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The surveillance programme for infectious salmon anaemia virus HPR0 (ISAV HPR0) in Norway 2023

REPORT 4/2024

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Content

Sammendrag

Den ikke-patogene varianten av infeksiøs lakseanemi-virus, ILAV HPR0, ble påvist ved seks av 75 settefisklokaliteter i forbindelse med prøvetakingen for overvåkingsprogrammet for ILAV HPR0 i 2023.

Siden det kun ble tatt ut prøver på ett tidspunkt hos hver av lokalitetene i løpet av kalenderåret, samt fra et begrenset antall kar, representerer trolig resultatet en underestimering av den faktiske prevalensen av ILAV HPR0 hos norske settefiskanlegg.

Summary

The non-pathogenic variant of the infectious salmon anaemia virus, ISAV HPR0, was detected in six of 75 hatcheries in the surveillance programme for ISAV HPR0 in 2023.

As the hatcheries were sampled only once in the calendar year and a limited number of tanks were sampled, this result is likely an underestimate of the true prevalence of ISAV HPR0 in Norwegian hatcheries.

Introduction

Infectious salmon anaemia (ISA) is a serious disease in salmon caused by ISA virus (ISAV), a virus within the *Orthomyxoviridae* family. The disease was first described in Atlantic salmon (*Salmo salar*) in Norway in 1984 and has since been reported in several countries (USA, UK, Canada, Faroe Islands and Chile). In Norway, the number of outbreaks peaked in 1990 with 80 cases. In the late 1980s and early 1990s several measures were implemented in order to combat and limit the spread of the disease. Since 1993, the annual number of outbreaks has varied between 1 and 25, and ISA is still a recurring challenge to the salmon farming industry in Norway (Sommerset *et al.*, 2024).

Infection with ISAV is listed by the World Organisation for Animal Health (WOAH), and ISAV HPRdel is notifiable within the EU, including Norway. In Norway, there is a legal obligation to report suspicion of ISA to the Norwegian Food Safety Authority (NFSA), and immediate restrictions on fish movement will be adopted. Following a suspicion, the NFSA performs fish sampling at the suspected site and submits the samples to the National Reference Laboratory for fish diseases, the Norwegian Veterinary Institute (NVI), for diagnostic investigation. If this investigation confirms an ISA diagnosis, the NFSA determines the official diagnosis for the site and makes decisions on the implementation of control measures such as the establishment of a containment area. ISA diagnoses are reported to the EU and the WOAH by the NFSA.

There are two main types of ISAV. The pathogenic type, termed ISAV HPR-deleted (ISAV HPRdel), is associated with ISA outbreaks, while the non-pathogenic type, termed ISAV HPR0, causes subclinical infections only. ISAV HPR0 is regarded as the origin of ISAV HPRdel through a deletion in segment 6 and a mutation or insertion in segment 5. While both types of ISAV are

reportable to the WOAH, ISAV HPR0 is not reported by Norway due to the absence of a notification requirement in the national legislation.

A vaccine containing ISAV HPRdel is commonly used with more than 100 million doses sold in 2023. Information about vaccination status is important when analysing samples from smolt for ISAV, as the vaccine virus may be detected several weeks after vaccination.

The surveillance programme for ISAV HPR0 in Norwegian hatcheries has been conducted since 2019.

Aim

The aim of the surveillance programme is to map the occurrence of ISAV HPR0 in hatcheries with Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) in Norway. Due to the new Animal Health Law in EU, the Norwegian strategy for combating and limiting ISA is currently under review. An overview of the ISAV HPR0-situation in hatcheries is an important part of the knowledge base for the new strategy.

Materials and methods

Hatchery selection was coordinated with the surveillance programme on *Gyrodactylus salaris*, with each hatchery being sampled every second year (50 % of hatcheries sampled per year). Sampling was conducted by the NFSA.

A total of 90 fish were sampled per hatchery. In each hatchery, ten tanks were randomly selected, and gill tissues were collected from nine fish per tank. All tanks in the facility were numbered consecutively, department by department. The total number of tanks were then divided by ten, and this number was used to choose tanks. In some cases, individual assessments had to be done to ensure that all departments were represented. It is important that the selection of tanks follow a formal predetermined procedure to ensure a random selection. Samples were taken from randomly selected, apparently healthy fish from each tank. Tissues from three fish in the same tank were pooled on RNAlater™, giving three samples per tank and 30 samples per hatchery. If the hatchery had less than ten tanks, all tanks were sampled, and the required number of samples were divided by the number of available tanks.

The samples were submitted to the Norwegian Veterinary Institute (NVI) for analysis for ISAV by real-time RT-PCR with primers and probe as described by Snow *et al*. (2006). To differentiate ISAV HPR0 from ISAV HPRdel, ISAV positive samples were further investigated by RT-PCR and sequenced with primers recommended by the World Organisation for Animal Health (WOAH, 2022) to determine the amino acids in the hypervariable region (HPR) of segment 6.

All results were made available to the NFSA through a shared database (EOS). If ISAV was detected, a separate report was sent to the NFSA. In addition, the NVI compiles a yearly report on the data to the NFSA.

Results

In total, 75 hatcheries were sampled. The geographic locations of sampled hatcheries and ISAV screening results are shown in Figure 1.

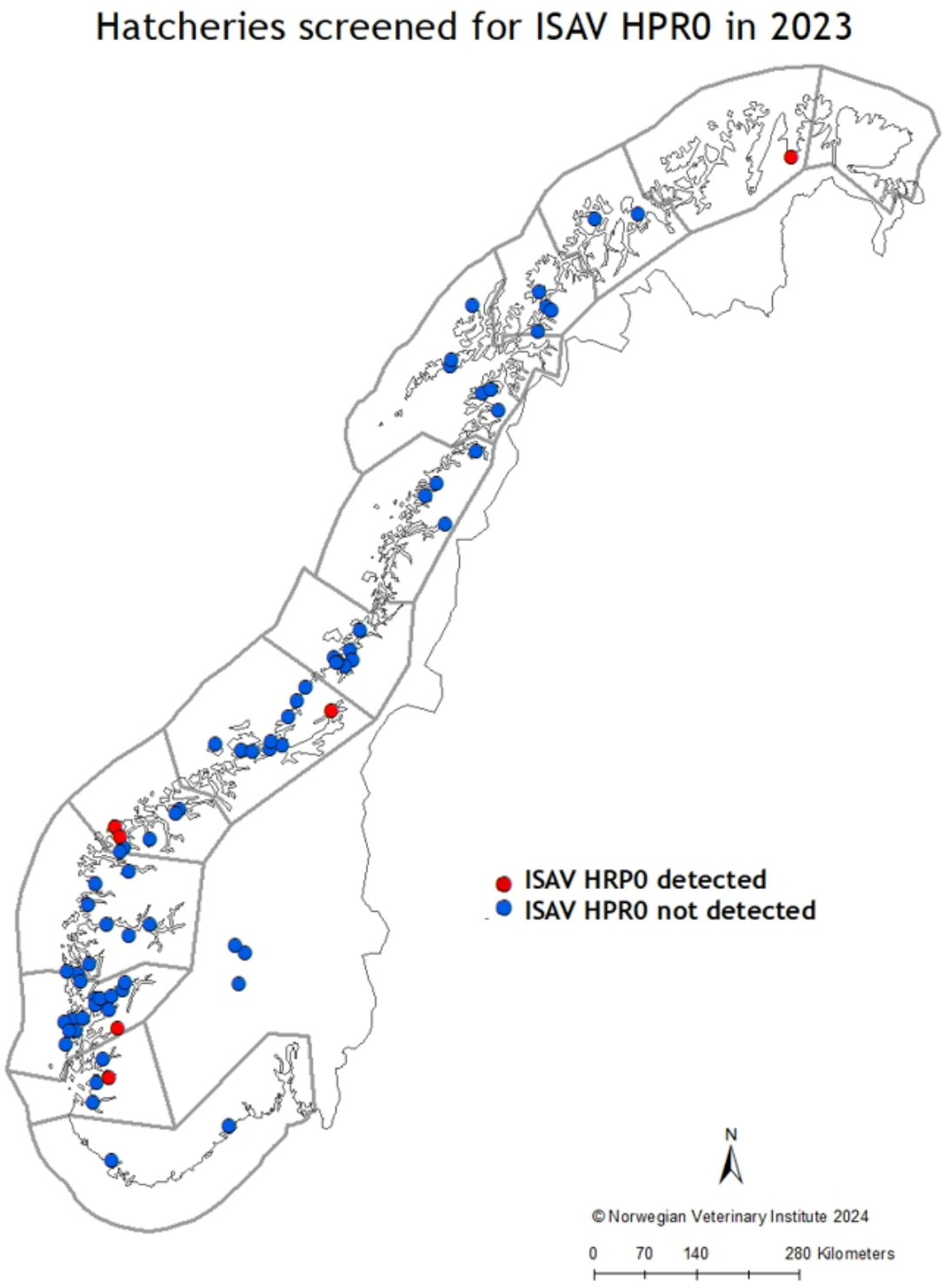

Figure 1: Geographic locations of sampled hatcheries and their ISAV HPR0 screening results

The sampling was performed at sixty-five hatcheries holding Atlantic salmon, seven hatcheries holding rainbow trout and three hatcheries holding both species. No locations with brown trout were sampled. The non-pathogenic variant of ISAV, ISAV HPR0, was detected in five Atlantic salmon hatcheries and one hatchery holding both species (only samples from Atlantic salmon were positive).

An overview of the sampled hatcheries regarding water flow is shown in Table 1, while details of the ISAV HPR0-positive hatcheries are shown in Table 2.

Table 1: Summary of the water flow used by the sampled hatcheries.

Hatchery technology*	Number of sampled hatcheries	Number (%) of HPR0-positive hatcheries
GS only	45	1 (2.2 %)
RAS only	19	2 (10.5 %)
GS and RAS	11	3 (27.3 %)

* GS = flow-through system, RAS = recirculation system.

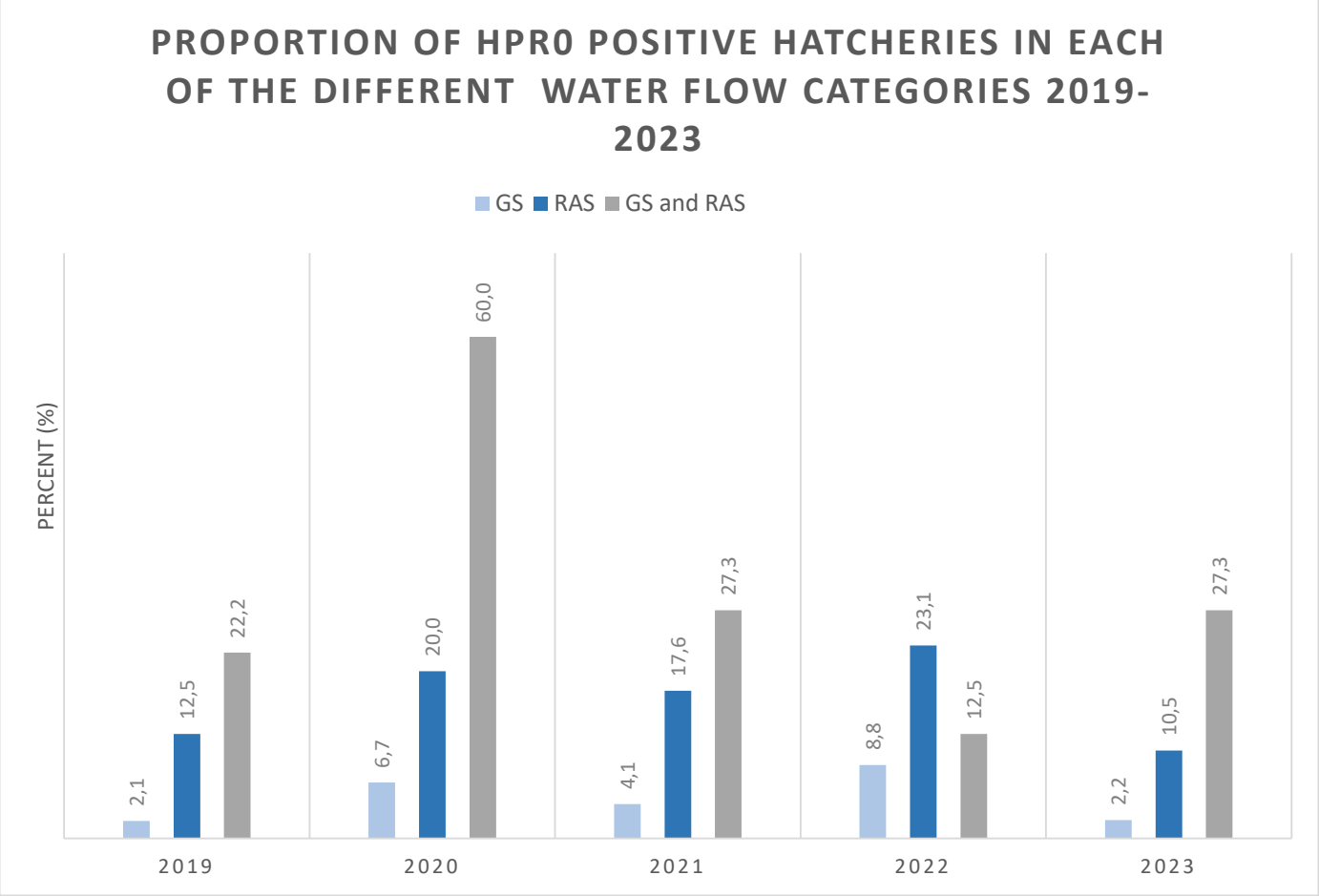

Figure 2: Summary of the water flow used by the sampled hatcheries from 2019 – 2023. More GS facilities than RAS facilities have been sampled (Table1), but the proportion that tests positive is greater in RAS and GS/RAS facilities

Table 2: Summary of data for ISAV HPR0-positive hatcheries and tanks.

Hatchery ID	Hatchery			Positive tanks		
	Technology*	No. tanks sampled	No. positive samples	No. positive tanks	Seawater addition	Average fish weight (g)
A	RAS/GS**	10	1	1	Yes	270
B	RAS	10	5	4	Yes	25
C	RAS	10	16	7	Yes	145
D***	RAS/GS**	10	3	2	No	153
E	GS	10	2	1	Yes	107
F	RAS/GS**	5	15	2	Yes	30

* GS = flow-through system, RAS = recirculation system
** Positive samples in RAS for A and D and in GS for F.
*** Farm D also produces rainbow trout, but samples from these tanks were negative.

Use of seawater (in all or some of the tanks) were registered at 29 locations where ISAV HPR0 was not detected.

Discussion

This report contains the results from the fifth year of the surveillance programme for ISAV HPR0 in Norwegian hatcheries. Eight percent of the hatcheries (six out of 75) tested positive for ISAV HPR0. ISAV HPR0-positive tanks were found in both recirculation- and flow-through systems, and 15 out of 17 of the positive tanks were run with seawater addition at the time of sampling. The hatchery that had ISAV HPR0-positive tanks without seawater addition was contacted in order to obtain information on previous seawater exposure of the sampled fish groups. The ISAV HPR0-positive fish groups had never been exposed to any seawater. As in previous years, more GS facilities than RAS facilities have been sampled (Table 1). However, the proportion of HPR0-positive hatcheries is greater among the RAS facilities and GS/RAS facilities.

The hatcheries were only sampled once in the calendar year, and a limited number of tanks were sampled per hatchery. As a result, it is likely that the results obtained in this surveillance programme is an underestimation of the true annual prevalence of ISAV HPR0 in Norwegian hatcheries. The apparent prevalence of ISAV HPR0 in 2023 (8 %) was relatively similar to that reported in the previous four years of the surveillance programme (7 % in 2019, 14 % in 2020, 10 % in 2021 and 11.5 % in 2022).

Approximately eighty-seven percent (65 out of 75) of the hatcheries sampled in 2023 were also sampled in 2021. Among the six hatcheries that tested positive for ISAV HPR0 in 2023, five were also tested in 2021. Two of these hatcheries tested positive for ISAV HPR0 in both years. Eight hatcheries tested positive for ISAV HPR0 in 2021, and six of these locations where retested in 2023. As already mentioned, two of these hatcheries also tested positive in 2023. The last two locations that tested positive in 2021, were retested in 2022 and were also then positive. Since 2019, a total of six hatcheries have tested positive for ISAV HPR0 twice. Among these, only two hatcheries have been sampled three times so far in the surveillance program. One of these hatcheries was ISAV HPR0 positive in 2019 and 2021, but negative in 2023. The

other hatchery was negative for ISAV HPR0 in 2019, but tested positive both in 2021 and 2023. No hatcheries has tested positive three times so far in the surveillance program.

The use of vaccines containing ISAV HPRdel may give a positive PCR-reaction due to the vaccine strain. This should be kept in mind when detecting ISAV HPRdel in samples from hatcheries.

Several ISAV HPR0-positive hatcheries have in recent years delivered smolt to sea sites with ISA outbreaks shortly after sea transfer, and the ISAV HPRdel at the sea site was found to be identical or closely related to the ISAV HPR0 detected in the respective hatchery based on sequences for segment 5 and segment 6 (Sommerset *et al.*, 2024). This suggests that ISAV HPR0 screening should be an important component of risk management measures in Norwegian hatcheries. The absence of a national overview of ISAV HPR0 detections makes it difficult to study and understand the actual level of risk posed by ISAV HPR0 presence in hatcheries.

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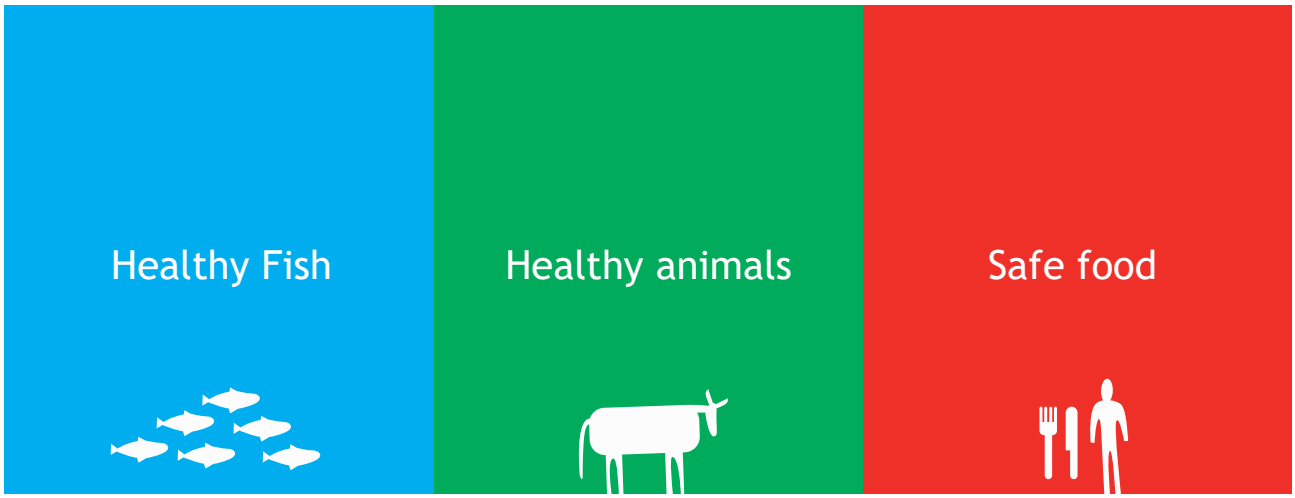

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