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Risk based health monitoring of wild finfish in Norway 2023

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Content

Sammendrag

I 2020 opprettet Veterinærinstituttet i samarbeid med Mattilsynet en portal for varsling av sykdom og dødelighet hos villfisk, den såkalte *syk villfisk portalen*. Hovedmålet med portalen er å forenkle rapporteringen slik at alvorlige sykdomstilfeller hos villfisk raskt blir oppdaget. De innmeldte sakene og det påfølgende diagnostiske arbeidet gir et innblikk i villfiskens helse. I tillegg identifiseres helseproblemer som kan være kandidater for videre oppfølging gjennom rettet overvåking. Varslingsportalen er en integrert del av helseovervåking av villfisk både i ferskvann og i det marine miljø.

Mattilsynets helseovervåkingsprogram for villfisk i 2023 var risikobasert og benyttet i all hovedsak prøvemateriale fra syk villfisk som ble innmeldt via syk villfisk portalen. I tillegg ble det undersøkt materiale fra andre kilder der villfisk hadde vist tegn til sykdom.

I det risikobaserte overvåkingsprogrammet ble det benyttet dyrking på utvalgte cellelinjer samt spesifikke PCR analyser. Virus ble ikke påvist i det undersøkte materialet.

Summary

A national portal for notification of diseases and mortality in wild fish was established in 2020 in cooperation between the Norwegian Veterinary Institute (NVI) and the Norwegian Food Safety Authority (NFSA).The main purpose of this portal is to facilitate early detection of serious diseases in wild fish by simplifying the reporting procedure. The reports themselves and the subsequent diagnostic work provide an insight into the health of wild fish. In addition, health problems that are candidates for further attention through surveillance and monitoring are identified. The notification portal has thus become an integral part of health monitoring of wild fish both in freshwater and in the marine environment.

In 2023, the notification portal was linked to the NFSA health monitoring program in order to investigate the presence of cultivable viruses in wild fish. Accordingly, samples were mainly collected during disease outbreaks in wild populations, but also from individual fish showing adverse behaviour or other signs of ill health.

No viral agents were detected by cultivation in selected cell lines, nor by specific qPCR.

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 (hereafter called the NFSA health monitoring programme). During the period from 2012 to 2021, NVI coordinated the programme in freshwater.

In 2020, NVI, in agreement with NFSA, launched a national portal for notification of diseases and mortality in wild fish, the so called *wild fish health portal*. The main purpose of the portal was to facilitate early detection of serious diseases in wild fish by simplifying the reporting procedure. The system also provides a general insight into the health of wild fish and highlight health problems that are candidates for further attention through surveillance and monitoring. The *wild fish health portal* is now an integral part of health monitoring of wild fish both in freshwater and in the marine environment.

In 2023, the NFSA health monitoring programme was linked directly to material provided by the *wild fish health portal*. The program investigates the presence of fish pathogenic cultivable viruses in dead and moribund wild fish reported to the system. Accordingly, samples were mainly collected during disease outbreaks in wild populations, but also from individual fish showing adverse behaviour or other signs of ill health.

Results are published in annual reports available on
https://www.vetinst.no/overvaking/health-monitoring-of-wild-fish

Aim

In 2023, the aim of the health monitoring programme was to investigate the presence of cultivable viruses in moribund wild fish.

Materials and methods

Materials

The risk based health monitoring programme comprised wild fish that due to clinical disease and mortality were submitted to NVI for examination. The majority of the fish were reported as dead or moribund in the wild fish health portal, and was collected from Finnmark County in the north all the way to Viken County in the south east near the border to Sweden as shown in Figure 1. The majority of the sampled fish, 89 %, were from salmonids, with Atlantic salmon (*Salmo salar*) as the dominating species. Smaller numbers of sea trout and brown trout (*Salmo trutta*) and pink salmon (*Oncorhynchus gorbuscha*) were also included. In addition, samples from European perch (*Perca fluviatilis*), common bream (*Abramis brama*) and Atlantic cod (*Gadus morhua*) were collected. An overview of investigated cases (species, location and main findings) included in the programme is listed in Appendix 1a-c.

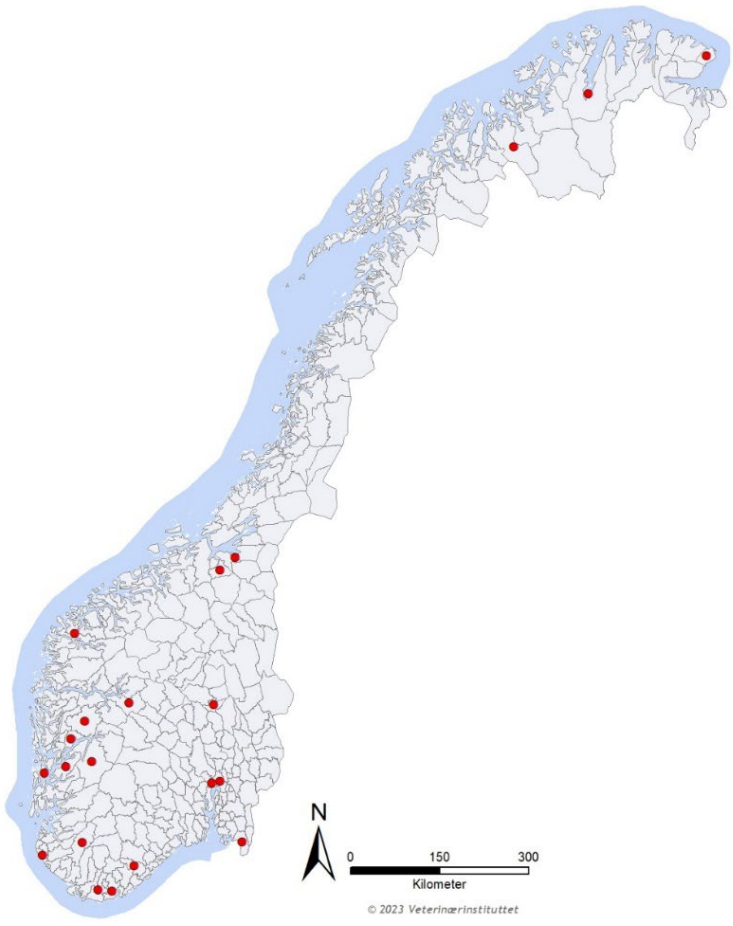


***Figure 1**. Map showing an overview of Norwegian municipalities with red dots indicating cases included in the 2023 risk based surveillance program for wild finfish (Ill.: Attila Tarpai).*

Virological examination

Finfish included are subject to gross pathological examination, histopathology, cultivation for bacteria, qPCR and immunohistochemistry, which together increases the probability of discovering infectious diseases. The virological examination is based on isolation of viruses in cell cultures and PCR.

Isolation of virus in cell culture

Tissue samples were investigated for the presence of virus by inoculation on a panel of cell lines according to the procedure described by the World Organization for Animal Health (WOAH) with minor modifications. The selected cell lines were bluegill fry cells (BF-2), epithelioma papulosum cyprini cells (EPC), Chum salmon heart cells (CHH-1) and Atlantic salmon gill cells (ASG-10). An in-house established epithelial cell line from Atlantic salmon skin was also used. The cell lines were selected partly based on their known susceptibility to a large number of viruses found in fish, and partly to represent various cell types and organs, with the purpose to increase the likelihood for detecting viruses in the tissue.

Samples from anterior kidney and/or myocardium and/or spleen were removed from the fish with sterile dissection tools and transferred to 30 ml Steriline® plastic tubes containing transport medium provided by NVI. If indicated by skin or gill lesions, samples from gills and skin were deposited in a separate tube with transport medium.

Tissue samples were transported frozen or on ice to NVI where they were homogenized, 1:10 (w/v), in transport medium. The homogenates were then clarified by centrifugation (2500 rounds per minute/ rpm), at 5℃. Aliquots of the resulting supernatants were transferred to Eppendorf tubes, then stored at -80 ℃ until inoculation.

One hundred and fifty (150) microliters of tissue homogenate supernatant was inoculated onto BF-2, EPC, CHH-1 and ASG-10 cells in a 24 well cell culture plate. In addition, supernatant from skin and gills samples were inoculated onto Atlantic salmon skin cells in 96 well cell culture plates. Inoculated cells were incubated at 15°C and examined for cytopathic effect (CPE) or possible toxicity. At 14 days post inoculation (dpi) the first passage (1p) cultures were subjected to one freeze/thaw cycle. Two passages were used and the total incubation period was four weeks per sample.

Polymerase chain reaction (PCR or qPCR) analyses

Tissue samples (anterior kidney, myocardium, spleen and gill) were removed from the fish with sterile dissection tools and transferred to 1.5 ml plastic Eppendorf tubes with 1 ml DNA/RNA stabilising reagent (RNAlater™) according to the manufacturer's recommendation.

In cases with high mortality or when indicated by pathological findings, samples were analysed with specific qPCR.

Results and discussion

Results
Cytopathogenic or toxic effect in cell cultures were not observed for any of the inoculated samples.
All qPCR-analyses for infectious hematopoietic necrosis virus (IHNV), viral haemorrhagic septicaemia virus (VHSV), infectious salmon anaemia virus (ISAV), salmonid alphavirus (SAV), cyprinid herpesvirus and piscine orthoreovirus (PRV-1) were negative for all samples analysed.

In conclusion, all investigations for viruses were negative.

Risk based surveillance
By examining moribund and dead fish the probability of discovering both known and hitherto unknown and undescribed cultivable fish pathogens increases. The program is based on cultivation for viruses, but is also assisted by gross pathological examination, histopathology, cultivation for bacteria, qPCR and immunohistochemistry, which together increases the probability of discovering infectious diseases. The risk based programme thus aims at being an important contributor to anticipatory preparedness, i.e. anticipating tomorrow's health problems in wild and farmed fish.

Virus infections
It is important to identify possible presence of severe listed viral diseases early in the process of a disease investigation. Specific qPCRs for infectious haematopoietic necrosis virus (IHNV) and viral haemorrhagic septicaemia virus (VHSV) were therefore used whenever indicated by high mortality or pathological findings. Specific qPCR for other viruses were also used when indicated by histopathological findings.

Cultivation on cell lines have limitations. The technique can only detect viable and cultivable viruses. Accordingly, viruses that are not cultivable, such as piscine orthoreovirus (PRV), piscine myocarditis virus (PMCV) and salmon gill poxvirus (SGPV) will not be detected in these cultivation analyses. In addition, sub-optimal handling of the samples prior to inoculation can also reduce virus viability giving false negative results.

Acknowledgements

The authors would like to thank every person and institution that were involved in providing wild fish for virus investigation. The authors also thank all pathologists and technical staff at the Norwegian Veterinary Institute for performing virus investigation with excellence.

References

1. Garseth, Åse Helen, Anne Berit Olsen, Britt Gjerset and Hilde Sindre (2023). Risk based health monitoring of wild finfish in Norway 2022. Surveillance program report. Norwegian Veterinary Institute report 10_2023

Appendix 1a Salmonids

*Appendix 1a displays a by county overview of diagnostic cases of salmonids included in the risk based surveillance program in 2023. Species include Atlantic salmon (Salmo salar), trout (Salmo trutta) and pink salmon (Oncorhynchus gorbuscha). *Samples from diagnostic cases from Q4 2022 were cultivated on cell-lines in 2023 and included in this years program. ¥ PCR analyses performed in the 2022 program (1).*

County	Location	Species	Fish	Virus samples	qPCR	Diagnosis	Comments
Troms & Finnmark	Komagelva	Pink salmon	8	8	40[π]	Diverse signs, parasitism, enlarged spleen, *Carnobacterium maltaromaticum*	July 2023. [π] Pink salmon health monitoring program including PCR analyses for infectious salmon anaemia virus, piscine orthoreovirus -1 (PRV-1) and *Renibacterium salmoninarum*. PCR analyses for IHNV/VHSV conducted for the NFSA IHN and VHS surveillance program.
	Lakselv	Pink salmon	1	2	5[π]	Yellow discoloration. Growth of diverse bacteria	
	Reisaelva	Brown trout	4	4	0	Bacterial sepsis	July 2023
Trøndelag	Homla, Malvik	Atlantic salmon	13	12*	0	*Saprolegnia parasitica*	October 2022. High mortality. Mixed secondary bacterial infections
	Gaula, Melhus	Atlantic salmon	1	1	0	Not defined, Parasites, tape worm	June 2023
	Stjørdalsvassdraget	Atlantic salmon	2	0	8	Inflammation in heart and skeletal muscle	August 2023

County	Location	Species	Fish	Virus samples	qPCR	Diagnosis	Comments
Vestland	Lærdal	Atlantic salmon	3	5	12	Saprolegnia, *Aeromonas hydrophila*,	August 2023, PCR analyses specific for ISAV, IHNV, VHSV, SAV
	Vosso	Atlantic salmon	5	5	0	Fin rot and cestoda	May 2023
	Vosso	Atlantic salmon	10	10	0	Fin rot and cestoda	January 2023
	Vosso	Brown trout	1	1	0	Fin rot and cestoda	January 2023
	Jondal	Sea trout	2	4	0	Myxozoa, pale gills, heart.	November 2022, Wild broodfish in genebank for wild salmon
	Strandadal	Sea trout	1	1	0	Myositis	November 2022, Wild broodfish in genebank for wild salmon
	Ådland	Sea trout	1	2	0	Parsites	November 2022, Wild broodfish in genebank for wild salmon
	Rosendal	Atlantic salmon	1	2	0	Parasites, myxozoa, cestoda	November 2022, Wild broodfish in genebank for wild salmon
Rogaland	Nordre-Varhaugelv, Hå Kommune	Atlantic salmon	4	5*	8¥	Saprolegniosis *Saprolegnia parasitica*	November 2022. High mortality. *A. hydrophila* mixed with *Lactococcus* sp. one fish. *Lactococcus* sp. one fish.

County	Location	Species	Fish	Virus samples	qPCR	Diagnosis	Comments
Agder	Tovdalselva, Kristiansand	Atlantic salmon	3	6	0	Aeromonas hydrophila sepsis Skin lesions	July 2023
	Mandalselva Lindesnes	Atlantic salmon	3	5	0	Saprolegniosis *Saprolegnia parasitica*	June 2023. Mixed secondary bacterial infections, Lactococcus, motile *Aeromonas* sp: *Yersinia ruckeri O1/not CC1*
	Mandalselva Lindesnes	Atlantic salmon	2	2*	4¥	Saprolegniosis *Saprolegnia parasitica*	November 2022. Mixed secondary bacterial infections, *Aeromonas* sp: *Yersinia intermedia.*
	Mandalselva Lindesnes	Atlantic salmon	1	2*	2¥	Saprolegniosis	November 2022. Mixed secondary bacterial infections, *Aeromonas* sp: *Yersinia intermedia.*
	Mandalselva Lindesnes	Brown trout	1	3*	2¥	Saprolegniosis	November 2022. Mixed secondary bacterial infections, *Aeromonas* sp: *Yersinia intermedia.*
	Sirdalsvatnet Sirdal	Brown trout	5	5*	20¥	Unknown	December 2022 Bacterial septicaemia and skin infections (including flavobacteriosis based on IHC)
Viken	Enningdalselva, Halden	Atlantic salmon	2	4	0	Red skin disease	May 2023 Red skin disease outbreak
	Aln, Oslo	Brown trout	1	2	0	Dermatitis	February 2023 Viral disease not detected
	Sandvikselva Bærum	Atlantic salmon	5	5*	0	*Saprolegnia parasitica*	October 2022 Mixed secondary bacterial infections.
	Fornebu, Bærum	Atlantic salmon	1	2*	0	Unknown	October 2022 Viral and bacterial disease not detected

Appendix 1b Non-salmonid freshwater and diadromous fish

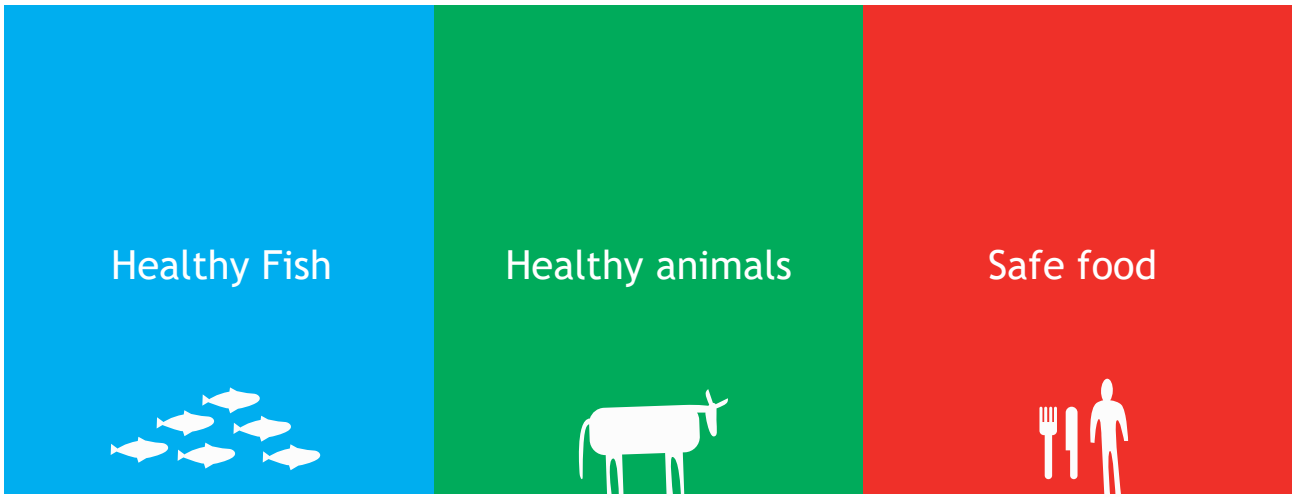
*Appendix 1b displays a by county overview of diagnostic cases from non-salmonid freshwater and diadromous fish included in the risk based surveillance program in 2023. Species include common bream (Abramis brama) and European perch (Perca fluviatilis). *Samples from diagnostic cases from Q4 2022 are cultivated on cell-lines in 2023*

County	Location	Species	Fish	# Virus samples	# qPCR	Diagnosis	Comments
Agder	Rosævatnet, Froland	European perch	3	6	0	Emaciation	October 2022 Wild-caught fish kept in tanks, research
Innlandet	Lågendeltaet/ Mjøsa, Lillehammer	Common bream	6	12	12	Neoplasia, hyperplastic/ hypertrophic skin lesions	June 2023 Wild fish observed and sampled by researchers at NMBU/NINA Lillehammer

Appendix 1c Marine fish

*Appendix 1c displays a by county overview of diagnostic cases from marine fish that were included in the risk based surveillance program in 2023. Species include Atlantic cod (Gadus morhua). *Samples from diagnostic cases from Q4 2022 are cultivated on cell-lines in 2023, ¥PCR analyses performed in the 2022 program (1).*

County	Location	Species	Fish	Virus samples	qPCR	Primary diagnosis	Comments
Vestland	Eidfjorden, Stad	Atlantic cod	1	1*	1¥	Mycobacteriosis *Mycobacterium salmoniphilum*	March 2022, Brain samples included in 2023 program.



Scientifically ambitious, forward-looking and collaborative- for one health!

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