PISCINE ORTHOREOVIRUS (PRV) IN THE PACIFIC NORTHWEST APPEARS TO BE OF LOW RISK TO WILD PACIFIC SALMONIDS

This summary report contains the most current information available on PRV risk to wild Pacific salmonids contributed by expert fish health practitioners and researchers in the Pacific Northwest

Prepared By

The Pacific Northwest Fish Health Protection Committee

By

T.R. Meyers
Alaska Department of Fish and Game
Juneau Fish Pathology Laboratory

Informational Report No. 10

September 2017

Summary Conclusion Based on Available Data: The ubiquitous nature of piscine orthoreovirus (PRV), its apparent historic presence in wild Pacific salmonid stocks in the Pacific Northwest and the lack of clear association with disease in Pacific salmonids suggest the virus poses a low risk to wild species of Pacific salmonids.

Why PRV in the PNW is of low risk regarding HSMI in wild Pacific Salmonids

1. The disease "heart and skeletal muscle inflammation" (HSMI) has not been reported in wild salmon populations in Norway or elsewhere and appears to only be a threat to farmed fish
2. While PRV causes HSMI in farmed Norwegian Atlantic salmon, high levels of PRV genetic material have been detected in asymptomatic wild and cultured salmonids with no evidence of HSMI disease
3. Histopathological lesions of HSMI were recently described as statistically correlated with the presence of PRV at one Atlantic salmon farm in British Columbia, Canada (BC) while other studies have detected the presence of PRV genetic material in wild and cultured Chinook, coho and pink salmon and steelhead trout from Washington State, BC and Alaska where years of surveillance have reported no presence of HSMI
4. Molecular testing of archived fish tissues in BC has shown that PRV was present in asymptomatic wild and farmed Pacific salmon since 1987 and may have been present as early as 1977 before Atlantic salmon were imported for aquaculture
5. HSMI has not been reported in Pacific salmon or steelhead in North America to date
6. Laboratory studies with Chinook and sockeye salmon have demonstrated that PRV is infectious and will persist for quite some time but does not cause fish mortality, HSMI, or any other apparent disease
7. Development of HSMI and HSMI-like diseases of farmed salmonids (Atlantic and coho salmon; rainbow trout) infected by PRV may be a result of different viral strains, host specific antiviral responses and environmental stressors that do not appear to be present or active for indigenous salmon on the Pacific Coast
8. **The presence of PRV genetic material in Pacific salmon tissues is not sufficient evidence for HSMI disease**

BACKGROUND

Piscine orthoreovirus (PRV), also known as Atlantic salmon reovirus, was identified in 2010 by next generation sequencing of tissues from farmed Atlantic salmon *(Salmo salar)* in Norway with the disease "heart and skeletal muscle inflammation" (HSMI) (1-3). HSMI was first described in 1999 in farmed Norwegian Atlantic salmon. In recent studies, Norwegian Atlantic salmon infected with gradient purified PRV preparations by injection and cohabitation were shown to replicate the virus and develop histopathological lesions diagnostic of HSMI establishing PRV to be the causative agent (4). Also, PRV variants have been associated with HSMI-like disease reported in farmed Norwegian rainbow trout and in farmed coho salmon in Chile and Japan (5-7). HSMI losses in Atlantic salmon have ranged up to 20% and the disease typically occurs 5-9 months after transfer of Atlantic salmon smolts from freshwater to seawater (8). The disease is diagnosed by histologic changes of mononuclear inflammation and necrosis of the heart and red skeletal muscle with absence of pancreatic lesions (9). Other studies have demonstrated that PRV replicates preferentially in the cytoplasm of red blood cells leading to inclusion body formation (10, 11) similar to erythrocytic inclusion body syndrome (EIBS) first reported in 1982 from cultured juvenile Chinook salmon in Washington State (12). Recent evidence indicates that EIBS in farmed Japanese coho salmon is caused by a new variant of PRV (PRV-2) (7) and that HSMI-like lesions

were also associated with this infection. This study suggests a relationship of PRV with EIBS as well as HSMI which will require further research for clarification. To date, there has been no surveillance for PRV-2 in the Pacific Northwest because the quantitative PCR method used to detect PRV would be unlikely to detect the PRV-2 variant (13).

Surveys using molecular diagnostic methods have shown that PRV genetic material is ubiquitous in asymptomatic wild and farmed Atlantic salmon in both the marine and freshwater environments in Norway (1, 14, 15). The high prevalence of PRV in asymptomatic fish raised earlier questions of whether PRV is the sole cause of the disease HSMI (14). However, recent challenge studies conclusively demonstrated causality of HSMI, at least for Norwegian PRV strains (4). PRV genetic material has also been detected in asymptomatic farmed Atlantic salmon in Chile and Ireland (16, 17); from hatchery Chinook, coho and pink salmon and steelhead trout in Washington State (18); from farmed Atlantic salmon and wild Chinook, coho and sockeye salmon in BC and from wild and hatchery coho and chinook salmon from Alaska (17, 19, 13). Despite PRV presence, there have been no published reports of HSMI disease in Ireland or US waters of the Pacific Northwest and Alaska. Conversely, one study has found evidence of HSMI histopathological lesions on a BC Atlantic salmon farm that were statistically correlated with the presence of PRV. This study reported only mild clinical signs with no associated elevation in fish mortality (20).

Molecular testing of archived fish tissues in BC has shown that PRV was present in wild and farmed salmonids since 1987 and may have been present as early as 1977 in one sample from steelhead trout (19). None of these tissues had histological lesions diagnostic for HSMI. These data suggest that PRV may have been present causing no disease in wild salmonids prior to the importation of Atlantic salmon for aquaculture purposes (19) and possibly much longer. Laboratory challenges of Chinook, sockeye and Atlantic salmon injected with PRV infected material from Pacific Northwest salmonids resulted in no significant mortality or clinical disease (21, 22). A second study of Chinook salmon also injected with PRV positive material from Pacific Northwest salmonids resulted in virus replication with transient cytoplasmic inclusion bodies in red blood cells causing no reduction in hematocrits and no fish mortality (23). Similar challenge studies in rainbow trout (23) also resulted in no direct mortality following injection with PRV infectious material. These experimental studies suggest PRV in the Pacific Northwest is of low virulence for rainbow trout, Chinook and sockeye salmon.

The ability of PRV to cause HSMI in Norwegian Atlantic salmon (4) is in contrast with laboratory challenge studies of BC Atlantic salmon that demonstrated high transmissibility of PRV but failure to elicit HSMI by either injection or cohabitation (22). Differences between Norwegian and Canadian studies may be confounded by challenge methodologies, PRV strain differences or other variables influencing stress and host disease resistance (4). Continuing genetic studies have shown that PRV has at least three major variants in salmonids: PRV (also called Genotype I) in Norwegian Atlantic salmon with two sub-genogroups 1a (also in BC Atlantic salmon; BC, WA, AK Pacific salmonids and Chilean coho salmon) and 1b (also in Chilean Atlantic and coho salmon); PRV-2 in Japanese farmed coho salmon causing EIBS; and a third variant (PRV-3 or Genogroup II) in Chilean coho salmon and Norwegian rainbow trout (5, 6, 7, 17).

In summary, it is likely that additional PRV genetic variants will be identified as typing efforts are expanded (24) that may include PRV-related viral species in non-salmonids (25). Whether HSMI disease will develop in a PRV-infected salmonid species appears to be influenced by PRV strain, specific host response and other environmental variables. The PRV strain present in indigenous Pacific salmon in the PNW, historically and experimentally, appears to be relatively benign and unable to produce significant disease or HSMI in native salmonids.

LITERATURE CITED

1. Palacios 6, Løvoll M, Tengs T, Hornig M, Hutchison S, et al. (2010) Heart and skeletal muscle inflammation of farmed salmon is associated with infection with a novel reovirus. PLoS One 5: e11487.

2. Mikalsen AB, Haugland 0, Rode M, Solbakk IT, Evensen *0* (2012) Atlantic salmon reovirus infection causes a CD8 T cell myocarditis in Atlantic salmon *(Salmo solar* L.). PLoS One 7: e37269.

3. Markussen T, Dahle MK, Tengs T, LøvolI M, Finstad ØS et al. (2013) Sequence analysis of the genome of piscine orthoreovirus (PRV) associated with heart and skeletal muscle inflammation (HSMI) in Atlantic salmon *(Salmo salar).* PLoS One 7: e70075.

4. Wessel Ø, Braaen S, Alarcon M, Haatveit H, Roos N, Markussen T, et al. (2017) Infection with purified *Piscine orthoreovirus* demonstrates a causal relationship with heart and skeletal muscle inflammation in Atlantic salmon. PLoS ONE 12(8): e0183781. https://doi.org/10.1371/journal.pone.0183781

5. Olsen AB, Hjortaas M, Tengs T, Hellberg H, Johansen R. (2015) First description of a new disease in rainbow trout (*Oncorhynchus mykiss* (Walbaum)) similar to heart and skeletal muscle inflammation (HSMI) and detection of a gene sequence related to piscine orthoreovirus (PRV). PLoS ONE. 10: e0131638. doi: 10.1371/journal.pone.0131638 PMID: 26176955.

6. Godoy MG, Kibenge MJT, Wang YW, Suarez R, Leiva C, Vallejos F, et al. (2016) First description of clinical presentation of piscine orthoreovirus (PRV) infections in salmonid aquaculture in Chile and identification of a second genotype (Genotype II) of PRV. Virol J 13: 98. doi: 10.1186/s12985-016- 0554-y PMID: 27296722.

7. Takano T, Nawata A, Sakai T, Matsuyama T, Ito T, Kurita J, et al. (2016) Full-genome sequencing and confirmation of the causative agent of erythrocytic inclusion body syndrome in coho salmon identifies a new type of piscine orthoreovirus. PLoS One 11 (10): e0165424.doi:10.1371/journal.pone.0165424

8. Kongtorp RT, Kjerstad A, Taksdal T, Guttvik A, Falk K (2004) Heart and skeletal muscle inflammation in Atlantic salmon, *Salmo solar* L: a new infectious disease. J Fish Dis 27: 351-358.

9. Kongtorp RT, Taksdal T, Lyngoy A (2004) Pathology of heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon *Salmo salar.* Dis Aquat Org 59: 217-224

10. Finstad Øs, Falk K, LøvoII M, Evensen 0, Rimstad E (2012) Immunohistochemical detection of piscine reovirus (PRV) in hearts of Atlantic salmon coincide with the course of heart and skeletal muscle inflammation (HSMI). Vet Res 43: 27.

11. Finstad ØS, Dahle MK, Lindholm TH, Nyman IB, LøvolI M, et at. (2014) Piscine orthoreovirus (PRV) infects Atlantic salmon erythrocytes. Vet Res 45: 35.

12. Leek SL. (1987) Viral erythrocytic inclusion body syndrome (EIBS) occurring in juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) reared in freshwater. Can J Fish Aquat Sci. 44: 685-688. doi: 10.1139/f87-083

13. Purcell MK, Powers RL, Evered J, Kerwin J, Meyers TR, Stewart B, Winton JR (in press) Molecular testing of adult Pacific salmon and trout (*Oncorhynchus* spp.) for several RNA viruses demonstrates widespread distribution of piscine orthoreovirus (PRV) in Alaska and Washington. J Fish Dis.

14. LøvoII M, Alarcon M, Bang Jensen B, Taksdal T, Kristoffersen AB, et at. (2012) Quantification of piscine reovirus (PRV) at different stages of Atlantic salmon *Salmo salar* production. Dis Aquatic Org 99: 7-12.

15. Garseth AH, Fritsvold C, Opheim M, Skjerve E, Biering E (2013) Piscine reovirus (PRV) in wild Atlantic salmon, *Salmo solar* L,, and sea-trout, *Salmo trutta* L., in Norway. J Fish Dis 36: 483-493.

16. Rodger HD, McCleary SJ, Ruane NM (2014) Clinical cardiomyopathy syndrome in Atlantic salmon, *Salmo salar* L. J Fish Dis doi: 10.1111/jfd.12186.

17. Kibenge Mi, Iwamoto T, Wang Y, Morton A, Godoy MG, et al. (2013) Whole-genome analysis of piscine reovirus (PRV) shows PRV represents a new genus in family Reoviridae and its genome segment S1 sequences group it into two separate sub-genotypes. Virology Journal 10: 10-230.

18. Miller KM, Teffer A, Tucker S, Li S, Schulze AD, Trudel M, Juanes F, Tabata A, Kaukinen KH, Ginther NG, Ming TJ, Cooke SJ, Hipfner JM, Patterson DA & Hinch SG (2014) Infectious disease, shifting climates, and opportunistic predators: cumulative factors potentially impacting wild salmon declines. Evolutionary Applications 7: 812-855.

19. Marty GD, Morrison OB, Bidulka J, Joseph T, Siah A (2015) Piscine reovirus in wild and farmed salmonids in British Columbia, Canada: 1974-2013. J Fish Dis 38: 713-728.

20. Di Cicco E, Ferguson HW, Schulze AG, Kaukinen KH, Li S, Vanderstiche R, Wessel Ø, Rimstad E, Gardner IA, Hammell KL, Miller KM (2017) Heart and skeletal muscle inflammation (HSMI) disease diagnosed on a British Columbia salmon farm through a longitudinal farm study. PLoS ONE 12(2): e0171471. doi:10.1371/journal. pone.0171471

21. Garver KA, Marty GD, Cockburn SN, Richard J, Hawley LM et al. (2016) Piscine reovirus, but not Jaundice Syndrome, was transmissible to Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), sockeye salmon, *Oncorhynchus nerka* (Walbaum), and Atlantic salmon, *Salmo salar* L. J Fish Dis 39: 117–128.

22. Garver KA, Johnson SC, Polinski MP, Bradshaw JC, Marty GD, Snyman HN, Richard J, Morrison, DB (2016) Piscine orthoreovirus from western North America is transmissible to Atlantic salmon and sockeye salmon but fails to cause heart and skeletal muscle inflammation. PLoS One, 11, e0146229.

23. Purcell MK, Garver KA, Winton JR (abstract) Risk assessment of piscine reovirus (PRV) infection in Pacific salmon and trout. Proceedings Seventh International Symposium on Aquatic Animal Health, August 31-September 4, 2014. Portland, OR

24. Garseth ÅH, Ekrem T, Biering E (2013) Phylogenetic evidence of long distance dispersal and transmission of piscine reovirus (PRV) between farmed and wild Atlantic salmon. PLoS One, 8, e82202

25. Sibley SD, Finley MA, Baker BB, Puzach C, Armién AG, Giehtbrock D, Goldberg TL (2016) Novel reovirus associated with epidemic mortality in wild largemouth bass (*Micropterus salmoides*). Journal of General Virology, 97, 2482-2487.