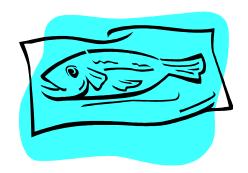


Sampling plan

Surveillance program with focus on certain fish diseases involved in disease-free status of Iceland

2024



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SURVEILLANCE PROGRAM WITH FOCUS ON CERTAIN FISH DISEASES INVOLVED IN DISEASE-FREE STATUS OF ICELAND



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AQUATIC ANIMALS

Disease-free statuses for Iceland

Pursuant to Article 280(1) of Regulation (EU) 2016/429 and Article 84(1) of Delegated Regulation (EU) 2020/689, the before existing disease-free statuses for Iceland were deemed approved in accordance with those Regulations.

The following disease-free statuses are currently in force in **Icelandic aquaculture**:

• IHN disease-free status; the whole territory of Iceland:

As first adopted by the EFTA Surveillance Authority's in College Decision No. 227/04/COL, dated 9 September 2004 and confirmed again under College Decision No. 036/16/COL, dated 9 February 2016.

• VHS disease-free status; certain compartments in Iceland:

MAST's application and Declaration of a VHS disease-free status of indipendent compartments in accordance with Council Directive No. 2006/88 was sent to the Authority on 25 November 2015 and entered into force on 2 May 2016.

The declared VHS-free indipendent compartments are the following three farming sites belonging to the Atlantic salmon breeding farm of Benchmark Genetics Iceland hf.: Vogavík, Kalmanstjörn and Kollafjörður.

• ISA (HPR-deleted) disease-free status; certain compartments in Iceland:

MAST's application and Declaration of an ISA disease-free status of indipendent compartments in accordance with Council Dir. 2006/88 was sent to the Authority on 22 March 2013 and entered into force on 7 July 2013.

The declared ISA-free (HPR-deleted) indipendent compartments are the following three farming sites belonging to the Atlantic salmon breeding farm of Benchmark Genetics Iceland hf.: **Vogavík**, **Kalmanstjörn** and **Kollafjörður**.



The aim of the control program

The purpose of the official surveillance program is to document the absence of IHN in the entire territory of Iceland and to maintain a status free from VHS and from infection with HPR-deleted ISAV in certain indipendent compartments of salmon broodfish farms. Some countries within the EEA (or parts thereof) have been given approval for national measures for Bacterial kidney disease (BKD), in accordance with the Commission Implementing Decision (EU) 2021/260. Targeted surveillance with regular sampling for BKD is an absolute demand for the farming sites within the ISA-free compartments that want to export to countries with these national measures for BKD.

Introduction - materials & methods -

All Icelandic fish farms have been included in the official national health control program since 1985. Screening of important virus and bacterial agents causing serious infectious diseases like Infectious salmon anaemia (ISA), Viral haemorrhagic septicaemia (VHS), Infectious haematopoietic necrosis (IHN) and Bacterial kidney disease (BKD) have been a big part of the surveillance program since the very beginning. The official surveillance also includes farms raising wild salmonids for restocking of rivers and lakes. This surveillance program has a riskbased approach, but the main focus is put on the broodfish farms. Infections which can be latent and diseases which do not have clear clinical symptoms are monitored by routine sampling. From 1993 European Union (EU) Directives on disease control measures have been followed. The surveillance is partly by regular "on-site" health inspections, under the supervision of the Veterinary Officer for Aquatic Animals, and partly by laboratory work conducted at the National Reference Fish Disease Laboratory at Keldur in Reykjavík (NRL). The NRL in Reykjavík has a close inter-laboratory collaboration with the EU Reference Laboratory on virus diseases in Denmark. In addition, a big part of the diagnostic services is performed by the Food and Veterinary Authority, Department of Fish and Animal Diseases in Torshavn, Faroe Islands and to some extent also by PatoGen Analyse A/S in Norway. All used laboratories are approved and accredited by an ILAC accredited agency to ISO 17025.

The sampling and diagnostic methods regarding viral examination are carried out along the lines given in the Commission Delegated Regulation (EU) N° 2020/689, including relevant amendments. Until spring 2009 the diagnostic methods were mainly based on culture on EPC, BF-2 and CHSE-214 cell-lines in the routine screening, in addition with clinical signs, gross pathology and histopathological examination of vital organs. An ELISA method has been used for the detection of BKD (*Renibacterium salmoninarum*) since 1991, with indirect fluorescent antibody test (IFAT) and/or qPCR methodology for confirmation. Since 2009 more and more routine samplings from farmed and wild fish are analysed by qPCR technique.



Monitoring of certain fish diseases by systemic sampling of farmed and wild fish population in Iceland

Disease-free status from Infectious haematopoietic necrosis (IHN)

Territory:	Period, date of change of status	
101110131	and/or other comment:	
Whole territory of Iceland	As first adopted by the EFTA Surveillance Authority's in College Decision No. 227/04/COL , on 9 September 2004 and confirmed again under College Decision No. 036/16/COL , on 9 February 2016.	

Infectious haematopoietic necrosis (IHN)

IHN has never been detected in Iceland. It is a notifiable disease, according to the Icelandic Act no. 25/1993 *on animal diseases and measures to control them*. Routine targeted samplings have been performed since 1985, both from wild and farmed salmonids. Iceland obtained formally disease-free status for IHN by the fish health authority of the European Union in 2004. Since 2017, IHN samples have also been analyzed by RT-qPCR, in addition to culture on cell-lines (Table 1 and 2a).

Sampling plan for 2024:

500 samples tested by qPCR and 1.000 samples examined with culturing on cell-lines.

Disease-free status from Viral haemorrhagic septicaemia (VHS)

Territory:	Period, date of change of status and/or other comment:
Compartments	MAST's Declaration of a VHS disease-free status of compartments in accordance with Council Directive No. 2006/88 was sent to the Authority on 25 November 2015 and entered
The declared VHS-free indipendent compartments are the following three farming sites	into force on 2 May 2016.
belonging to the Atlantic salmon breeding farm of Benchmark Genetics Iceland hf.: Vogavík, Kalmanstjörn and Kollafjörður.	Pursuant to Article 280(1) of Regulation (EU) 2016/429 and Article 84(1) of Delegated Regulation (EU) 2020/689, the before existing disease-free statuses for Iceland were deemed
	approved in accordance with those Regulations.



Viral haemorrhagic septicaemia (VHS)

VHS is a notifiable disease, according to the Icelandic Act no. 25/1993 on animal diseases and measures to control them. Routine targeted samplings have been performed since 1985. VHS-virus was for the first time detected in lumpfish of wild origin in Iceland in October 2015. The detection was made in a marine research farm owned by the Icelandic state and had no connection to the salmonid aquaculture. The lumpfish VHS-virus was sequenced by the European Reference Laboratory for Fish Diseases in Denmark and blasted towards other known genotypes. The results showed a totally new appearance of VHSV subtype, most likely a highly host specific and a unique variant for lumpfish.

Iceland obtained formally disease-free status for VHS by the fish health authority of the European Union in 2004. Following the virus detection in the wild lumpfish in 2015 the disease-free status was temporarily suspended. After stamping out of the lumpfish in the respective landbased research farm, Icelandic authorities started up with a new process of achieving VHS-free status. At that time, the authority decided to declare the three indipendent compartments of the broodfish farms breeding Atlantic salmon in the country. This recognition was confirmed on 2 May 2016. Since 2017, VHS samples have also been analyzed by qPCR, in addition to culture on cell-lines (Table 1 and 2b).

Sampling plan for 2024:

1.000 samples tested by qPCR and 1.000 samples examined with culturing on cell-lines.

Table 1. *No. of samples analyzed for IHNV and VHSV since 1985 (cell-culture)*:

	Number of	Number of	Number of
Year	<u>individuals</u>	<u>farms</u>	<u>positive</u>
	sampled	sampled	<u>farms</u>
1985	1.214	-	0
1986	5.591	-	0
1987	9.121	-	0
1988	10.503	-	0
1989	4.854	-	0
1990	6.831	-	0
1991	5.603	-	0
1992	2.763	-	0
1993	949	-	0
1994	610	16	0
1995	775	18	0
1996	601	17	0
1997	945	21	0
1998	806	19	0
1999	860	17	0
2000	696	15	0
2001	706	15	0
2002	533	12	0
2003	885	13	0
2004	1.109	16	0
2005	725	13	0
2006	524	13	0
2007	669	16	0
2008	812	15	0



2009	963	15	0
2010	1.220	13	0
2011	310	12	0
2012	335	12	0
2013	394	12	0
2014	432	12	0
2015	753	13	1*
2016	1.155	12	0
2017	1.127	13	0
2018	966	12	0
2019	1.178	13	0
2020	1.509	11	0
2021	1.046	13	0
2022	935	12	0
2023	1.002	11	0

^{*} VHS-virus positive **lumpfish of wild origin** in 1 farm.

Diagnostic method: EPC and BF-2 cell-lines are used routinely.

Laboratory: Institute for Experimental Pathology at Keldur in Reykjavik, Iceland.

Table 2a. *Number of samples analyzed for IHNV (qPCR)*:

Year	Number of <u>individuals</u> sampled	Number of <u>farms</u> sampled	Number of <u>negative</u> samples	Number of positive samples
2017	22	2	22	0
2018	636	3	636	0
2019	228	3	228	0
2020	481	3	481	0
2021	209	3	209	0
2022	483	6	483	0
2023	583	4	583	0

Diagnostic method: qPCR.

Laboratory: Institute for Experimental Pathology at Keldur in Reykjavik, Iceland and Food & Veterinary Agency, Department of Fish & Animal Diseases in the Faroe Islands.

Table 2b. *N*° *of samples from VHS-free compartments analyzed for VHSV (qPCR)*:

Year	Number of <u>individuals</u> sampled	Number of <u>farms</u> sampled	Number of <u>negative</u> samples	Number of positive samples
2017	206	3	206	0
2018	743	3	743	0
2019	360	3	360	0
2020	666	3	666	0
2021	270	3	270	0
2022	459	3	459	0
2023	572	3	572	0

Diagnostic method: qPCR.

Laboratory: Institute for Experimental Pathology at Keldur in Reykjavik, Iceland and Food & Veterinary Agency, Department of Fish & Animal Diseases in the Faroe Islands.



Disease-free status from infection with highly polymorphic region deleted Infectious salmon anaemia virus (HPR-deleted ISAV)

Territory:	Period, date of change of status and/or other comment:
Compartments	MAST's Declaration of an ISA (HPR-del) disease-free status of compartments in accordance with Council Dir. 2006/88 was
The declared ISA-free (ISAV HPR-deleted) indipendent compartments are the following	sent to the Authority on 22 March 2013 and entered into force on 7 July 2013.
three farming sites belonging to the Atlantic salmon breeding farm of Benchmark Genetics Iceland hf.: Vogavík, Kalmanstjörn and	Pursuant to Article 280(1) of Regulation (EU) 2016/429 and Article 84(1) of Delegated Regulation (EU) 2020/689, the
Kollafjörður.	before existing disease-free statuses for Iceland were deemed approved in accordance with those Regulations.

Infectious salmon anaemia (ISA)

Infection with ISAV is a WOAH/OIE listed infection and ISAV HPR-del is notifiable within the EU and also according to the Icelandic Act no. 25/1993 on animal diseases and measures to control them. There are two main types of ISAV; one virulent type with varying pathogenicity associated with ISA outbreaks termed HPR-deleted ISAV (ISAV HPR-del) and the other type regarded as non-pathogenic, causing subclinical infections, termed ISAV HPRO. Positive testing for ISAV HPRO is not notifiable according to the EU legislation. The disease was described for the first time in Atlantic salmon (*Salmo salar*) in Norway in 1984 and has since been reported in several countries: UK, USA, Canada, Faroe Island, Chile and now also in Iceland.

ISA (HPR-del) was for the first time detected in a sea-cage farm on the east coast of Iceland in late Nov. 2021. The second outbreak occurred within the same company, but in a neighboring fjord system in May 2022. Infection tracing and sequencing of the virus revealed that the first outbreak was so-called primary outbreak, due to a mutation of a local non-pathogen ISAV-HPRO variant. The virus isolates from the second outbreak were identical to previously sequenced isolates from the first outbreak.

The location of the sea-cage farming sites on the east coast is far away from the landbased and closed farm sites belonging to Benchmark Genetics Iceland and have nothing to do with Benchmark broodfish sites. There are no contacts or relationship between those two companies. The broodfish sites of Benchmark are located close to the capital city of Reykjavík on the southwest of Iceland, approx. 700 km away from the ISAV detection on the east coast of Iceland (see map on page 10). The salmon in the affected area was stamped out and for security reasons the fish in the whole fjord system were slaughtered and the area put on at least 90 days fallowing period.

Benchmark Genetics Iceland hf. is the only broodfish farm of origin which is approved for exporting live Atlantic salmon eyed eggs from Iceland. The farm has a history of being free of notifiable virus diseases since founded in 1991. But, with intensive viral screening of the broodstock some few samples have been found to be positive for the low/non-pathogen ISAV (HPR0). It must be emphasized that the õIcelandic strainö of the HPRO variant has never been



detected by sequential examination in other countries, despite extensive export of eyed salmon eggs throughout the world since 1996. However, all eggs and milt from these parents are being destroyed by an official veterinarian for security reasons. In this context, it is worth emphasizing that Benchmark Genetics Iceland's broodstock sites are being visited almost weekly all year around by a fish health veterinarian in connection with the stripping which gives a unique possibility to monitor the development of fish health in the core establishments distributing eggs to domestic and foreign companies. That close contact to the fish, opening almost every single male and female, gives the authority valuable information about the general health situation at any time.

Sampling plan for 2024:

11.000 samples tested by qPCR.

Table 3. Number of samples from the three indipendent compartments of Benchmark Genetics Iceland analyzed for **ISAV** the last 13 years:

	Number of	Number of	Number of
Year	<u>individuals</u>	HPR0 positive	HPR-deleted
	sampled	samples*	positive samples
2011	8.206	67*	0
2012	8.230	52*	0
2013	10.777	118*	0
2014	10.310	60*	0
2015	13.566	49*	0
2016	12.794	39*	0
2017	12.396	31*	0
2018	10.246	55*	0
2019	6.793	12*	0
2020	6.491	4*	0
2021	7.681	21*	0
2022	11.722	25*	0
2023	12.063	9*	0

^{*} Low/non-pathogen ISAv (HPR0).

Diagnostic method: qPCR.

Laboratory: Institute for Experimental Pathology at Keldur in Reykjavik, Iceland and Food & Veterinary Agency, Department of Fish & Animal Diseases in the Faroe Islands.



Bacterial kidney disease (BKD)

Bacterial kidney disease, caused by *Renibacterium salmoninarum*, has a sporadic occurrence in Iceland since first diagnosed in 1968. It is a notifiable disease, according to Act no. 25/1993 *on animal diseases and measures to control them*. Routine samplings have been performed since 1985. All Atlantic salmon breeding farms in Iceland have been BKD-free since founded. Breeding farms have, in conjunction with the surveillance in ISA-free compartments, also performed intensive targeted surveillance for *R. salmoninarum*. Some countries within the EEA have been given approval for national measures for BKD by the fish health authority of the European Union. In order to export salmon eggs to these countries, targeted surveillance with regular sampling for BKD must be conducted in an ISA-free compartment/zone.

Sampling plan for 2024:

Atlantic salmon in breeding farms: 3.000 samples tested by qPCR and 200 by ELISA.

Table 4. Number of samples from the three indipendent compartments of Benchmark Genetics *Iceland analyzed for BKD* the last 13 years:

Year	Number of individuals	Number of farms	Number of positive
	sampled	sampled	<u>samples</u>
2011	1.447	3	0
2012	820	3	0
2013	923	3	0
2014	1.446	3	0
2015	1.609	3	0
2016	702	3	0
2017	2.336	3	0
2018	2.114	3	0
2019	2.612	3	0
2020	2.769	3	0
2021	2.199	3	0
2022	3.322	3	0
2023	4.063	3	0

Diagnostic method: ELISA (enzyme-linked immunosorbent assay) and qPCR.

Laboratory: Institute for Experimental Pathology at Keldur in Reykjavik, Iceland and Food & Veterinary Agency, Department of Fish & Animal Diseases in the Faroe Islands.



