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Prevalence of piscine orthoreovirus and salmonid alphavirus in sea-caught returning adult Atlantic salmon (*Salmo salar* L.) in northern Norway

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Abstract

Heart and skeletal muscle inflammation (HSMI) caused by piscine orthoreovirus (PRV) and pancreas disease (PD) caused by salmonid alphavirus (SAV) are among the most prevalent viral diseases of Atlantic salmon farmed in Norway. There are limited data about the impact of disease in farmed salmon on wild salmon populations. Therefore, the prevalence of PRV and SAV in returning salmon caught in six sea sites was determined using real-time RT-PCR analyses. Of 419 salmon tested, 15.8% tested positive for PRV, while none were positive for SAV. However, scale reading revealed that 10% of the salmon had escaped from farms. The prevalence of PRV in wild salmon (8%) was significantly lower than in farm escapees (86%), and increased with fish length (proxy for age). Sequencing of the S1 gene of PRV from 39 infected fish revealed a mix of genotypes. The observed increase in PRV prevalence with fish age and the lack of phylogeographic structure of the virus could be explained by virus transmission in the feeding areas. Our results highlight the need for studies about the prevalence of PRV and other pathogens in Atlantic salmon in its oceanic phase.

KEYWORDS
aquaculture, piscine orthoreovirus, salmonid alphavirus, wild salmon

1 | INTRODUCTION

Viral diseases represent a serious problem in Atlantic salmon (*Salmo salar* L.) farming in Norway, often leading to substantial economic losses. Heart and skeletal muscle inflammation (HSMI) and pancreas disease (PD) are among the most common viral diseases in Norwegian aquaculture, with 101 and 138 cases, respectively, reported in 2016 (Hjeltnes, Bornø, Jansen, Haukaas, & Walde, 2017). PD caused by salmonid alphavirus (SAV) is endemic in salmon farmed in central and western Norway, but uncommon in northern Norway. In contrast, HSMI caused by piscine orthoreovirus (PRV) is widespread in Norwegian salmon farms along the Norwegian coastline (Kristoffersen, Bang Jensen, & Jansen, 2013; Wessel et al., 2017).

Disease outbreaks in salmon farms may lead to increased infection and disease risks at neighbouring farms and in wild fish populations, and there is an increasing public concern of disease impacting wild salmon populations in Norway (Svåsand et al., 2017). Data are collected annually on the frequency and geographical distribution of disease outbreaks in fish farms (Hjeltnes et al., 2017). Correlating such data from regions differing in farming intensities and disease profiles with pathogen prevalence in local wild salmon populations would thus help to address this concern. However, pathogen prevalence data alone as an indicator of infection pressure have

limitations (Mcvicar, 1997). Virulent pathogens may cause disease in wild salmon, leading to direct mortality or increased susceptibility to predation and therefore rendering them less catchable. Therefore, when screening wild stocks for infections, we are normally able to collect non-diseased infected fish such as individuals that have recently acquired infections or have survived an infection and become "carriers."

An overall prevalence of PRV in wild Atlantic salmon caught in Norwegian rivers between 2007 and 2009 was 13.4% (Garseth, Fritsvold, Opheim, Skjerve, & Biering, 2013). However, as the river-caught salmon examined had been kept in tanks for weeks or months prior to sampling and testing, PRV prevalence might have been overestimated due to horizontal virus transmission among the fish. Conversely, there is a possibility that the physical condition of salmon most heavily infected with PRV is compromised, rendering them less likely to ascend the rivers and decreasing virus prevalence in river-caught fish (Miller et al., 2014).

Northern Norway has fjords with a very low salmon farming intensity (Data S1). This will change in the future, and therefore, it is important to obtain quantitative as well as qualitative data on the virus occurrence in wild salmon in that region (Anonymous 2015).

To address this, real-time RT-PCR was used to determine the prevalence of PRV and SAV in sea-captured returning wild adult salmon from six sea locations along the northern coast of Norway. The study aimed to investigate virus prevalence and genotype diversity in wild salmon in relation to regional farming intensity and disease frequency as well as fish sex and sea-age prior to river entry.

2 | MATERIALS AND METHODS

2.1 | Salmon

A total of 419 Atlantic salmon were caught by commercial fishermen at six sites distributed across three counties in northern Norway (Finnmark [Sites A Bugøynes, B Nordkapp and C Alta], Troms [Site D Månes] and Nordland [Sites E Andøy and F Stamnes]) selected on the basis of them differing in fish farming intensity and HSMI outbreak frequency (Figure 1, Data S1). Salmon were caught between 13 June and 23 August 2012 using nets and fish traps. Scale samples from each fish were used to determine whether the salmon was wild or had escaped from a farm, and to estimate its sea-age. The fish were frozen at −20°C as soon as possible after capture.

2.2 | Tissue sampling

Fish lengths and weights were recorded, and heads were sawed from frozen fish posterior to the pericardial cavity. While still frozen, heart (ventricle) tissue samples were removed aseptically and transferred to tubes on dry ice and sent to commercial laboratory for RNA extraction and PCR testing (PatoGen Analyse AS). The head and abdomen of the fish were then thawed and inspected visually to identify sex and evidence of external or internal lesions or other signs of disease.

2.3 | SAV and PRV PCR testing

RNA was extracted from the tissue samples and tested for the presence of SAV and PRV RNA at PatoGen Analyse AS using in-house methods and real-time RT-PCR assays as described previously (Madhun et al., 2015). The SAV PCR test targeted the nsP1 gene (Hodneland & Endresen, 2006) and detects both SAV subtypes (2 and 3) found in Norway (Hjortaas et al., 2013). The PCR test employed a method described previously (Glover, Sørvik, Karlsbakk, Zhang, & Skaala, 2013; Palacios et al., 2010) and detected the PRV-*Salmo salar* (PRV-Ss) virus variant (Hauge et al., 2017). Samples giving a C_t (cycle threshold) value <37.0 were considered positive. Elongation factor 1-alpha was used as reference gene to assess the quality of RNA in the tissue, and a C_t value <21 was considered to be satisfactory.

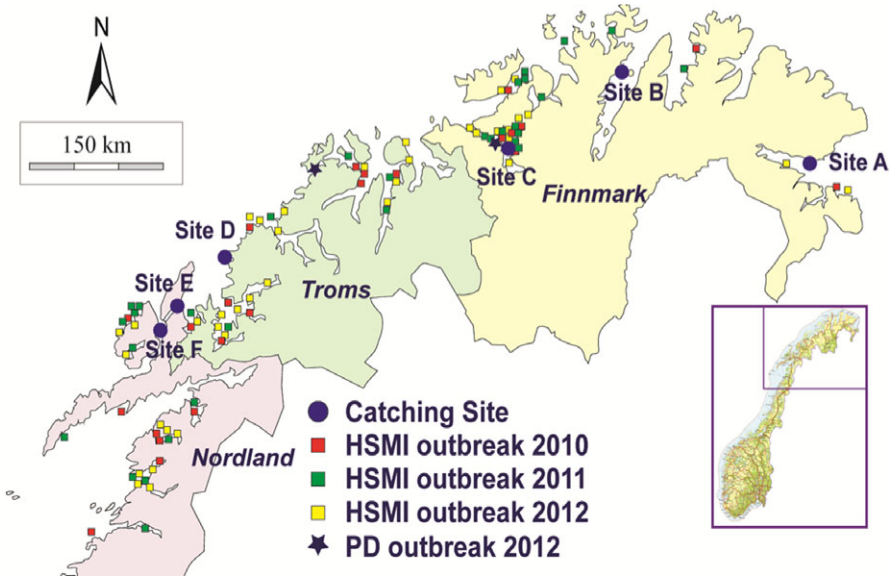

FIGURE 1 Map showing the 6 salmon capture locations in 2012 and the locations of HSMI and PD outbreaks at fish farms between 2010 and 2012 [Colour figure can be viewed at wileyonlinelibrary.com]

2.4 | Sequence analysis

Samples from Atlantic salmon showing PRV real-time RT-PCR C_t values <30 were analysed using two sets of PCR primers (S1 No 1 and S1 No 2) amplifying 601- and 596-bp overlapping fragments representing a region of 1,016 bp in S1 segment as previously described (Garseth, Ekrem, & Biering, 2013). Briefly, cDNA was synthesized from RNA using SuperScript® VILO™ cDNA Synthesis kit (Life Technologies) according to the manufacturer's recommended protocol and cDNA was then amplified by PCR using Gotaq Flexi DNA Polymerase (Promega) according to recommended kit protocol.

A portion of the PCR was electrophoresed in a 1% Seakem LE agarose gel (BioNordica) containing GelRed stain (VWR), and remaining DNA was purified using ExoSAP-IT® PCR Product Clean-Up (VWR). DNA was subjected to bidirectional cycle sequencing using the PCR primers and Big-Dye version 3.1 reagent and sequenced using a 3730XL Analyzer (Applied Biosystems) at the MBI Sequencing Facility, University of Bergen. Each PRV PCR-positive sample was amplified and sequenced twice to increase sequence length and quality. Genogroup assignment was mostly based on partial (837 nt) S1 sequences and the phylogenetic analysis described in Garseth, Ekrem et al. (2013), but a few sequences were shorter and assigned based on sequence identity. The S1 sequences obtained in this study have GenBank accession numbers MG720339–MG720377.

2.5 | Statistical analysis

The probability of PRV infection in a fish at a capture site was assessed using a Bernoulli generalized linear mixed model (GLMM) in which capture location was used as random intercept. Covariates incorporated sex (male vs female numbers), origin (wild vs escaped salmon numbers), time of capture (days after salmon capture commenced) and fish length as a proxy for sea-age (see Data S2).

TABLE 1 The origin and percentage of PRV-positive salmon from the six sampling sites in northern Norway

	Number and origin of salmon			Number of PRV-positive salmon	
County Area	Total	Wild	Escaped (% total)	Wild (% of wild)	Escaped (% of escaped)
Finnmark					
Site A	167	165	2 (1)	9 (5)	1 (50)
Site B	29	25	4 (14)	2 (8)	3 (75)
Site C	63	60	3 (5)	4 (7)	3 (100)
Troms					
Site D	104	85	19 (18)	7 (8)	16 (84)
Nordland					
Site E	34	25	9 (26)	2 (8)	8 (89)
Site F	22	17	5 (23)	6 (35)	5 (100)
Total	419	377	42 (10)	30 (8)	36 (86)

3 | RESULTS AND DISCUSSION

3.1 | Characteristics of the studied salmon

None of the 419 salmon captured at any of the six sampling sites spanning the northern coastline of Norway showed external or internal lesions or other signs of disease. Scale examination identified 42 (10%) to be escapees from farms (Table 1). Such a proportion was not unexpected considering that hundreds of thousands farmed salmon are reported to have escaped annually in most years between 2000 and 2015 (Anonymous 2017) and is consistent with a 10% prevalence in Nordland, Troms and Finnmark counties determined from examining over 20,000 salmon sampled during the main returning period in 2011–2012 (Niemelä et al., 2014).

One sea-winter (one SW) wild fish dominated at sites A, B, C and D and two SW fish dominated at sites E and F. A few three SW (n = 9), four SW (n = 6) and previous spawner fish (n = 8) were also identified but as numbers of these were skewed to specific sites and therefore only data on one SW and two SW fish were used in statistical analysis (see Data S2).

3.2 | Salmonid alphavirus prevalence

Salmonid alphavirus was not detected by real-time RT-PCR in heart from any of the wild salmon or farm escapees examined, indicating a prevalence <0.89% at 95% probability. There were only two PD outbreaks in the northern counties (Nordland, Troms and Finnmark) between 2010 and 2012 (Figure 1). The probability of wild salmon being exposed to SAV from farms escapees was therefore low both when leaving the coast as smolts (2010–2011) and when returning as adults in 2012. The absence of SAV in the salmon tested is also consistent with previous findings that SAV infections are uncommon in wild salmonids irrespective of farming intensity or the frequency of PD outbreaks at the locations examined (Biering et al., 2013; Garseth et al., 2015; Madhun et al., 2016; Plarre & Nylund, 2014).

3.3 | Piscine orthoreovirus prevalence

Piscine orthoreovirus was detected by real-time RT-PCR in 16% of the salmon examined (C_t values 23.0–36.7, mean 30.2). Overall PRV prevalence was 8% in returning wild salmon and 86% in farm escapees (Table 1). The statistical analyses showed that the probability of infection increased with fish length (proxy for sea-age) irrespective of their origin (Figure 2, Data S2).

The prevalence (86%) of PRV in escapees was significantly ($p < .01$) higher than that detected in salmon escapees (55%) examined from 36 Norwegian rivers between 2007 and 2009 (Garseth, Fritsvold et al., 2013). However, PRV was detected at a similar prevalence (79%) in 129 farm escapees trapped in the Etna river in western Norway during 2014 (Madhun et al., 2017). The high prevalence of PRV infection in salmon escapees from both coastal and river systems in Norway highlights the potential for them acting as vectors for infection transfer to wild salmon (Garseth, Biering, &

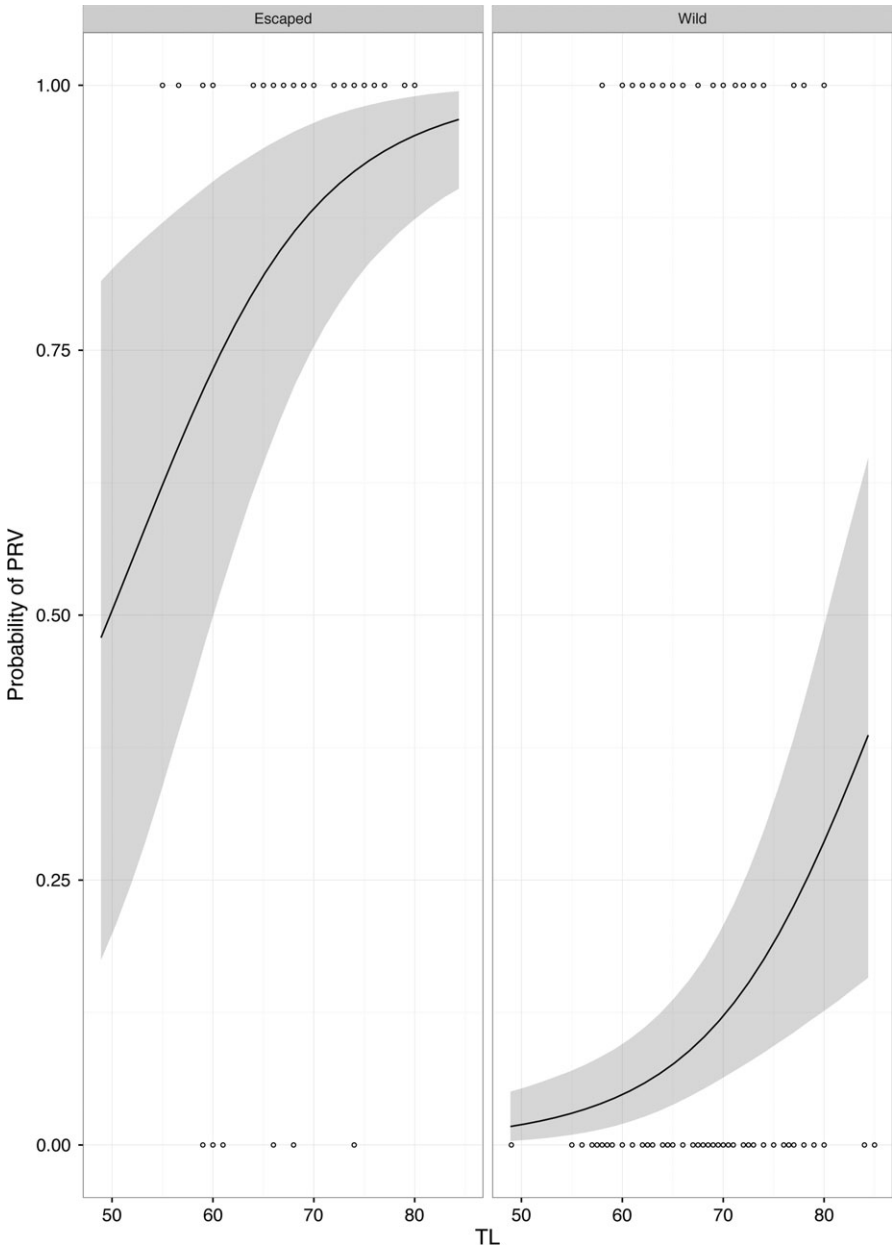

FIGURE 2 Probability of PRV being detected by real-time RT-PCR in relation to fish total length (TL) and the origin (wild or farm escapee) of fish. The figure shows the posterior mean fitted values and 95% credible intervals using the R program (see Data S2)

Aunsmo, 2013; Garseth, Fritsvold et al., 2013; Madhun et al., 2015, 2017).

PRV was detected in wild salmon at a low prevalence (8%), which is not significantly different from that (12%) found in wild salmon ($n = 66$) examined from three rivers in the same region between 2007 and 2009 (Garseth, Fritsvold et al., 2013). Some of the river-caught fish in that study were cohabitated in tanks for weeks or months prior to sampling, introducing the possibility of prevalence increase due to PRV transmission in the tanks (Garseth, Fritsvold et al., 2013). On the other hand, virus prevalence could be underestimated if PRV-infected fish have poorer performance and are less likely to ascend the rivers (Miller et al., 2014).

Statistical analysis suggested that sea-age increases the likelihood of PRV infection in the salmon, as reported previously (Garseth, Biering et al., 2013). This might be expected due to increased exposure of both farmed and wild salmon to virus from various potential sources as they age. Earlier studies have shown that the risk for farm fish to be infected with viruses increases with increased time in net-pens, hence sea-age (Jansen et al., 2010; Jensen et al., 2013; Kristoffersen et al., 2013).

Wild salmon may be exposed to the viruses prevalent in salmon farms in the fjords when migrating past as smolt or returning as adults. However, they may be exposed already in the rivers as juveniles, both from virus-infected farm escapees and from spawning wild salmon. PRV infection was detected in both parr from a river and in migrating smolt from three fjords located in western Norway (Svåsand et al., 2017). Little is known about virus prevalence and transmission among salmon in the oceanic feeding areas. However, the finding that spending more years at sea increases the risk for wild salmon of being PRV-infected could be an effect of PRV

TABLE 2 PRV genotypes (based on S1 sequences) detected in wild salmon and farm escapees across the six sampling sites (A–F) in northern Norway, allocated to genogroups (1–4) of Garseth, Ekrem et al. (2013)

	Escaped farm salmon				Wild salmon			
Genogroup	1	2	3	4	1	2	3	4
Site A	1				1	1		
Site B	2							1
Site C	1	1					1	
Site D	5	6	1	3	4	1		
Site E	4	1						
Site F	1[a]	1		1[a]		2		1
Total	14	9	1	4	5	4	1	2

[a]PRV S1 RNA segment sequence from the same fish with mixed genogroup 1 and 4 signals (likely double infection).

infections being acquired by wild salmon in the feeding areas. Relevant to this, PRV was detected at higher prevalence in returning adult salmon (15%) than in leaving smolts (5%) in a fjord in western Norway (Svåsand et al., 2017). Another possibility is that PRV infection may affect life-history traits of wild salmon by for example delaying the age of returning (due to reduced growth or delayed maturation) or increasing the mortality of fish that mature early (Michalakis & Hochberg, 1994; Van Der Most, De Jong, Parmentier, & Verhulst, 2011; Vollset, Barlaup, Skoglund, Normann, & Skilbrei, 2014). Therefore, more knowledge about both the prevalence of virus infections in wild salmon in feeding areas and the effect of PRV infection on behaviour, growth and maturation of wild salmon is needed.

We did not observe any association between sex and PRV prevalence in the fish. This is at variance with the results from Garseth, Biering et al. (2013), who found that males were twice as likely to be infected as females (Garseth, Biering et al., 2013). Whether this apparent difference is real or related to factors such as that our salmon were caught at sea rather than river, originate from different geographical regions, were captured in different years or sampled directly rather than after tank rearing is not known.

3.4 | PRV genotypes

Sequences of an 837 nt region of the σ3 protein gene segment S1 RNA were obtained from all 39 fish in which PRV was detected by real-time RT-PCR at a C_t value <30. In the current study, sequences from 12 wild salmon and 27 farm escapees were compared with previously published sequences (Garseth, Ekrem et al., 2013). All PRV groups (1–4) were detected in wild salmon and farm escapees (Table 2). PRV Group 1 was the most common (19/39 = 49%) in both wild salmon and farm escapees. The low number of sequences available from each site made it difficult to compare the occurrence of different PRV genogroups in wild salmon and farm escapees.

The PRV genogroups detected in our study were consistent with those previously identified in salmon captured from rivers in the same region (Garseth, Ekrem et al., 2013). Results from both studies suggest a lack of a regional pattern in the phylogenetic structure of PRV. This could be due to prolonged and extensive movements of salmon for aquaculture or stock enhancement purposes, and frequent virus exchange between farmed and wild fish (Garseth, Ekrem et al., 2013). However, PRV transmission among salmon in the oceanic feeding areas could also cause or contribute to the lack of a phylogeographic pattern. Salmon populations from different regions of Europe and from North America may interact (Gilbey et al., 2017; Olafsson et al., 2016; Thorstad, Whoriskey, Rikardsen, & Aarestrup, 2010) and have the potential for pathogen exchange (Besnier et al., 2014; Fjørtoft et al., 2017). Data about virus prevalence in salmon from different age classes in the oceanic feeding areas of the North Atlantic may provide information about such transmission.

3.5 | The association between PRV prevalence and collection site

In the current study, salmon were captured from sites differing in farming intensity and frequency of HSMI outbreaks (Data S1, Figure 1). However, statistical analyses identified no significant relationship between PRV prevalence and site. Therefore, similar to previous reports (Garseth, Fritsvold et al., 2013; Marty, Morrison, Bidulka, Joseph, & Siah, 2015), our study has shown no association between salmon farming and the prevalence of PRV infection in wild salmon.

In summary, based on a unique material consisting of sea-caught returning salmon, we report PRV and SAV prevalence in wild salmon. The prevalence of PRV was low and SAV was not detected in the tested fish. PRV prevalence was not significantly higher in salmon caught in areas with high farming intensities. We highlight the need for studies on viral infections in Atlantic salmon from the oceanic feeding areas.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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