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Content

Summary

Piscine orthoreovirus-3 (PRV-3) is present in farmed rainbow trout and in wild sea trout in Norway. In 2021, the health monitoring programme for wild salmonids investigated the presence of PRV-3 in wild freshwater resident brown trout, and in freshwater based inland aquaculture of rainbow trout and brown trout in Norway.

PRV-3 was detected in brown trout in a stock enhancement hatchery in the County of Innlandet and in wild brown trout from four lakes. Two of these lakes were stocked with brown trout from the PRV-3 positive hatchery. Two of the PRV-3 positive wild brown trout were lacking the adipose fin, indicating that they were released from the hatchery. The results indicate that PRV-3 is present in freshwater resident brown trout, but also that the occurrence can be associated with release from stock enhancement hatcheries. Accordingly, the study strongly suggest that virus can be spread by stocking of infected fish in the wild.

Sequencing of PRV-3 from the PRV-3 positive stock enhancement hatchery and the lakes could provide stronger evidence of this virus spread. Histopathological investigations would provide information regarding the health effects of PRV-3 infection in brown trout.

Introduction

In 2012, the Norwegian Veterinary Institute (NVI) and the Institute of Marine Research (IMR) were commissioned by the Norwegian Food Safety Authority (NFSA) to carry out annual health monitoring of wild anadromous salmonids in Norway. NVI coordinates the programme in freshwater and publishes the results in annual reports available on https://www.vetinst.no/overvaking/health-monitoring-of-wild-fish

Heart and skeletal muscle inflammation (HSMI) is one of the most common viral diseases of farmed Atlantic salmon in Norway [1]. Piscine orthoreovirus-1 (PRV-1) was first associated with HSMI in Atlantic salmon in 2010 [2], and in 2017 the causal relationship between PRV-1 and HSMI was firmly established [3]. Variants of PRV have since been described and linked to disease conditions in other species. Erythrocytic inclusion body syndrome (EIBS) in Coho salmon (*Oncorhynchus kisutch*) is linked to PRV-2 [4], and PRV-3 is also associated with disease in Coho salmon [5] as well as HSMI-like lesions in rainbow trout (*Oncorhynchus mykiss*) [6, 7, 8]. Experimental infection studies have confirmed the causal relationship between PRV-3 and HSMI-like disease seen in rainbow trout [8], and furthermore that the virus is better adapted to rainbow trout than Atlantic salmon. PRV-3 has been found at all levels of the marine rainbow trout production cycle, including brood fish, hatcheries and after sea transfer [6]. In 2017, health monitoring of wild salmonids showed that PRV-3 was common in wild sea trout, while the virus was not found in wild freshwater resident brown trout [9, 10].

In Norway, farming of rainbow trout takes place as two nearly separated productions: the large-scale marine production along the coast and small-scale freshwater-based inland aquaculture. The productions use eggs and fry from the same suppliers.

Norway has a small commercial inland production of brown trout for consumption, and a larger production of brown trout for stock enhancement in lakes and rivers. Reports from continental Europe provide evidence that PRV-3 is present in inland farming of rainbow trout and brown trout [7, 11, 12, 13], while information regarding the presence of the virus in Norwegian inland aquaculture and stock enhancement hatcheries is lacking.

Aim

The overall purpose of the programme is to investigate the sources and occurrences of disease-causing agents in wild salmonids, including Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*).
In 2021, the health monitoring programme investigated the presence of piscine orthoreovirus-3 (PRV-3) in wild freshwater resident brown trout, and in inland aquaculture of rainbow trout and brown trout in Norway.

Materials and methods

Inland aquaculture and stock enhancement hatcheries
Eleven fish farms were included in the study of which six produce rainbow trout and five produce brown trout. Two of the rainbow trout farms are hatcheries that receive fertilised eggs from AquaGen, two of the on-growing farms receive fry from these hatcheries and one receive fry from a hatchery not included in the study. The five farms that produce brown trout comprise three stock enhancement hatcheries, a commercial hatchery and a commercial on-growing farm producing brown trout for consumption.

In each fish farm, authorised fish health personnel collected samples from heart, kidney and spleen from 30 fish in RNAlater™ for real-time RT-PCR. Aliquots of kidney samples were sent to Pharmaq Analytiq for detection of PRV-3 by real-time RT-PCR, while the remaining samples were included in the NFSA surveillance programme for infectious hematopoietic necrosis virus (IHNV) and viral haemorrhagic septicaemia virus (VHSV) and analysed at the NVI.

Wild freshwater resident brown trout
Samples from wild brown trout were collected at seven locations (lakes).

Samples from Lake Selbusjøen comprised wild-caught brown trout used as broodfish by a stock enhancement hatchery. After capture, these brown trout were held in a net cage in the outlet of River Nea before stripping and the following euthanasia and post mortem examination. Brown trout from the lakes Femunden, Kangsvatnet and Snåsavatnet were captured by the Norwegian Institute for Nature Research (NINA), as part of fish monitoring in large lakes under

the EU Water Framework. These fish were captured by trawling and net fishing (NordicBG series), then frozen at -20 ºC and thawed before sampling.

Personnel from one of the stock enhancement hatcheries captured 20 brown trout from each of two lakes that were stocked with hatchery-reared brown trout and a nearby lake with natural recruitment. The hatchery personnel sampled scales in particular envelopes and kidney samples in RNAlater™ from these wild brown trout. Body length, weight, sex and absence or presence of the adipose fin was recorded on the scale sample envelopes. Presence of the adipose fin is recorded because this fin is removed in hatchery-reared brown trout to distinguish them from naturally recruited conspecifics. Samples were sent to NVI for quality control and preparation for PCR. All kidney samples in RNAlater™ were then sent to Pharmaq Analytiq for PCR for PRV-3.

Results

Inland aquaculture and stock enhancement hatcheries

Table 1 displays an overview of the fish farms that were included in the 2021 health monitoring programme and results from the PRV-3 specific PCR.

In a stock enhancement hatchery in the County of Innlandet, all the 30 sampled brown trout were PCR positive for PRV-3 (Ct-values 15.7-30.6, mean 21.8). All other locations and fish were PCR-negative.

***Table 1**. Overview of study samples from inland aquaculture and stock enhancement hatcheries, including results from PRV-3 specific PCR.*

Farm	Species	Production	County	No. samples	No. positive samples (ct-value range)
1	Rainbow trout	Hatchery	Innlandet	30	0
2	Rainbow trout	Hatchery	Innlandet	30	0
3	Rainbow trout	On growing	Innlandet	30	0
4	Rainbow trout	On growing	Innlandet	30	0
5	Rainbow trout	On growing	Innlandet	29	0
6	Rainbow trout	On growing	Innlandet	30	0
7	Brown trout	Hatchery	Vestland	30	0
8	Brown trout	On growing	Rogaland	30	0
9	Brown trout	Stock enhancement	Innlandet	30	30 (15.7-30.6, mean 21.8)
10	Brown trout	Stock enhancement	Innlandet	30	0
11	Brown trout	Stock enhancement	Troms & Finnmark	30	0
	Total			**329**	**30**

Wild freshwater resident brown trout

Table 2 displays an overview of the locations included in the 2021 health monitoring programme, including results from PRV-3 specific PCR. PRV-3 was detected in altogether eight brown trout from four of the seven lakes studied. The adipose fin was absent in the two PRV-3 positive brown trout from Lake 2, implying that they were released from the PRV-3 positive hatchery.

***Table 2**:. Results from PCR-analyses of kidney tissue from brown trout captured in lakes Femunden, Selbusjøen, Kangsvatnet, Snåsavatnet and three lakes in the County of Innlandet that were stocked with brown trout from a PRV-3 positive facility. Table shows number of fish tested, number of PCR-positive fish, and Ct-values*

Location	County	No. samples	No. positives	Ct-values
Femunden	Innlandet	22	0	
Selbusjøen	Trøndelag	41	2	31.8 and 32.2
Snåsavatnet	Trøndelag	46	0	
Kangsvatnet	Trøndelag	40	0	
Lake 1	Innlandet	20	3	18.3, 28.8 and 29.8
Lake 2	Innlandet	20	2*	29.6 and 29.6
Lake 3	Innlandet	20	1	33.1
Total		**209**	**8**	

*The adipose fin was absent (removed), indicating that the fish were released from the hatchery.

Discussion

The cross sectional study was limited in size both with regards to number of locations included and the number of fish from each location. Nevertheless, all brown trout were PCR-positive for PRV-3 in a stock enhancement hatchery in the County of Innlandet, and the Ct-values indicated a high viral load. In experimental transmission studies, heart lesions consistent with those observed in HSMI in Atlantic salmon were present in both shedders and cohabitant brown trout [14]. Investigation of histopathological lesions in PRV-3 positive brown trout is necessary to further evaluate the health effects for infected wild and hatchery reared brown trout.

PRV-3 was also detected in lakes stocked with brown trout from the PRV-3 positive hatchery. The absence of the adipose fin in two of the PRV-3 positive brown trout suggests that they were released from the PRV-3 positive hatchery. Sequencing and phylogenetic analyses that are necessary to provide evidence of virus spread during release of hatchery reared brown trout is out of scope for the health monitoring programme.

It is not known whether brown trout or rainbow trout is the original host of PRV-3, but it is well known that the virus is present in these species in the marine environment in Norway and in inland aquaculture in continental Europe. Investigation of how the virus could have been introduced to the stock enhancement hatchery revealed no contact with rainbow trout farms or anadromous brown trout. There are no recorded observations of feral (escaped or released)

rainbow trout in the water source upstream the hatchery, nor in the lakes where the hatchery captures their broodfish (www.artsdatabanken.no).

The virus was also found in Lake Selbusjøen, and similar to the aforementioned lakes, hatchery reared brown trout are released in Selbusjøen. The hatchery that releases brown trout in Selbusjøen was not included in the study.

Despite being present in the marine rainbow trout production, PRV-3 was not detected in the rainbow trout farms included in the study. Rainbow trout farming in the Valdres region is based on introduction of disinfected eggs to the hatcheries. This represents a higher level of biosecurity than introduction of post-hatch stages of fish, for instance fry. To date, studies confirming vertical transmission of PRV-3 in rainbow trout has not been published. However, studies conducted by the Gene bank for wild Atlantic salmon in Norway indicate that PRV-3 is not transmitted vertically in brown trout (sea trout) (Gåsnes & Garseth, unpublished work).

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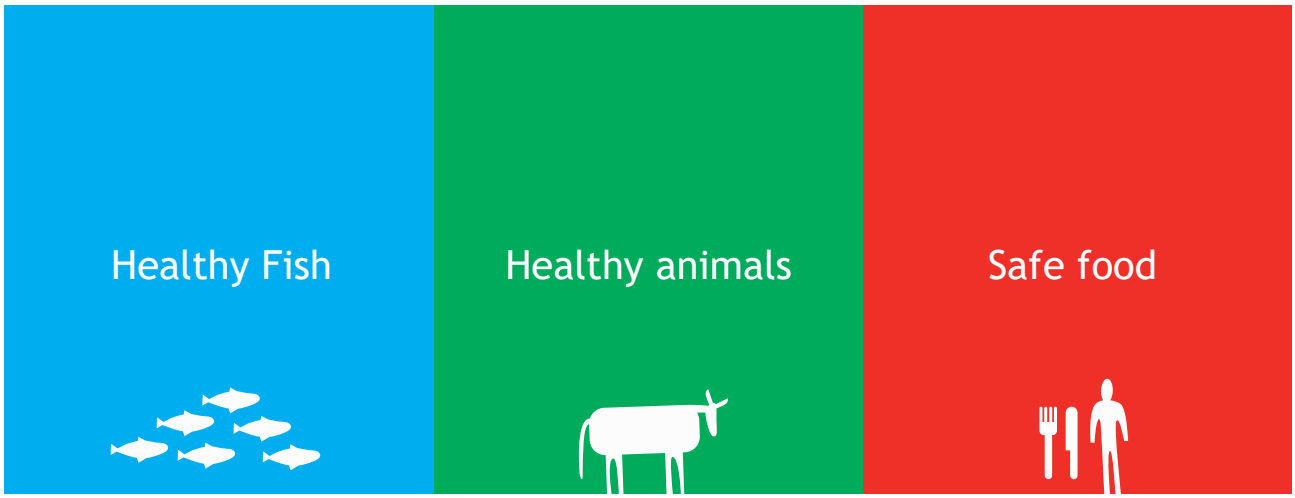

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