studies are distinctly different compared to the experimental challenges completed in Norway, where PRV leaves the red blood cells, infects other tissues, (primarily heart and skeletal muscle), inducing a severe host response.

Multiple variables, such as host genetics and husbandry factors, limit direct comparisons of laboratory studies in BC and Norway. Therefore, Garver (DFO) and Rimstad (Norwegian University of Life Sciences) labs are conducting a side-by-side comparison of BC PRV and Norway PRV using the same lab, and fish. The group is also planning a simultaneous challenge – conducted in Norway and Canada. The two labs have exchanged purified PRV stocks, to directly compare PRV from both locations. Trials have begun.

Results will focus on virulence of strain, differences in susceptibility, and immune response in hosts, and differences in host strains (e.g. broodstock selection) Specifically:

- Pathology:
    - Can BC PRV cause lesions in Norwegian fish?
    - Can Norway PRV cause lesions in BC and St John River fish?
- Viral load: Quantity and tissue location
    - BC PRV vs Norway PRV, presence in plasma?
- Antiviral response
    - Do Norwegian fish respond to BC PRV?

Studies will also be conducted to look at PRV transmission and infection dynamics

- In BC preliminary surveys suggest PRV infection in Atlantic Salmon predominantly appear following transition to seawater. Studies will assess:
    - Marine reservoirs –
        - Temporal and spatial analysis of infections
        - Assess whether it is localized to aquaculture areas or ubiquitous in salmonid and non-salmonid hosts
    - Farm to farm/wild spread investigations
    - Infectious dose
    - Virus stability in the environment

Pacific Salmon diseases from around the world which are associated with the presence of PRV (presented at the workshop by Dr. Miller)

Norway
Farmed rainbow trout experienced a disease, termed HSMI-like, that clinically includes anorexia, lethargy, and modest mortality (up to 21%) in freshwater hatcheries and up to 4 months after saltwater transfer. Gross signs include haemorrhages, ascites, anemia, bulging eyes and jaundice. Pathologically, most fish show inflammatory lesions in the heart, mostly observed in the spongy layer, but necrotic lesions in hepatocytes and

some cardiomyocytes and red muscle fibres are also observed, along with increased circulatory neutrophils in kidney and spleen and haemosiderosis in spleen indicative of red blood cell lysis.

This disease is associate with another new strain of PRV, PRV-3 (aka PRV-Om), observed in high load across all five farms sampled with HSMI-like disease, and none without (Olsen et al. 2015).

IP-injection and cohabitation challenge studies show that the PRV-3 strain can replicate in both rainbow trout and Atlantic Salmon, but the response of both the transcriptome and of inflammatory lesions in the heart occurred earlier, stronger and for longer in rainbow trout. Unlike PRV-1 in Atlantic Salmon, it appears this disease is more acute, and the virus subsequently clears (Hauge et al. 2017).

Chile

Farmed rainbow trout and Coho Salmon have been diagnosed with disease closely resembling the HSMI-like disease in farmed rainbow trout in Norway, also in association with PRV-3. It is unclear, however, whether PRV-1 may also be involved as most farms contain mixed infections of both strains.

Clinical signs of the disease in Coho Salmon include lethargy and morbidity. Jaundice, pale heart, ascites, and blood clots in the abdominal cavity can occur. Pathologically, myocarditis is restricted to the spongy layer of the heart, similar to rainbow trout in Norway, with major hepatic necrosis in fish with high viral load and erythrophagocytosis in kidney and spleen (Godoy et al. 2016).

Japan

In a Japanese study on farmed Coho Salmon (Takano et al. 2016), outbreaks of disease on farms, caused by PRV-2 (as confirmed in challenge studies using purified virus), included erythrocytic inclusion bodies (EIBs, viral factories observed in blood cells), jaundice (assumed to be caused by excesses of bilirubin), severe anemia, and often mild transitory HSMI-like heart lesions, mostly confined to the spongy layer of the myocardium. Clinically, fish were off feed. Cumulative mortality on farms can be up to 23%. They have found that EIBS is a higher risk in fish fed large amounts of food for rapid growth.

In this disease, the virus does not localize as readily in the heart, but rather is more abundant in the kidney and liver compared to cases of Atlantic Salmon infected with PRV-1, as also demonstrated by in-situ hybridization results (Miller, DiCicco); necrotic lesions in liver and kidney are the most evident pathological lesions in affected Coho Salmon. Authors established that protective immunity could occur in previous evidence of disease.

British Columbia

A disease classified as jaundice/anemia also occurs in farmed Chinook Salmon, with approximately 5% of audit fish diagnosed with this disease throughout BC, and 14% overall on the west coast of Vancouver Island (audit data from 2011-2013). These fish also carry PRV-1a, the only genotype of PRV detected in BC and the same strain that causes HSMI. Injection of an organ homogenate from farmed jaundice Chinook Salmon into naïve Chinook, Sockeye and Atlantic Salmon failed to reproduce the jaundice disease (Garver et al. 2015).

Drs. DiCicco and Miller further discussed in situ hybridization studies they have conducted on farmed Chinook in BC, described below.

Clinically, jaundice Chinook Salmon can be off feed and often have a pale liver. Pathologically, hepatic and renal interstitial cell necrosis are the most compelling lesions, but transient heart inflammation, often occurring before necrotic lesions in liver and kidney, can occur in the spongy layer of the heart. As with the other previously described diseases in Pacific Salmon, erythrophagocytosis in kidney and spleen and hemosiderin in the spleen, and anemia, are likely the result of lysis of affected red blood cells in fish with the virus confined to red blood cells, there is no sign of disease in any tissues, and, as in Atlantic Salmon, no significant response in the host. However, unlike Atlantic Salmon, higher loads of PRV do not appear to be as tolerated, as 80% of fish with overall tissue loads in excess of 1000 copies per µl of blood show evidence of disease (50% in Atlantic Salmon), and at a copy number of 10,000, all fish are diseased. Also unlike Atlantic Salmon, the red blood cells are lysed (as can be seen in in-situ analysis), and virus and haemoglobin becomes highly abundant in the spleen, kidney and liver, where macrophages also come in to engulf the damaged and destroyed red blood cells. Immunohistochemical staining of haemoglobin shows huge excess in the kidney tubules, which become necrotic, but the virus is also present in the affected hepatocytes and kidney tubules. Heart lesions are fleeting, restricted to the spongy layer, and generally occur before necrosis of liver and kidney. The researchers speculate that this data may indicate some correlation with PRV-1a and this Pacific Salmon disease, which also closely resembles diseases in Pacific Salmon in Japan, Norway, Chile, and Washington State.

US Northwest (Presented by Purcell)

Intra-erythrocytic inclusion bodies (EIBS) were first described in 1981 Chinook Salmon (Columbia River) and were typically observed, with anemia and co-occuring with other diseases (BKD, fungal, bacterial coldwater disease). Chinook and Coho Salmon appear most susceptible to EIBs. The syndrome is not commonly diagnosed in recent years. Passing the agent (PRV-1) from infected, but not diseased, Atlantic Salmon did not recreate the anemia condition in naïve Chinook Salmon. However, when challenges were performed with PRV-infected tissues obtained from individuals, which were diagnosed with EIBs, there was resultant development of EIBs.

Whether EIBs in BC is a feature of one or multiple distinct diseases is not well understood. Viral erythocytic necrosis, now established to be cause by a DNA virus, ENV, also shows evidence of EIBs.

Knowledge Gaps Identified for Further Investigation

A number of key areas for further investigation were identified by workshop participants.

PRV in BC:

- Is there a physiological measurable effect (or harm) to Pacific Salmon infected with PRV, and specifically PRV-associated jaundice/anemia, i.e. an effect on fitness?
- Further investigation is needed on lysis of red blood cells in Pacific Salmon (resulting in anemia/jaundice) but not in Atlantic Salmon (Workshop participants held different views on this).
- Communicate what a normal cellular/immune response is in salmon to a viremia, in comparison to response to PRV.
- Determine if PRV is spread vertically (via eggs)
- Research on how to culture the virus.
- Research on how to de-activate the virus.
- Developing a strategy for delaying PRV infection, and determining how fish are infected in freshwater. (The source in freshwater is unknown).
- How prevalent is PRV in wild fish (salmonids and non-salmonids)?
- Full genome sequencing of PRV infecting wild salmon or salmon without disease symptoms is likely to be a prerequisite for identifying genome markers linked to virulence differences.

HSMI in Atlantic Salmon & Jaundice/EIBs in Chinook & Coho in BC:

- If a case definition cannot be agreed upon in the literature, a working definition should be provided in future publications (Case definition was not agreed upon by workshop participants). EIBs, jaundice, cardiac inflammation and a host viral response are not unique to PRV infection and to HSMI.
- Describe why PRV generates HSMI in some Atlantic salmon and not others, what mechanism are involved.
- There was concern expressed that fish health audit samples of farm-raised salmon are not targeting outbreak events. Mortality events that may be related to HSMI should be studied to get a better sense of the impacts.
- While RT-qPCR can screen PRV infection without killing fish, a PRV-positive result does not reliably predict harm (HSMI or other disease). Lethal sampling and sampling of recently moribund fish is presently the only means of diagnosing harm if PRV/HSMI is suspected. Histology remains the only standard for

diagnosing HSMI. How can we use other tools (e.g. viral disease diagnostic panel) for HSMI without lethal sampling?
- How do we determine/assess if harm is being caused by this disease in wild fish?
- There is a need for wild fish surveys to determine prevalence in all life stages of wild salmonid species and if there are any other hosts.

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