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Health monitoring of wild anadromous salmonids in freshwater in Norway 2017

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Health monitoring of wild anadromous salmonids in freshwater in Norway

Content

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Introduction

Heart and Skeletal Muscle Inflammation (HSMI) is one of the most common viral diseases of farmed Atlantic salmon *Salmo salar* L. in Norway [1]. Piscine orthoreovirus (PRV) (hereafter referred to as PRV1) was first described in association with HSMI in Atlantic salmon in 2010 [2], and in 2017 the causal relationship between PRV and HSMI was firmly established [3]. Recently new variants of PRV have been described and linked to several disease conditions. Erythrocytic inclusion body syndrome (EIBS) in Coho salmon *Oncorhynchus kisutch* Walbaum has been linked to a variant called PRV2 [4]. A third variant, called PRV3, has been described in association with HSMI-like lesions in Coho salmon Chile [5] and rainbow trout *Oncorhynchus mykiss* Walbaum in Norway [6, 7]. Experimental infection studies have confirmed the causal relationship between the PRV3 and HSMI-like disease seen in rainbow trout [8], and furthermore that this virus is better adapted to rainbow trout than Atlantic salmon. Epidemiological investigations and PCR based surveillance programs show that PRV3 is present at all levels of the rainbow trout production cycle, including brood fish, hatcheries and after sea transfer [6].

Aim

In 2017, the objective of the health monitoring program in freshwater was to investigate the occurrence of PRV3 in wild salmonids.

Materials and methods

The study sample comprised wild-caught brood fish of Atlantic salmon, landlocked salmon *Salmo salar* L., Arctic char *Salvelinus alpinus* L. and sea trout *Salmo trutta* L. from stock enhancement hatcheries and the Genebank for wild Atlantic salmon (**Table 1**). Fish from the same river were held together in tanks from a few days up to 6–7 weeks before stripping and tissue sampling. Additional samples of trout were obtained during rotenone treatment in the Vefsna region in 2011(sea trout), one lake in Trøndelag in 2010 (brown trout) and two lakes in Sogn & Fjordane in 2016 (brown trout). All Atlantic salmon were classified as wild based on scale reading and genetic tests [9-13]. Heart and kidney samples were fixed in RNAlater™ and sent to Norwegian Veterinary Institute for PRV3 specific real-time RT-PCR analysis. A selection of positive samples from sea trout were verified by repeated real-time RT-PCR on re-extracted nucleic and conventional RT-PCR followed by gel electrophoresis and sequencing with primers and conditions as previously described [6]. The obtained sequences were compared with PRV3 sequences in the GenBank database. Selected samples from both sea trout and Atlantic salmon were also examined for the presence of PRV1 [14].

Table 1. Overview of study sample including species, morphology, year and number of counties, watercourses and fish.

Species		Counties	Water-courses	Fish	Years
Anadromous	Atlantic salmon *Salmo salar* L.	4	15	220	2016
	Seatrout *Salmo trutta* L.	2	19	197	2011, 2016
	Arctic char *Salvelinus alpinus* L.	1	2	11	2016
Non-anadromous	Landlocked salmon *Salmo salar* L.	1	1	40	2015
	Brown trout *Salmo trutta* L.	2	3	79	2010, 2016

Results

PRV3 was present in wild sea trout from 13 of 19 investigated rivers and in both regions included in the study (Table 2). Ct-values ranged from 22.5 to 39.6. The PCR-results for two samples from Vefsn and two samples from Hardanger were verified, and the sequences showed 99–100% identity with PRV3 previously detected in rainbow trout in Norway (GenBank accession number LN680851). None of the examined samples from sea trout were positive for PRV1.

Four of 220 samples from wild Atlantic salmon had a positive real-time RT-PCR reaction, but with Ct-values approaching the detection limit of the method (Range 34.6–40.0). Positive results were not confirmed by sequencing. Two of these salmon were also positive for PRV1. Positive samples were from three rivers in the Hardanger region.

Table 2. Overview of results from real-time RT-PCR analyses for PRV3 in sea trout (anadromous trout *Salmo trutta* L.). PCR results are presented as the proportion of test-positive among tested (test-positive/No. tested).

Region	River	2011	2016	Ct-value (range)
Nordland	Drevja	0/14		
	Fusta	0/6		
	Hundåla	2/30		22.5–29.8
	Vefsna	1/10		31.6
Hardanger	Austrepoll		2/13	32.0–35.2
	Fjæra		0/3	
	Granvin		4/12	29.0–36.2
	Jondal		4/9	28.9–37.6
	Omvik		5/9	33.0–36.3
	Opo		0/1	
	Osa		4/9	31.6–35.1
	Rosendal		2/11	26.8–31.0
	Sima		2/15	28.6–35.4
	Steinsdal		4/25	31.7–39.6
	Strandadal		0/8	
	Uskedal		1/9	36.3
	Ådland		0/8	32.8
	Ænes		2/4	29.5–31.6
	Øyreselva		1/1	33.4
Fish positive/tested Rivers positive/tested		3/60 2/4	31/137 11/15	22.5–39.6

Discussion and Conclusion

PRV3 was present in sea trout from altogether 13 of 19 rivers and in both the northern and south-western region. Overall, the per-river sample sizes in this study were low. Consequently, the minimum detectable prevalence in each of these rivers is quite high, indicating that the study underestimates the actual occurrence of PRV3 in sea trout [15]. Accordingly, we can conclude that PRV3 is a common virus in sea trout in both regions. None of the selected PRV3 positive sea trout were positive for PRV1. In previous studies, 0–3 % of sea trout were PRV1 positive [16-19]. Consequently, the PRV3 survey has revealed a much higher prevalence, higher viral levels, and furthermore, that the PRV3 sequences group together with PRV3 from rainbow trout.

Altogether, four Atlantic salmon were PRV3 positive (Ct-values 34.6–40.0). The salmon came from three different rivers in the Hardanger region, and were not held in tanks with PRV3 positive sea trout. A recent study has demonstrated that Atlantic salmon are less susceptible for PRV3 than rainbow trout [8].

PRV3 was absent in non-anadromous salmonids. This could mean that salmonids in non-anadromous water courses in Norway are of minor importance as a reservoir, but this should be studied further.

None of the 11 anadromous Arctic char were virus positive. Due to the sample size this result remains inconclusive.

PRV3 is present at all stages of the marine rainbow trout production cycle (Olsen et al., 2015), but this study reveals that wild sea trout could also be a reservoir or source. Rainbow trout and the trout (*Salmo trutta* L.) are both well-known globetrotters, the former introduced to fish farms worldwide, and the latter released for recreational fishing purposes worldwide.

The risk of introducing PRV3 to farms through intake of freshwater from non-anadromous sources may be negligible. On the other hand, the use of freshwater from anadromous water sources may constitute a risk of introducing PRV3 to freshwater facilities. At sea, infected farmed rainbow trout constitute a significant reservoir that could infect sea trout in the vicinity.

In conclusion, piscine orthoreovirus 3 (PRV3) is a common virus in sea trout. Wild Atlantic salmon are less susceptible to the virus, which may explain the low prevalence and viral loads recorded in wild specimen in this study. The absence of PRV3 in non-anadromous salmonids indicates that the virus may be linked to the marine environment in Norway, although the virus has been detected in non anadromous trout (farmed) in France [20]. Further studies, including the presence of PRV3 in inland-farmed rainbow trout and brown trout near these farms, are warranted.

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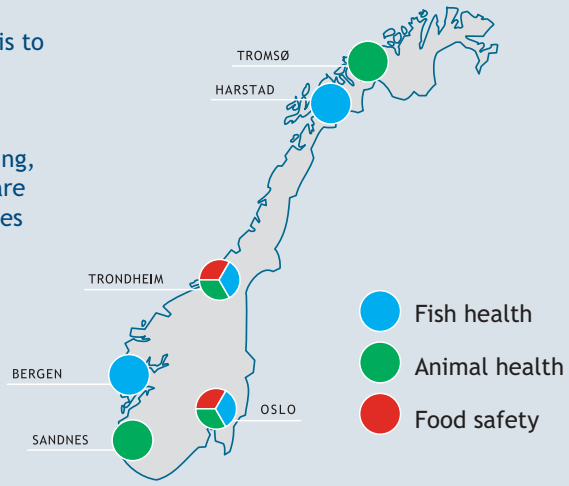

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