[Media Backgrounder: Norway's Infectious Salmon Aquacalypse – Going Global Since 1984](#) (August 2021)

Intrafish reported in August 2021:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

Grieg Seafood to cull 1 million salmon, delay stocking Eastern Canada farms after ISA discovery

The company made the decision to delay stocking in its Newfoundland operations out of a 'precautionary approach' after the virus discovery, but says its 2025 harvesting target remains unchanged.

3 August 2021 14:58 GMT UPDATED 4 August 2021 18:54 GMT

By Dominic Welling and Drew Cherry

Grieg Seafood Newfoundland on Tuesday said it will cull one million fish and postpone the first transfer of fish to sea until Spring 2022 after finding a positive sample of infectious salmon anemia (ISA) at its smolt site.

The decision is taken based on a precautionary approach and to reduce risk, the company said, noting that it plans to develop salmon farming operations in Placentia Bay "gradually and responsibly."

During a routine sampling of the fish scheduled to transfer to sea from the Marystown facility this summer, one fish provided a suspect detection of ISA.

Scottish Sea Farms acquires Grieg Seafood Hjaltland UK

Read more

Over the last few weeks, 295 additional samples have been collected and analyzed by Provincial Veterinary Authorities, said Grieg, and all samples have provided negative results and no ISA was detected.

However, Placentia Bay is a promising area for salmon farming with no known history of ISA so Grieg Seafood Newfoundland will not risk introducing the virus into the environment, it said.

As a result, almost one million fish that was scheduled for sea transfer this summer, will be culled, the company announced.

All of these fish are in the same recirculating aquaculture system (RAS) as the one fish with the detection, and the company "would not have been able to maintain its fish health and welfare standard in sea should the virus exist in this fish group," it said.

A thorough review has been initiated to find out why an ISA detection occurred, and measures will subsequently be put in place to avoid similar occurrences in the future, said Grieg.

Virus found in British Columbia wild salmon can be traced back to Norwegian farms, new study finds

Read more

The financial impact of the postponement is minor, the company said, as the first group of fish had few individuals compared to regular operations.

During the spring and summer of 2022, around 3 million fish are due to be transferred to sea, in accordance with the original schedule.

These eggs and fish are currently growing well in a separate building in the Marystown facility. The fish will be harvested in 2023 and 2024.

RELATED NEWS

'Their story needs to be told': Executive behind Grieg's Skuna Bay sees gold in California trout farmer
Markets
18 July 2021 12:53 GMT

European Commission says price-fixing lawsuit against farmed salmon giants is compromising its investigation
Legal
18 July 2021 5:00 GMT

Cermaq pleads again with Canadian government for crucial salmon transfer approval
Legal
9 July 2021 20:21 GMT

Canada denies Cermaq's fish transfer request; now fish farmer will likely have to kill 1.5 million salmon
Legal
8 July 2021 18:02 GMT

Ranking: Salmon farming companies' earnings per kilo plummet in first quarter
Analysis
21 May 2021 8:31 GMT

[Note that Scottish Sea Farms announced in July 2021 that it was buying Grieg Seafood's Scottish salmon farms]

Fish Farmer reported in July 2021:

Fish Farmer

For all the latest industry news, markets and jobs in aquaculture

News

ISA cases continue to mount

By Vince McDonagh - 20th July 2021

Two more suspected cases and one confirmed outbreak of infectious salmon anaemia (ISA) have been discovered on the north Norwegian coast.

The first suspected is at sea site 31757 at Kasterberget in Ibestad, in the Troms and Finnmark municipality where the company Kleiva Fiskefarm is engaged in salmon production.

The second is at the Stabben sea site 34297 in Tjeldesund municipality, also in Troms and Finnmark, where Ellingsen Seafood farms salmon.

In both instances the ISA suspicion was based on the results of PCR tests carried out on samples of fish. The Norwegian Food Safety Authority said it will carry out its own follow up tests to see if the suspicions are correct.

If ISA is confirmed it could order the companies to empty the sites in a move which normally proves costly for salmon companies.

The proven outbreak is at a hatchery in Salangen municipality, again in the Troms and Finnmark region which appears to have suffered more cases than in any other part of the country. Salangfisk AS farms on the site.

A suspected outbreak was first reported on 5 July and the food safety authority took its own samples four days later. It has now confirmed ISA.

The usual restrictions have been put in place to limit spread of the disease including the implementation of a control zone and a ban on the movement of fish. The authority said it may order the site to be emptied.

In the past 10 days, eight suspected or confirmed cases of the disease have been reported to the authority.

Intrafish reported in June 2021:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

ISA taking a bit out of Norway salmon prices

An abundance of smaller fish and quiet summer demand has put continued downward pressure on prices.

25 June 2021 13:01 GMT *UPDATED 25 June 2021 13:01 GMT*

By Robert Nedrejord and Marthe Njåstad in Bergen

Norwegian salmon prices have continued to soften amid an abundance of fish harvested and processed early as a result of the spread of infectious salmon anemia ISA in some Norwegian farms.

On Friday, there was a wide variation in prices cited by market sources.

Chilean recovery of Chinese salmon market still a long way off

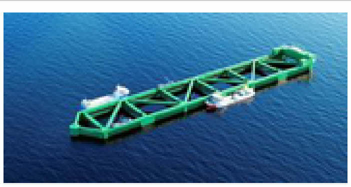
Read more

One exporter told of a general fall of NOK 5-6 (€0.49-0.59/$0.59-0.71)/ kg on all sizes from last week.

Prices for the main industry sizes range between NOK 48-63 (€4.73-6.21/$5.65-7.43)/kg.

"A lot of fish have appeared, and it is quiet for summer, so it is difficult to sell this week. There are probably many processing who will shut down for a few weeks holiday," the exporter said.

"In a way, it will be another week we have to pay to sell fish, following many in recent times," he said.



Nordlaks can't convert developmental licenses at its Havfarm 1 offshore site, throwing project in jeopardy

Read more

A second exporter also reports a similar general fall in prices and a lot of small fish in the market, which affects prices.

A producer in the north highlighted an abundance of small fish and the widespread processing of salmon affected by ISA.

"A lot of salmon is slaughtered between 2-4 kilos. It pulls prices down. It has taken with it the price of fish between 4 and 5 kilos as well," the producer said.

RELATED NEWS

Icelandic salmon farmers Ice Fish Farm, Laxar begin 'strategic discussions' toward potential merger
Aquaculture
23 June 2021 10:04 GMT

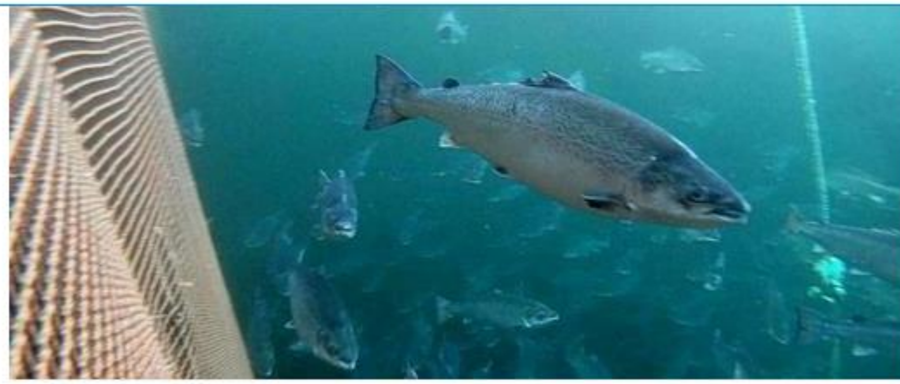
Grieg-backed land-based salmon farmer Proximar inks $6 million supply contract
Aquaculture
23 June 2021 8:48 GMT

AquaMaof: 'Salmon is just the tip of the iceberg' for RAS
Aquaculture
23 June 2021 6:17 GMT

US salmon market growing at unprecedented level

Intrafish reported in May 2021:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species



Grieg Seafood suspects deadly infectious salmon anemia outbreak at Norway salmon farm

The site in question contains 840,000 fish.

7 May 2021 10:20 GMT *UPDATED 7 May 2021 13:03 GMT*

By Ann Eileen D Nygård in **Bergen**

Global salmon producer Grieg Seafood suspects infectious salmon anemia (ISA) in one of its salmon cages in Stangnes, Hammerfest, in northern Norway.

The site is in a known area for ISA, and is already subject to several restrictions, said the company in a press release.

Offshore or land-based? Why this exec thinks open ocean farming holds far less risk of

The site holds 840,000 salmon -- approximately 2,400 metric tons -- with an average weight of 2.9 kilos. Grieg had already planned for the site to be harvested this summer, but will now bring the harvest date forward.

"Grieg Seafood aims to improve the fish health and welfare of the salmon we breed, and we work hard to reduce the risk of ISA," said Grieg Seafood Finnmark's public relations manager Roger Pedersen.

RELATED NEWS

ISA suspected at Norway Royal Salmon site
Aquaculture
18 February 2021 12:14 GMT

ISA confirmed at Yadran salmon farming site in Chile
Aquaculture
28 January 2021 15:01 GMT

Fish Farmer reported in April 2021:

Fish Farmer

For all the latest industry news, markets and jobs in aquaculture

HOME NEWS WHAT'S NEW JOBS OPINION EVENTS FEATURES READ FISH FARMER MEDIA PACK

ALL SECTIONS SEARCH

News

ISA outbreak reported in Nordland region

By Vince McDonagh - 19th April 2021

A potentially worrying outbreak of Infectious Salmon Anaemia (ISA) has been reported in the Nordland region of Norway.

The Norwegian Food Safety Authority is currently dealing with at least three suspected or confirmed cases in one of the country's main salmon farming areas. Last Thursday it received reports of a suspected outbreak at sea site 36037 Klipen where Nova Sea AS and Tomma Laks AS operate farm sites. The findings are based on early scientific PCR analysis samples. The authority says it plans to take follow-up samples soon so that the Veterinary Institute can verify suspicions.

ISA has also been confirmed at sea site 11138 Skalsvika in Meløy municipality, where Nova Sea also has farming operations, and there has been a suspected ISA outbreak at the sea site 38517 Måvær in Lurøy municipality, where Lovundlaks farms.

Similar tests will be carried out in order to confirm or dispel earlier suspicions. In order to limit the spread of infection, strict travel and other restrictions have been imposed on the site, including a ban on the movement of fish without a special permit.

Meanwhile, the authority says ISA has been confirmed at a research station at Gildeskal municipality in Nordland run by GIFAS, a privately owned research business. GIFAS reported its suspicions towards the end of March and follow up testing has now established that the disease is present.

ISA outbreaks – suspected or confirmed – usually mean that infected salmon cages have to be emptied and the fish destroyed. It also means the farms in question cannot export to a number of countries, most notably China.

An official inquiry into the high number of ISA cases last year is currently under way. The disease, while contagious and costly for those farms affected, is not harmful to humans.

A factsheet published by the World Organization for Animal Health reports:

Infectious Salmon Anemia

What is Infectious Salmon Anemia?

Infectious salmon anaemia (ISA) is a disease of farmed Atlantic salmon (*Salmo salar*) caused by the *orthomyxovirus* infectious salmon anaemia virus (ISAV), which is related to the viruses causing influenza. ISA causes anemia (as the name suggests), lethargy, and poor health and can result in significant mortality.

ISAV has been identified in healthy salmon in the wild, and other members of the salmon family (trout and Arctic Char) can also be infected by the virus, but disease outbreaks have only been seen in farmed Atlantic salmon.

ISA is a disease listed in the *OIE Aquatic Animal Health Code* (2009) and countries are obligated to report incidences of the disease to the OIE according to Chapter 1.1 of the code.

Where is the disease found?

The disease was first described in Norway in 1984 and has since been found in the United Kingdom (Scotland) in 1998, in Canada in New Brunswick in 1996 and Nova Scotia in 1998, in the United States in 2000 and in the Faroe Islands in 2000. ISA virus was first reported in Chile in 1999, and classical disease was first reported in Chile in 2007. While it has been controlled in the United States, it remains present in Norway, Canada and Chile. In Scotland the disease was eliminated in 2000, but reappeared in late 2008.

How is the disease transmitted and spread?

Risk factors for the spread of ISA are proximity to infected fish, transfer of fish from infected to clean areas, and common use of boats or equipment.

ISA is transmitted by contact with infected live salmon or biological materials containing the virus, such as animal wastes or discharges from normal culture operations, and slaughter facilities. Research has shown that boats servicing the aquaculture industry have spread the disease between aquaculture sites. The virus can survive in seawater but is killed by ultraviolet light and by disinfectants.

Infected fish may transmit the disease weeks before they show apparent signs of infection, with the virus found in blood, gut contents, urine, and mucus of infected salmon. The transfer of smolts (young fish) from one site to another has therefore led to the transfer of disease. Moreover, fish that survive outbreaks may continue to shed viral particles for more than a month into the surrounding water.

Sea lice, a common parasite of farmed salmon have also been shown capable of spreading the disease.

The European Union Reference Laboratory for Fish and Crustacean Diseases reported in April 2021:

European Union Reference Laboratory for Fish and Crustacean Diseases
NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

ISA

Infectious salmon anaemia (ISA)

Aetiology

Infectious salmon anaemia (ISA) is an orthomyxovirus infection of sea-farmed Atlantic salmon (*Salmo salar*) inducing a systemic and lethal condition characterised by severe anaemia and variable haemorrhages and necrosis in several organs. ISA virus can also be isolated from disease free rainbow trout. The virus belongs to the family Orthomyxoviridae and has recently been classified as the type species of the new genus *Isavirus*. ISAV is related to influenza viruses, but has a number of unique characteristics that distinguish it from other known orthomyxoviruses from warm-blooded animals. ISA virus is serologically fairly homogenous and can be identified using MAb.

ISA was first detected in Norway in the early 1980s. The viral aetiology was first established in the early 1990s. The disease has subsequently been detected in Canada, Scotland, Faroe Islands, USA and Chile. ISA is classified as a non-exotic disease in Council Directive 2006/88/EC.

Symptoms and pathology

ISA outbreaks occur mainly in spring at temperatures between 5 and 15 °C. Sick fish are lethargic, they sink to the bottom or cling to the net sides using they mouth, often sick fish place themselves vertically with their head in the water surface. Under experimental conditions the incubation period is usually 10-20 days. In infected populations under field conditions the infection can remain hidden for months before the disease breaks out. A disease outbreak can have either an acute course with high mortality or be more long-drawn with increase in daily mortality, which may continue for several months.

Typical findings are pale gills, bulging eyes and distended belly. Late in the disease course bleeding in the skin, loss of scales and bloody, swollen anus can be observed. Spleen and liver are often strongly swollen, dark and with petechial haemorrhages. Such may also occur in the perivisceral adipose tissue and peritoneum. The heart is pale because of anaemia and haematocrit values are very low (haemolytic anaemia). By histopathology haemorrhagic necrosis in the liver can be observed. The macroscopic changes are darkening, bleeding, especially at the abdomen and anaemic gills. When opening the fish typical observations are dark liver, pale heart and pale gills.

Diagnosis and differential diagnosis

Diagnosis is based on a combination of clinical, pathological and clinical chemical findings. ISA virus is isolated from organ material from infected fish by cultivation in specific cell cultures and identified by RT-PCR or immunofluorescence. The disease might be mistaken for bacterial septicaemia (vibriosis, furunculosis) and viral haemorrhagic septicaemia (VHS).

EU DIAGNOSTIC MANUAL

- ISA diagnostic methods

OIE MANUAL

- ISA

Fig 1. Atlantic salmon parr experimentally infected with ISAV HPRΔ by immersion. Extensive haemorrhage in the right eye.

Fig 2. Atlantic salmon parr experimentally infected with ISAV HPRΔ by immersion. Haemorrhage in the left eye, gill anaemia and severe liver congestion.

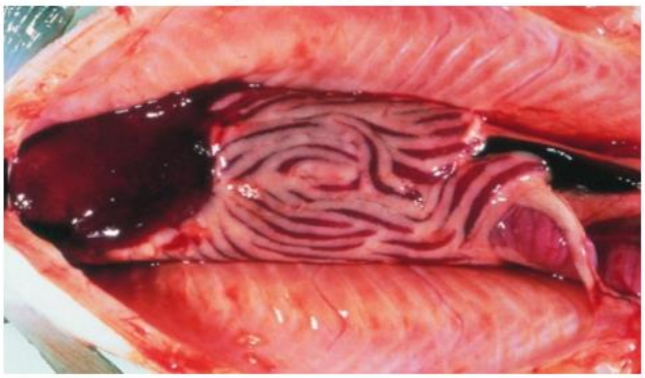
A factsheet published by the Australian Government in 2020 reported:

Australian Government
Department of Agriculture, Water and the Environment

Infection with HPR-deleted or HPR0 infectious salmon anaemia virus (ISAV)

Also known as infectious salmon anaemia (ISA)
From *Aquatic animal diseases significant to Australia: identification field guide*, 5th edition

Figure 1 Atlantic salmon (*Salmo salar*) with ISA



Note: Gross internal signs of ISA include dark liver, ascites and enlarged spleen.
Source: T Poppe

Signs of disease

Important: Animals with this disease may show one or more of these signs, but the pathogen may still be present in the absence of any signs.

Disease signs at the farm, tank or pond level are:

- mortality rate up to 100%
- fish congregating near the surface
- fish gasping at the surface
- lethargy
- loss of appetite.

Gross pathological signs are:

- pale gills and heart
- swollen abdomen
- exophthalmos (popeye), bleeding eyes
- fin rot
- ecchymotic (bruise-like) skin haemorrhages

- scale-pocket oedema
- swollen and dark liver, kidney and spleen (early sign); liver may be almost black
- petechial (pinpoint) haemorrhages in internal fat, peritoneum and skeletal muscle
- dark red intestinal wall mucosa
- ascites (fluid in the abdominal cavity)
- surface haemorrhages on liver.

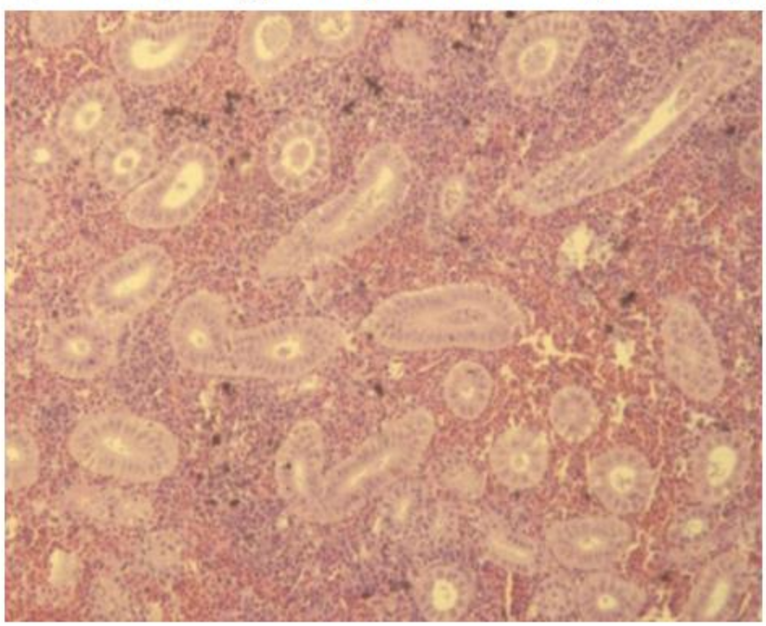
Microscopic pathological signs are:

- renal interstitial haemorrhage and tubular necrosis
- branchial lamellar and filamental congestion
- congestion of the intestine and pyloric caecae
- perivascular inflammation and focal necrosis in liver.

Disease agent

ISA is caused by infection with the pathogenic highly polymorphic region (HPR)-deleted infectious salmon anaemia virus (ISAV), or the non-pathogenic HPR0 (non-deleted HPR) ISAV, a single stranded RNA virus classified within the genus *Isavirus* within the family *Orthomyxoviridae*. Infection with HPR-deleted ISAV may cause severe disease in Atlantic salmon (*Salmo salar*). However, Detection of HPR0 ISAV has never been associated with clinical signs of disease in Atlantic salmon.

Figure 3 Histopathology of kidney of Atlantic salmon (*Salmo salar*) with ISA



Note: Renal interstitial haemorrhage.
Source: T Poppe

Intrafish reported in October 2020:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

New Mowi ISA finding could impact 1 million fish

Two separate samples at two different Newfoundland farms have shown the presense of the ISA virus.

12 October 2020 4:21 GMT UPDATED 12 October 2020 11:50 GMT

By Rachel Mutter

Mowi's Northern Harvest Sea Farms suspects the presence of the Infectious Salmon Anaemia (ISA) virus at its McGrath Cove North and Ironskull Point marine sites off the south coast of Newfoundland, Canada.

In each case, the suspect detections occurred in one Atlantic salmon from one cage at each site that was sampled through the provincial government's Department of Fisheries, Forestry, and Agriculture's s aquatic animal health surveillance program.

The cage at the McGrath Cove North site from which the suspect detection occurred contains approximately 185,000 fish, and the marine site is comprised of three cages containing a total population of approximately 560,000 fish.

The cage at the Ironskull Point site from which the suspect detection occurred contains approximately 225,000 fish, and the marine site is comprised of three cages containing a total population of approximately 500,000 fish.

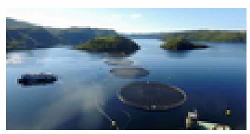
Northern Harvest Sea Farms is working with federal and provincial authorities and following best practices in the handling of these farms. These sites have been quarantined, and the company is following all regulatory requirements.

At the end of August Northern Harvest confirmed the presence of the ISA at its Cinq Island Cove marine site, also located off the south coast of Newfoundland.

The company was forced to harvest 200,000 salmon.

The new infection comes five months after Newfoundland Fisheries Minister Gerry Byrne reinstated 10 aquaculture site licences belonging to Northern Harvest Sea Farms, a division of Mowi Canada East, in the Fortune Bay region on the south coast of Newfoundland and Labrador.

The operation has been riddled with problems over the past year in particular. Last year Byrne suspended Northern Harvest's 10 farming licenses, citing the company's failure to disclose to the department in a timely manner all relevant information relating to a salmon mass mortality event at the sites. (Copyright)



Cooke salmon farming operation forced to remove 485,000 fish after deadly virus strikes again

Read more

Nearly 50,000 salmon escaped from storm-hit Mowi Scotland site

Read more

Expert fears new ISA 'horror show' on Norway's salmon farms

Read more

RELATED NEWS

Virus rears its ugly head at Cooke-owned trout farm
Salmon
4 September 2020 8:01 GMT

Mowi harvests 200,000 salmon after ISA confirmed at Canada farming site
Salmon
31 August 2020 6:27 GMT

A Freedom of Information disclosure by the Scottish Government in October 2020 included:

OFFICIAL SENSITIVE- COMMERCIAL

From: <REDACTED>
<REDACTED>
13 March 2020

Cabinet Secretary for Rural Affairs and Tourism

TEMPORARY SUSPENSION OF EXPORTS LIFTED FOR SALMON AND RAINBOW TROUT OVA FROM NORWAY

Purpose

1. To advise that the Norwegian Food Safety Authority has decided to lift its suspension on some compartments of ova with effect from 6th March.

Priority

2. Routine.

Background

3. We previously supplied advice regarding the temporary suspension of exports of salmon and rainbow trout ova from Norway. The suspension has been in place since June 2019, following an internal audit by the EFTA surveillance authority (responsible for assessing control systems related to food and feed safety). A number of issues with the Norwegian official control system were identified which meant that the Norwegian Food Safety Authority (NFSA) could not provide a reliable list of Infectious Salmon Anaemia (ISA) disease free compartments. Scotland is free from ISA, and therefore no imports from Norway could be accepted unless an attestation of disease freedom was signed by Norwegian Authorities.

4. Mr Ewing raised this issue at a meeting with Mr Nesvik, the Norwegian Fisheries Minister, at AquaNOR in August and again when he met him in November 2019. The NFSA has been working hard to address the concerns raised in the audit report however this has taken longer than expected. The NFSA had previously indicated that they were hopeful to commence exports from November 2019 to meet the start of Norway's normal peak in ova exports. This time line was not met. Part of the process they were working to involved establishing a new list of ISA free compartments, along with discussions with EFTA to determine whether the issues raised had been satisfactorily resolved. Correspondence suggested that some areas would lose their ISA free status, and therefore the areas open to trade with Scotland will reduce. A risk of delay, pending discussions with EFTA, remained.

5. The confirmation by the Norwegian Food Safety Authority that they have lifted their self-imposed suspension on the following compartments as from 6th March 2020 will be very much welcomed by most the farmed salmon industry in Scotland.

6. The ISA-free zones and compartments are as follows:

- Compartment 18000 - Rimstad, Company AquaGen
- Compartment 12917 – Sjølseng – Hall 6, Veksthall 1 og 2, Company AquaGen

- Compartment 12917 – Sjølseng - Hall 2, Company AquaGen

Consequently, the NFSA can recommence issuing health certificates for live ova produced at these compartments and exports to the UK can resume.

Communications

7. The SSPO has been advised of the lifting on the ban from these compartments and I suggest that the following form of words may be used in relation to any enquiries on this matter:

We welcome the decision by the Norwegian Food Safety Authority to lift their self-imposed suspension of health certification of live aquaculture animals from certain ISA-free zones and compartments from 6 March 2020. This means live ova produced in specific compartments can now be certified for export to Scotland.

Recommendation

8. You are invited to note this development.

<REDACTED>
<REDACTED>
Marine Scotland
Ext <REDACTED>
13 March 2020

Copy List:	For Action	For Comments	For Information		
			Portfolio Interest	Constit Interest	General Awareness
Cabinet Secretary for the Environment, Climate Change and Land Reform			X		

Scottish Government data published in October 2020 - sourced from the Scottish Fish Farm Production Survey 2019 – detailed how foreign imports of ova (especially from Norway) have flooded 'Scottish' salmon farms:

Table 18: Source, number (000's), previous year's estimate of ova laid down to hatch during 2010-2019 and projected production for 2020

Year	In house broodstock	Out-sourced GB broodstock	GB wild broodstock	Foreign ova	Total	Previous year's estimate
2010	13,744	26,220	0	29,657	69,621	61,011
2011	15,664	14,630	0	34,322	64,616	54,526
2012	18,556	9,981	0	34,700	63,237	55,723
2013	16,996	8,263	0	41,315	66,573	49,249
2014	14,418	2,725	10	53,684	70,837	48,149
2015	6,479	223	10	61,463	68,175	65,284
2016	5,884	4	0	58,458	64,346	59,604
2017	6,228	360	0	59,158	65,746	60,673
2018	8,780	200	0	61,499	70,479	67,374
2019	5,516	1,724	75	63,931	71,246	71,571
2020						70,598

The number of ova laid down to hatch was 71.2 million, an increase of 0.8 million (1.1%) on the 2018 figure. The majority of the ova (89.7%) were derived from foreign sources, this being an increase of 2.4 million (4.0%) on the 2018 figure. Supplies derived from GB broodstock decreased by 1.7 million, an 18.5% decrease on the 2018 figure. In 2019, 75,000 ova from GB wild broodstock were laid down to hatch, ova derived from wild stocks are generally held and hatched for wild stock enhancement by the aquaculture industry in cooperation with wild fisheries managers.

Imports and Exports

Table 22a: Source and number (000's) of salmon ova, fry, parr and smolts imported during 2010-2019 derived from health certificates

Import Year	Ova				Fry, Parr and Smolts	
	EU Member States	EFTA		Total	EU Member States	EFTA-Norway
		Iceland	Norway			
2010	2,150	0	26,533	28,683	452	0
2011	3,400	0	35,851	39,251	800	0
2012	10,134	0	23,849	33,983	0	0
2013	10,700	2,719	35,044	48,463	55	0
2014	5,218	3,813	49,831	58,862	1,602	1,748
2015	4,815	8,978	45,926	59,719	2,118	365
2016	5,444	5,324	38,602	49,370	1,956	0
2017	7,000	13,883	37,025	57,908	2,012	0
2018	7,250	10,116	48,430	65,796	1,700	0
2019	10,184	26,352	23,673	60,209	297	0

Georgia Edkins
@Georgia_Edkins

Record numbers of foreign salmon eggs are being imported by Scottish fish farms - @TheGAAIA in today's Scottish Mail on Sunday:

10

By Georgia Edkins

90% of Scottish salmon 'ISN'T from Scotland'

66m eggs shipped in from abroad

KNOWN as the king of fish, the Scottish salmon is prized by diners around the world.

But campaigners warn that millions of salmon sold by the country's fish farms should not be regarded as Scottish at all.

For fish farms are importing record numbers of foreign salmon eggs – mostly from Norway and Iceland – to boost stocks.

It is thought that around 90 per cent of salmon eggs hatching in Scotland are foreign.

More than 65 million foreign eggs, or ova, were shipped to Scottish fish farms last year, up from 57.9 million in 2017.

The foreign ova are hatched in Scotland and the fish reared in sea cages. Once they have been harvested and packaged, they are marketed as Scottish, despite originally hailing from abroad.

Fish farmers insist importing eggs is vital to grow the salmon industry, which they hope will double in value by 2030.

But critics claim the figures are evidence of 'food fraud' and that consumers are being duped into believing they are buying completely Scottish fish. Some also fear foreign ova could lead to the spread of devastating viral diseases.

Last night, campaigners called for an end to the 'deceptive' use of foreign salmon eggs.

Scottish Salmon Watch's Don Staniford told The Scottish Mail on Sunday: 'This is deceptive marketing and it is a salmon scandal.

'Twenty years ago Scottish salmon came from domestic eggs but the industry are ramping up the number of eggs. Scottish salmon should now be renamed Norwegian salmon with "Made in Norway" stamped on the packaging.

'They are trading on Scotland's good image. We want the importing of eggs to stop.'

Salmon egg imports are monitored by the Scottish Government through its Marine Scotland Directorate. In the first three months of this year 27.4 million eggs were shipped into the country.

Mr Staniford said: 'Importing eggs is a public health hazard. Consumers are thinking salmon is a healthy product but it is sourced from disease-laden farms.'

In 2017, one of the biggest Norwegian fish egg exporters to Scotland suffered an outbreak of infectious salmon anaemia (ISA).

This virus causes severe anaemia in fish, which can develop pale scales and abnormal swimming patterns. The import of eggs to Scotland only resumed once the Norwegian exporter became ISA-free again.

But there is no statutory duty to sample eggs before they are introduced into Scottish waters, according to the Scottish Government.

However, Hamish Macdonell, director of strategic engagement for the Scottish Salmon Producers' Organisation, said: 'All imported eggs are subject to stringent legal controls to ensure their highly regulated biosecurity.

'All of the eggs used by Scottish salmon farmers hatch and complete their life cycle in Scotland. Scottish provenance is defined by the environment in which the fish are grown.

'The number of eggs imported has gone up, from 59.7 million in 2015 to 65.8 million in 2018, a rise of 10 per cent, which is in line with the industry's ambition to achieve 5 per cent year-on-year growth.'

KING OF FISH: Scottish salmon

'Should be renamed as Norwegian'

9:21 AM · Jul 7, 2019

A Freedom of Information disclosure by the Scottish Government in August 2020 reported:

Scottish Government
Riaghaltas na h-Alba
gov.scot

Search site

About Topics News Publications Statistics and research Consultations Blogs

Home › Publications ›

PUBLICATION - FOI/EIR RELEASE

Imports of Salmon eggs and Infectious Salmon Anaemia (ISA) since 16 December 2019: EIR release

Published: **3 Sep 2020**

Directorate: Marine Scotland Directorate

Part of: Economy, Marine and fisheries, Public sector

Information request and response under the Environmental Information (Scotland) Regulations 2004

FOI reference: FOI/202000058422

Date received: 8 Jul 2020

Date responded: 7 Aug 2020

From: <REDACTED> (MARLAB) <<REDACTED>@gov.scot>

Sent: 01 April 2020 15:51

To: <REDACTED>(MARLAB) <<REDACTED>@gov.scot>; <REDACTED> (MARLAB) <REDACTED>@gov.scot>; <REDACTED> <REDACTED>@scotland.gsi.gov.uk>; <REDACTED> <REDACTED>@gov.scot>

Cc: <REDACTED> (MARLAB) <REDACTED>@gov.scot>; <REDACTED>(MARLAB) <REDACTED>@gov.scot>; <REDACTED>(MARLAB) <REDACTED>@gov.scot>; <REDACTED>(MARLAB) <REDACTED>@gov.scot>

Subject: Import of salmon ova from Norway

Hi all

I have received notification from Aquagen that a consignment of 100,000 salmon ova from their Rimstad site in Norway are due to arrive on 8th of April following approval of the site as ISA free by Mattilsynet. These are elite ova that will be used for broodstock in the UK in 3-4 years. The ova are going to be delivered to Rysa Incubation Unit in Orkney and ongrown with Cooke Aquaculture.

Aquagen are taking the opportunity to import elite ova to cover production in 3-4 years and while there are still flights available.

From: <REDACTED> (MARLAB)
Sent: 21 January 2020 10:02
To: <REDACTED> <<REDACTED>@gov.scot>
Subject: ova

Hi <REDACTED>

Some notes on ova imports and Aquagen

60.2 million salmon ova imported into Scotland in 2019 (figures from health certificates) - 26.3 million from Iceland (Stofnfiskur), 23.7 million from Norway (Aquagen, Grieg Seafood Rogaland and Mowi Norway) and 10.2 million from Republic of Ireland (Mowi Ireland).

We have not received any consignments from Norway since May 2019. The number of ova imported from Iceland in 2019 has increased to fill the gap caused by the issues in Norway (increased from 10.1 million in 2018 to 26.3 million in 2019).

Aquagen now have a salmon hatchery in Dumfries, Scotland (Holywood Breeding Centre) which they will use to supply Scottish industry (not sure if this will be exclusively or if there will be some imports from Aquagen Norway as well to Scottish industry, will depend on demand). <REDACTED> – Out of scope

<REDACTED> – Out of scope

A FOI Dossier - Norwegian Ova Banned Due to ISA published by Scottish Salmon Watch in February 2020 (sourced from a FOI disclosure by the Scottish Government on 14 February 2020) included:

From: [Redacted] SG staff
Sent: 22 November 2019 13:19
To: [Redacted] SG staff
Cc: Palmer MR (Mike) <Mike.Palmer@gov.scot>; [Redacted]<[Redacted]@scotland.gsi.gov.uk>
Subject: RE: Briefing and Itinerary - Mr Ewing's visit to Norway 24-26 November

In very short summary, there were some serious short falls found with regard to Norway's ability to provide assurance that the compartments they are trading from are free from infectious salmon anaemia. The suspension was recommended until Norway could put forward a justifiable list of ISA free compartments. That process has been delayed on two occasions. The NFSA was due to submit their suggest list to EFTA on 1 November. They did not meet this deadline and we have just had feedback from the Commission that they are still working to bring together documentation. We should press that this list is finalised urgently and shared with us when it is submitted. The suspension will not be lifted until the list is provided to EFTA and they are satisfied Norway can assure that they are free from ISA.

The industry accepts that regulatory short comings in Norway have been exposed and that the ban is in place in order to protect the interests of trading partners and Scotland's ISA free disease status. For minimal impact, the industry would like to see the ban lifted by January at the latest. If the Norwegian Minister suggests that this date will not be met, we will engage in further contingency planning discussions with industry in order to reopen previously used broodstock sites, which would allow the stripping of fish in Scotland and create an alternative ova supply.

[Redacted]
[Redacted]

Marine Scotland – Aquaculture, Crown Estate, Recreational Fisheries, EMFF and Europe
[Redacted]@gov.scot
Web: http://www.scotland.gov.uk/marinescotland
Mail: Scottish Government, 1B North, Victoria Quay, Edinburgh EH6 6QQ
gov.scot

From: [Redacted]@gov.scot
Sent: 06 November 2019 22:28
To: [Redacted]@scottishsalmon.co.uk; [Redacted]@scottishsalmon.co.uk
Subject: OVA - for producers

Hi [Redacted]

We discussed the current suspension of ova exports at the Framework meeting yesterday. Obviously SSF, Mowi and SSC are up to date from Framework meetings, but could you please circulate to all so everyone is clear on the current picture?

Message from Scottish Government Aquaculture Policy Unit;

A meeting of the EU's Standing Committee on Plants, Animals, Food and Feed took place on 24 October. One of the agenda items was the temporary suspension of ova exports from Norway in relation to ISA certification;

- Norway confirmed that it is still working towards a deadline of 1 November to provide documents to EFTA for review and to re-establish a list of ISA free compartments. They have confirmed that they will not resume exports without positive feedback from EFTA.
- EFTA confirmed that it will review documents submitted in cooperation with Commission services. EFTA have planned a follow up mission in 2020 to ensure that guarantees have been fulfilled by Norway.
- The suggested ISA disease free compartment list will be shared with the Commission when documents are submitted to EFTA.
- Member States wish to discuss the topic at the next ScoPAFF meeting scheduled for 21-22 November.
- The Commission requested that Norway keeps it updated with any plans to resume trade and to confirm that it will not resume certification before the next committee meeting.

This means that **certification will not resume until 21/22 November at the earliest**, pending acceptance of each ISA free area put forward and its related documentation. There is the potential for further delay.

From: [Redacted]@gov.scot
Sent: 06 November 2019 21:33
To: Cabinet Secretary for the Rural Economy <CabSecRE@gov.scot>; Minister for Trade, Investment and Innovation <MinisterTII@gov.scot>
Cc: Cabinet Secretary for the Environment, Climate Change and Land Reform <CabSecECCLR@gov.scot>; Minister for Rural Affairs and the Natural Environment <MinisterRANE@gov.scot>; Higgins K (Kate) <Kate.Higgins@gov.scot>; Dobson L (Leanne) <Leanne.Dobson@gov.scot>; Palmer MR (Mike) <Mike.Palmer@gov.scot>; [Redacted]@gov.scot>; Voas S (Sheila) <Sheila.Voas@gov.scot>; [Redacted]@gov.scot>; [Redacted]@gov.scot>; [Redacted]@gov.scot>; [Redacted]@gov.scot>; [Redacted]@gov.scot>; [Redacted]@gov.scot>; [Redacted]@gov.scot[Redacted]@gov.scot>; [Redacted]@gov.scot>; [Redacted]@gov.scot>; [Redacted]@gov.scot>; [Redacted]@gov.scot>; Communications Rural Economy & Environment <CommunicationsRuralEconomy&Environment@gov.scot>; [Redacted]@gov.scot>; [Redacted]@gov.scot>; [Redacted]@gov.scot>; Black G (Graham) <Graham.Black@gov.scot>; Director of Marine Scotland Mailbox <Directormarinescotland@gov.scot>; [Redacted]@scotland.gsi.gov.uk>; Mcdonnell T (Tim) <Tim.Mcdonnell@gov.scot>; Moffat C (Colin) (MARLAB) <Colin.Moffat@gov.scot>; [Redacted]@gov.scot>; [Redacted]@gov.scot>
Subject: 6 November update - OVA - RE: Update - To note - TEMPORARY SUSPENSION OF EXPORTS OF SALMON AND RAINBOW TROUT OVA TO SCOTLAND FROM NORWAY - Aquaculture

PS Cabinet Secretary for the Rural Economy

Mr Ewing will wish to be aware that a meeting of the Farmed Fish Health Steering Group took place yesterday. The industry remain concerned about the further delay to the resumption of ova exports from Norway. [Redacted] (Mowi) and [Redacted] (Scottish Sea Farms) expect that they can source stock from elsewhere until the end of the year, but may face ova sourcing problems in Q1 in 2020 if the situation is not resolved. The SSPO were also present at the meeting and we will pass a further update to them to distribute to their membership to ensure that all producers are up to date with the situation as it changes.

Scottish Sea Farms will hold contingency planning discussions with Scottish Government's Fish Health Inspectorate around the potential use of authorised broodstock sites in Scotland to source stock for the production of additional ova in Scotland next year if required.

We will meet with the CEO of AquaGen Norway and the MD of AquaGen Scotland on 4 December. Earlier this year, AquaGen purchased an egg production facility in Dumfries from Scottish SeaFarms. They have been upgrading the facility and working through some water quality issues which are affecting production at the site; https://aquagen.no/en/2019/03/01/aquagen-buys-scottish-sea-farms-freshwater-hatchery/

We will keep Ministers updated

From: [Redacted]@defra.gov.uk
Sent: 05 July 2019 16:06
To: [Redacted]@trade.gov.uk; [Redacted]@cefas.co.uk
Cc: [Redacted]@fco.gov.uk; [Redacted]@defra.gov.uk; [Redacted]@gov.scot; [Redacted]@cefas.co.uk; [Redacted]@trade.gov.uk; [Redacted]@mobile.trade.gov.uk
Subject: RE: Norwegian - EU Trade Barrier

Dear [Redacted]

Apologies for the slow response.

I discussed with [Redacted] (Scottish govt), who had more detailed information on the issue:

She confirmed that there is a temporary suspension on the movement of fish and ova from Norway to countries free from Infectious Salmon Anaemia (including the GB health zone). It is her understanding that this temporary suspension has been in place since June. The suspension has been put in place voluntarily following a meeting between the Norwegian Food Safety Authority and the EFTA surveillance authority on 29 May, where issues were raised with the Norwegian documentation system which lists ISA free compartments.

The Norwegian Food Safety Authority (NSFA) has written to all exporters in Norway to state that no export licenses will be issued until resolved, and that they expect that to take 3 – 4 weeks. [Redacted] will check on progress with Norwegian colleagues in the coming days, who are confident that they will be able to resolve the situation and keep disruption to a minimum. [Redacted] is correct in that Scottish gvt is not privy to the exact cause of the suspension, as the NSFA are working to address issues raised before the final findings are published. We are led to believe that they are largely administrative in nature, but we cannot verify this. The Scottish fish farming industry is of course heavily reliant on the import of Norwegian ova and we are monitoring the situation very closely.

More widely, the systems in place in Europe to protect aquatic animal health have not changed and the processes to enforce current fish health legislation (EC 2006/88) remain. Member states may submit disease freedom declarations to SCOPAFF and each member state has the opportunity to comment on these. This is a long standing process which is unaffected by upcoming changes to animal health law (which will largely regulates aquatics in the same way once on force).

[Redacted] will update you on the outcome of discussions with the Norwegian authorities as soon as she has more information.

Intrafish reported in August 2020:

IntraFish | Aquaculture | Fisheries | Processing | Markets | Feed | Opinion | Species

Mowi harvests 200,000 salmon after ISA confirmed at Canada farming site

The disease means 200,000 fish will have to be harvested.

31 August 2020 8:27 GMT *UPDATED 31 August 2020 18:33 GMT*

By Rachel Mutter

Mowi-owned Northern Harvest Sea Farms (NHSF) has confirmed the presence of Infectious Salmon Anaemia virus (ISA) at its Cinq Island Cove marine site located off the south coast of Newfoundland in Canada.

NHSF is removing all Atlantic salmon raised in the same cage with the two fish identified as positive by the Provincial Government's aquatic animal health surveillance program.

The cage contains approximately 200,000 Atlantic salmon, and the marine site is comprised of three cages containing a total of approximately 600,000 Atlantic salmon.

Fish from all other cages at the site have tested negative.

The quarantine order issued by the Department of Fisheries, Forestry, and Agriculture for Cinq Island Cove remains in effect.

The infection comes three months after Newfoundland Fisheries Minister Gerry Byrne reinstated 10 aquaculture site licences belonging to Northern Harvest Sea Farms, a division of Mowi Canada East, in the Fortune Bay region on the south coast of Newfoundland and Labrador.

The operation has been riddled with problems over the past year in particular. Last year Byrne suspended Northern Harvest's 10 farming licenses, citing the company's failure to disclose to the department in a timely manner all relevant information relating to a salmon mass mortality event at the sites.

Expert fears new ISA 'horror show' on Norway's salmon farms

Read more

Mowi CEO may face tough choice: Where to cut 1,500 jobs

Read more

A flood of new funds are investing in aquaculture,

RELATED NEWS

Chilean salmon farmer Camanchaca loses over 100,000 fish
Aquaculture
31 August 2020 5:40 GMT

Higher production costs blow hole in Mowi Chile's Q2 earnings
Finance
31 August 2020 4:15 GMT

Surging retail demand makes Mowi's value-added division a standout in otherwise bleak quarter
Processing
28 August 2020 22:08 GMT

Mowi looks to offload 50% stake in wellboat JV
Aquaculture
28 August 2020 9:39 GMT

Mowi drops salmon harvest guidance, plans further staff cuts as profit plunges 50%
Finance
28 August 2020 8:10 GMT

Intrafish [reported in July 2020](#):

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

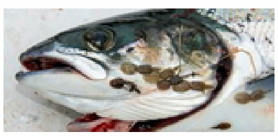
ISA cases surge past 2019 levels in Norway's salmon farms

Seven months in, cases have already almost doubled numbers for the whole of last year.

24 July 2020 5:38 GMT *UPDATED 24 July 2020 5:38 GMT*

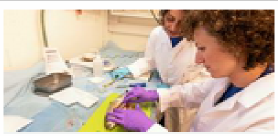
By **Ole Jacob Stronen Riise** in **Bergen**

Infectious salmon anemia (ISA) cases in the Norwegian farmed salmon industry have already surpassed the total 10 confirmed cases from last year.



Scientists patent new method for preventing sea lice on farmed salmon

Read more



Gas, disease and puberty: Team battles to find answers to land-based salmon farming's biggest challenges

Read more

So far, the Norwegian Food Safety Authority has confirmed 12-13 cases in 2020, and in the past month alone, more suspicions of ISA outbreaks have been reported in Troms and Finnmark.

If the outbreaks are confirmed, the industry will have totalled 19 cases this year, which is already almost double last year's confirmed cases, Torfinn Moldal, a researcher at the Veterinary Institute told **IntraFish**.

ISA suspicions are usually reported based on the detection of viruses by PCR and any signs of disease among the fish in the cages, Moldal said.

"It is too early to say anything about why we see this increase," Moldal said.

"When we examine samples, we sequence the virus and look for relationships to variants sequenced during other outbreaks. We are also looking for geographical location of outbreaks."

The Norwegian Food Safety Authority is in close contact with the Norwegian Veterinary Institute to map possible causal links and routes of infection, Bjarne Bjorshol, a senior adviser in the fish health and fish welfare department of the Norwegian Food Safety Authority told **IntraFish**.

"At present, we do not want to speculate on the background for the relatively strong increase in the number of ISA detections, we will have to come back to that later," Bjorshol said.

Half of the diseases are transmitting between sites, the other half are accidental, Moldal said.

The Global Aquaculture Alliance reported in June 2020:

Global Aquaculture Alliance

Aquaculture Directory

ABOUT MEMBERSHIP THE

Health & Welfare

Salmon ova transport ban between Norway and Scotland, brought on by ISA, slowly ending

1 June 2020
By Nicki Holmyard

Viral disease has been impacting sector for four decades

When AquaGen Scotland acquired its own salmon hatchery last spring, the bold move saw the company take a leading position as the only specialist breeding center for salmon ova in Scotland.

It also turned out to be a timely acquisition, as the import of salmon eggs from Norway to Scotland was suspended shortly thereafter, in May 2019, when concerns were raised about the surveillance of Infectious Salmon Anaemia (ISA), a viral disease affecting Atlantic salmon (*Salmo salar*).

The ban is only now, a year later, beginning to be lifted slowly, as different production zones satisfy the Norwegian Food Safety Authority (NFSA) of their ISA-free status.

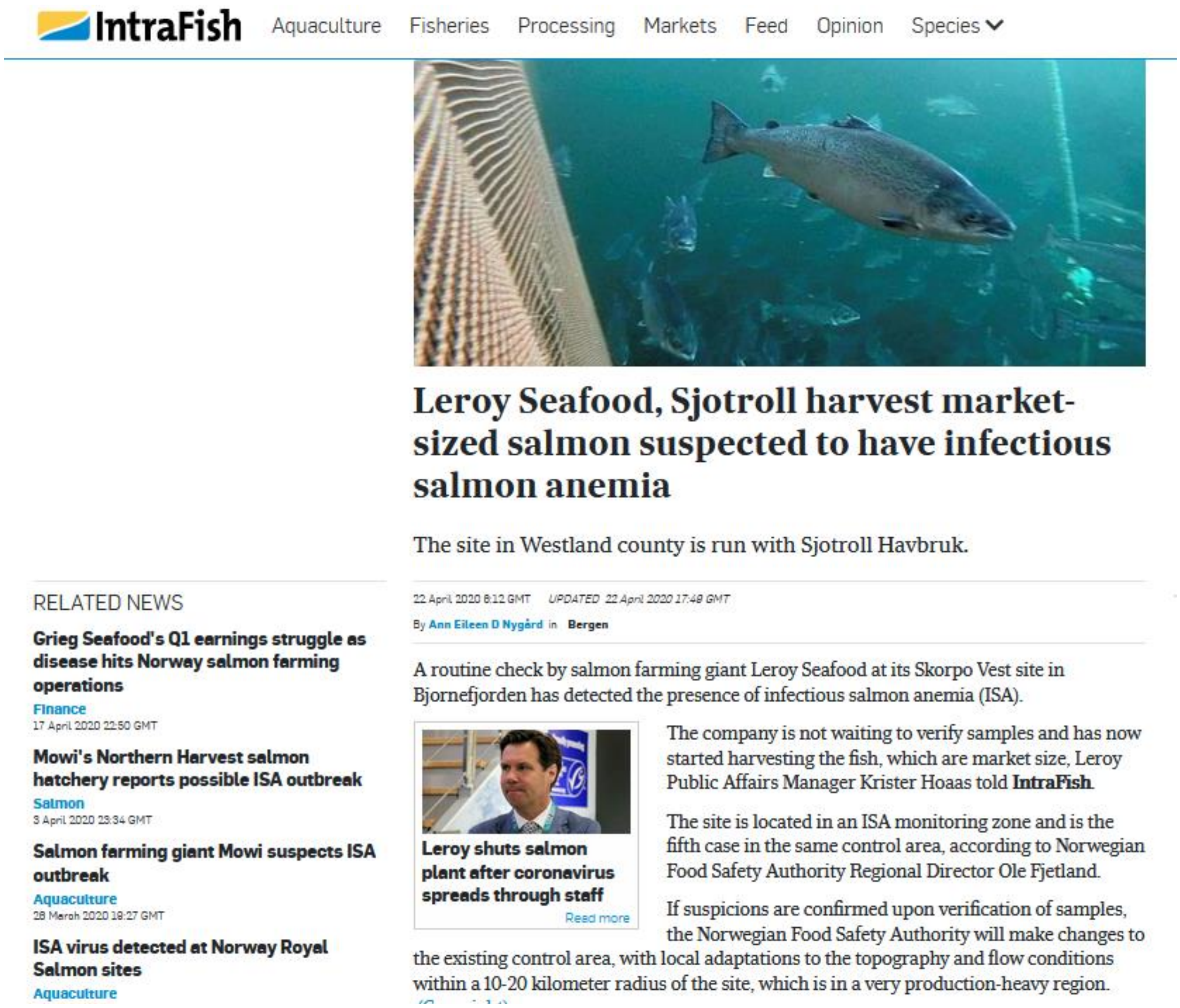
The trade restrictions followed a mission to Norway by the European Free Trade Association's (EFTA) Surveillance Authority (ESA), to evaluate animal health controls in relation to aquaculture.

ESA regularly undertake on-the-spot inspections to ensure that the EFTA Countries apply European Economic Area (EEA) food and feed safety, and animal health and welfare legislation correctly, and have carried out more than 60 inspections since 2006.

ISA first emerged in Norway in 1984, but has since been periodically reported in Canada, the United States, the Faroe Islands, Ireland and Scotland. The virus causes severe anaemia in fish but is not harmful to humans. There are currently no treatments for ISA, which can cause major losses, both from the disease and the culls put in place to contain it.

AquaGen Scotland's parent company in Norway had been a key supplier to the Scottish market since 2016, and the ban was a cause of concern for CEO Nina Santi, in terms of loss of market share and loss of access to ova for Scottish salmon farmers. Norwegian suppliers have at times, accounted for up to 90 percent of salmon ova used there.

Intrafish reported in April 2020:



IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

Leroy Seafood, Sjotroll harvest market-sized salmon suspected to have infectious salmon anemia

The site in Westland county is run with Sjotroll Havbruk.

22 April 2020 8:12 GMT *UPDATED 22 April 2020 17:48 GMT*

By Ann Eileen D Nygård in **Bergen**

A routine check by salmon farming giant Leroy Seafood at its Skorpo Vest site in Bjornefjorden has detected the presence of infectious salmon anemia (ISA).

Leroy shuts salmon plant after coronavirus spreads through staff
Read more

The company is not waiting to verify samples and has now started harvesting the fish, which are market size, Leroy Public Affairs Manager Krister Hoaas told **IntraFish**.

The site is located in an ISA monitoring zone and is the fifth case in the same control area, according to Norwegian Food Safety Authority Regional Director Ole Fjetland.

If suspicions are confirmed upon verification of samples, the Norwegian Food Safety Authority will make changes to the existing control area, with local adaptations to the topography and flow conditions within a 10-20 kilometer radius of the site, which is in a very production-heavy region.

RELATED NEWS

Grieg Seafood's Q1 earnings struggle as disease hits Norway salmon farming operations
Finance
17 April 2020 22:50 GMT

Mowi's Northern Harvest salmon hatchery reports possible ISA outbreak
Salmon
3 April 2020 23:34 GMT

Salmon farming giant Mowi suspects ISA outbreak
Aquaculture
28 March 2020 19:27 GMT

ISA virus detected at Norway Royal Salmon sites
Aquaculture

[Note that Scottish Sea Farms are owned by SalMar and the Lerøy Seafood Group ASA of Norway via the company Norskott Havbruk]

The Ferret reported in February 2020:

Imports of salmon eggs from Norway have been banned over fears a deadly viral disease could spread to Scotland, we can reveal.

New documents disclose that exports of Norwegian eggs were banned last June following an inspection by the European Free Trade Association (EFTA), a ban that remains in place.

The Scottish Government says it is pressing Norway to resolve the situation and that the fish farming industry has an "adequate" supply of eggs.

Millions of salmon eggs, also known as ova, have been imported from Norway to Scottish salmon farms in recent years but there have been concerns over Infectious Salmon Anaemia (ISA), a viral disease affecting Atlantic Salmon.

The Norwegian Government has been contacted for a comment.

Under European Union legislation urgent action must be taken to contain an outbreak of ISA. EU rules will remain in place until the end of this year. Two outbreaks of ISA in Scotland in 1998-99 and 2008-09 were successfully eradicated.

Scottish Salmon Watch [reported in February 2020](#):

Scottish Salmon Watch, 24 February 2020

['Nightmare Scenario': Norwegian Salmon Egg Exports Banned Due to Disease Risks](#)

- Critical EFTA surveillance report lead to ban in June 2019 (still in place in Feb 2020)
- Infectious Salmon Anaemia cripples Norway leaving companies importing eggs from Stofnfiskur in Iceland (no imports from Norway after 22 May 2019 shipment to Mowi)

Freedom of Information documents and data disclosed by the Scottish Government to Scottish Salmon Watch on 14 February 2020 via [FOI-19-02663](#) [1] reveal:

- In June 2019, exports of Norwegian salmon eggs (ova) were banned due to the risk of spreading Infectious Salmon Anaemia (ISA) following a critical inspection by the European Free Trade Association's Surveillance Authority (ESA) in May 2019 [2]

Final report

EFTA Surveillance Authority's mission to

Norway from 20 to 29 May 2019

in order to evaluate animal health controls

in relation to aquaculture

- "Norway is unable to ensure that farmed fish/shellfish sent for export to other EEA states will not affect the health of farmed fish/shellfish in those receiving countries," [explained the Scottish Government in a letter to Scottish Salmon Watch on 14 February 2020](#). "As of the date of this communication, the suspension remains in place as corrective measures are taken and implemented."

- "ESA found that Norway must improve the controls of diseases in farmed fish/shellfish that will be traded in the EEA," [reported ESA in a press release dated 30 September 2019](#). "Currently, Norway cannot fully ensure that farmed fish/shellfish sent from Norway to other EEA-states does not affect the health of farmed fish/shellfish in the receiving countries."

- "At the time of the mission there was no reliable system in place in Norway enabling identification of farms which have been granted ISA-free status," detailed the ESA report dated 23 September 2019. "Moreover, in the majority of cases, such status has been granted without or with very limited involvement of the NFSA [Norwegian Food Safety Authority] staff prior to the stage when the formal application is forwarded to

the NFSA. The lack of official verification by the NFSA of surveillance activity undertaken to prove freedom from ISA casts significant doubt on the reliability of the statements included in the declaration of free status for compartments submitted by the NFSA since it is not in a position to ascertain the accuracy of the information being certified or ensure that no conflict of interest compromises the process."

"Norway has submitted several declaration for dependent Infectious Salmon Anaemia (ISA)-free compartments: i.e. sites which are dependent on the health status of the surrounding water," detailed the ESA report published in September 2019. "However, in these cases Norway does not apply additional disease surveillance activities to confirm that the sea waters surrounding element of the dependent compartment (e.g. neighbouring salmon farm or susceptible species of wild fish) can also be considered free of ISA. The mission team considers that due to the lack of surveillance in surrounding waters and the absence of any additional measures to prevent the introduction of ISA to sea sites declared free of ISA, such dependent compartments should not be declared and certified for intra-EEA trade and export to third countries as ISA-free compartments."

Conclusion:

34. There is currently no reliable definitive list of ISA-free compartments and zones publicly available for Norway. The information currently available in Norwegian legislation and on the NFSA's website is inaccurate and contradictory. This, combined with the use of inconsistent terminology, has the potential to mislead officials and interested parties regarding which areas in Norway are disease free and from which certification and trade of live fish and products thereof may take place.

Conclusions

89. Due to delays in withdrawing ISA free status, compartments that no longer fulfil the requirements of ISA-free status still appear on the list of ISA-free compartments in the relevant Norwegian legislation. This precludes the possibility of relying on that list to ascertain conclusively that aquaculture animals originate from ISA free areas.

- FOI documents cited "serious short falls" & "regulatory short comings in Norway"
- "We cannot accept exports from Norway until authorities are able to attest to ISA disease freedom," admitted an internal Scottish Government memo in November 2019

- "Yet another nightmare scenario and example of why we are rigorous in our implementation of the regulations and adherence to surveillance and control requirements to evidence and maintain disease status," said the Scottish Government in October 2019

A scientific paper published in Frontiers of Veterinary Science in January 2020 reported:

ORIGINAL RESEARCH article

Front. Vet. Sci., 15 January 2020 | https://doi.org/10.3389/fvets.2019.00481

Infectious Salmon Anemia and Farm-Level Culling Strategies

Lars Qviller*, Anja B. Kristoffersen, Trude M. Lyngstad and Atle Lillehaug

Norwegian Veterinary Institute, Oslo, Norway

Infectious salmon anemia (ISA) is an infectious disease, and outbreaks must be handled to avoid spread between salmon sea farms. Intensive culling at infected farms is an important biosecurity measure to avoid further spread but is also a costly intervention that farmers try to avoid. A lack of action, however, may lead to new outbreaks in nearby salmon sea farms, with severe impacts on both economy and animal welfare. Here, we aim to explore how a time delay between a detected outbreak and the culling of both infected cages and entire farms affects the further spread of the disease. We use a previously published model to calculate how many salmon sea farms were directly infected in each outbreak. To investigate the effect of culling on the further spread of disease, we use the number of months elapsed from the detected outbreak to (a) the first cage being depopulated, and (b) to the entire salmon sea farm being depopulated as predictors of how many new farms the virus was transmitted to, after controlling for contact between the farms. We show that the lapse in time before the first cage is depopulated correlates positively with how many new salmon sea farms are infected, indicating that infected cages should be culled with as little time delay as possible. The model does not have sufficient power to separate between culling of only cages assumed to be infected and the entire farm, and, consequently, provides no direct empirical evidence for the latter. Lack of evidence is not evidence, however, and we argue that a high probability of spread between cages in infected salmon sea farms still supports the depopulation of entire farms as the safest option.

1. Introduction

The production of farmed Atlantic salmon is a growing industry that has developed into a large international business over the last few decades. Norway is currently the largest actor in this industry, with an annual production of about 1.2 million metric tons and with somewhere between 350 and 450 million salmon in marine farms along the Norwegian coast at any time during the last 4 years (1). An average Norwegian salmon sea farm is stocked with almost one million smolt (calculated using data gathered from https://www.fiskeridir.no). The continuously increasing volumes have led to concerns about environmental and pathogenic impacts (1, 2). The current political aim of a quintupling of the aquaculture production by 2050 (2, 3) will lead to an increase in fish populations susceptible to pathogenic disease agents, and more efficient multiplication and dissemination of such agents may be a consequence. It is therefore of great importance to develop effective disease control measures and to evaluate their effect.

Marine culture of salmonids usually takes place in production units consisting of between one and 15 cages in proximity to each other. The cages may be constructed as open net pens outlined by steel squares that are welded together or by individual plastic rings, typically with a circumference of 160 m. The cages are held in a limited area, managed together by a central fleet with workers, equipment, and a shared system for fodder distribution. Such production units are commonly referred to as salmon sea farms. Each such farm must be approved by the authorities and registered in the Norwegian aquaculture register.

Infectious salmon anemia (ISA) is a serious viral disease in farmed Atlantic salmon, and it may also infect rainbow trout. It is caused by virulent HPR-deleted strains of the ISA virus (4), possibly evolving from non-virulent strains of the ISA virus, which is called ISAV-HPR0 (5, 6). The disease is listed as notifiable by the World Animal Health Organization (OIE, 4). An outbreak of ISA develops slowly, but the majority of the fish in an infected population may succumb during the production cycle. Cumulative mortalities as high as 90% have been reported in farms (4, 7–9). The disease is contagious, and while there is an ongoing debate over whether it can transmit vertically (4, 9, 10), its ability to spread horizontally is well established (4, 9, 11, 12). The disease can spread to other salmon sea farms, using pathways such as passive transmission in the water or with contaminated equipment, boat traffic, or the movement of fish (12–18).

The disease has even shown its potential to destroy entire salmon farming industries, tragically exemplified with the epidemics in Chile in 2007–2009 (18, 19) and in the Faeroe Islands in 2000–2005 (20). The Norwegian aquaculture industry was the first to experience challenges with ISA, with the first outbreak described in 1984 (8). As many as 80 new infected farms were reported in 1990, at the peak of historical outbreaks in Norway (21). The ISA problem in Norway in the nineties was mitigated through regulations including compulsory fish health controls, disinfection in hatcheries, regulation of live fish transportation, "all-in-all-out" (only one generation of fish at the same time in each salmon sea farm), and following between generations (9). Due to its status as a listed disease, outbreaks of ISA call for mandatory disease control measures to be taken by the Norwegian Food Safety Authority. These measures include establishing a disease control zone with surveillance of fish populations, culling of infected cages or entire farm populations, and ensuring a period of coordinated fallowing of the entire zone after depopulation (4, 22).

Although the contemporary issue with ISA outbreaks in Norway is much less severe than in the nineties, the industry still faces between zero and seven outbreaks (~ 0.7% of each generation) with unknown source of infection, every year on average (23). These initial outbreaks may cause local epidemics of secondary outbreaks. Aldrin et al. (12) have developed a transmission model that uses factors such as geographic distances between salmon sea farms, genetic similarities between virus isolates, and the number of fish in both the susceptible and infected salmon sea farms. This model can be used to describe local epidemics in order to substantiate whether the outbreak in one farm has spread to another. Note that this model (or any model derived from it) did not include the effects of water currents. The model infers infectious contact over 6 months prior to the outbreak, and we assume that hydrodynamic patterns even out over the period. Further, the mechanisms behind transmission of ISA are not known in enough detail to include spatiotemporal movements of the water.

There is evidence suggesting that all cages in a salmon sea farm with ISA are highly likely to contract the disease if one cage is infected and that the functional epidemiologic unit of an ISA outbreak is the entire salmon sea farm and not the single cage (19).

Due to the slow development of the disease, the individual salmon sea farm may occasionally benefit if production is continued, despite the increased mortality. It is therefore not surprising that some farms want to delay the culling of the population, sometimes by almost a year, in order for the fish to reach harvesting size and thereby reduce economic losses. However, if neighboring salmon sea farms are infected, the total losses may increase correspondingly, and if the disease is not managed, the situation may escalate. A key element in preventing the disease from further spread is the early removal of infected populations, but the economic cost of such actions often leads to culling only of cages with clinical signs and/or confirmed diagnosis (18, 24). Such a practice is highly questionable, as fish are known to be infectious several weeks or even months before displaying any clinical signs (9, 12, 15).

A factsheet on ISA published by the Scottish Government in December 2019 reports:

Scottish Government
Riaghaltas na h-Alba
gov.scot

About | Topics | News | Publications | Statistics and research | Consultations | Blogs

Home › Publications ›

PUBLICATION - FACTSHEET

Diseases of wild and farmed Finfish

Published: 17 Dec 2019

Directorate: Marine Scotland Directorate

Part of: Marine and fisheries

Some fish and shellfish diseases of particular significance in Scotland.

Infectious Salmon Anaemia (ISA)

Infectious salmon anaemia (ISA) is an infectious viral disease of Atlantic Salmon (*Salmo salar L.*). The disease was first reported in Norway in 1984, but has since been reported in Canada, the USA, the Faroe Islands, Ireland and Scotland. Both outbreaks of ISA in Scotland in 1998-99 and 2008-09 were successfully eradicated. Atlantic salmon is the only susceptible species known to develop clinical disease, but ISA virus can replicate in rainbow trout (*Oncorhynchus mykiss*) and sea trout (*Salmo trutta L.*).

Where and When Might it Occur?

In Norway, cases of ISA have occasionally been reported in fresh water farms but generally in hatcheries which use part-sea water. The overwhelming majority of cases occur in farmed fish in sea water. The virus has been detected in wild fish but cases of clinical disease have only been reported in farmed fish.

Diagnosis

The causative agent of ISA is an orthomyxovirus. The presence of the virus is confirmed by histopathological changes consistent with ISA, immunohistochemistry, isolation and identification techniques and real-time reverse transcription polymerase chain reaction (RT-PCR) followed by nucleic acid sequence analysis.

Control

The virus can be transmitted through water, but the highest risk factors for spread of disease are movement of live fish, discharge of untreated blood and contact with infected vehicles and equipment.

ISA is a notifiable disease within Great Britain. ISA – infection with genotype HPR-deleted of the genus Isavirus (ISAV) is listed as a non-exotic disease under Annex IV, Part II of Council Directive 2006/88/EC (as amended in 2014/22/EC). Great Britain is an approved zone for this disease, and to maintain this disease-free status, all farms holding susceptible species of fish are routinely inspected for clinical signs of the disease. Under EU legislation action must be taken to contain any outbreak, to eradicate sources of infection and to protect other fish farms by:

- Compulsory slaughter and disinfection of infected farms
- Strict movement controls on suspect farms
- Placing farms in the vicinity of an outbreak under surveillance

There are no treatments for ISA and no licensed vaccines in the EU. Vaccine trials in Canada have yielded equivocal results.

Cases of clinical disease may be characterised by severe anaemia, ascites (accumulation of fluid in the body cavity), haemorrhage in internal organs and darkening of the liver.

More information

- The Infectious salmon anaemia (ISA) virus
- World Organisation for Animal Health, Aquatic Manual - Chapter 2.3.5 Infection with Infectious Salmon Anaemia Virus

The World Organization for Animal Health reported in 2019:

CHAPTER 2.3.5.

INFECTION WITH HPR-DELETED OR HPR0 INFECTIOUS SALMON ANAEMIA VIRUS

1. Scope

Infection with infectious salmon anaemia virus (ISAV) means infection with the pathogenic agent highly polymorphic region (HPR)-deleted ISAV, or the non-pathogenic HPR0 (non-deleted HPR) ISAV of the Genus *Isavirus* of the Family *Orthomyxoviridae*.

IHPR-deleted ISAV may cause disease in Atlantic salmon (*Salmo salar*), which is a generalised and lethal condition characterised by severe anaemia, and variable haemorrhages and necrosis in several organs. The disease course is prolonged with low daily mortality (0.05–0.1%) typically only in a few cages. Cumulative mortality may become very high for a period lasting several months if nothing is done to limit disease dissemination (Rimstad et al., 2011).

Detection of HPR0 ISAV has never been associated with clinical signs of disease in Atlantic salmon (Christiansen et al., 2011). This virus genotype replicates transiently and has mainly been localised to the gills. A link between non-pathogenic HPR0 ISAV and pathogenic HPR-deleted ISAV, with some outbreaks potentially occurring as a result of the emergence of HPR-deleted ISAV from HPR0 ISAV has been suggested (Cárdenas et al., 2014; Christiansen et al., 2017; Cunningham et al., 2002; Gagné & LeBlanc, 2018; Mjaaland et al., 2002).

2.3.2. Prevalence

In net pens containing diseased fish, the prevalence of HPR-deleted ISAV may vary widely, while in adjacent net pens (without diseased fish) ISAV may be difficult to detect, even by the most sensitive methods. Therefore, for diagnostic investigations it is important to sample from net pens containing diseased fish.

There is increasing evidence that the prevalence of the nonpathogenic HPR0 ISAV varients may be high in Atlantic salmon production areas. HPR0 ISAV in Atlantic salmon appears to be seasonal and transient (Christiansen et al., 2011). HPR0 ISAV have also been detected in wild salmonids (Rimstad et al., 2011).

2.3.3. Geographical distribution

Initially reported in Norway in the mid-1980s (Thorud & Djupvik, 1988), infection with ISAV in Atlantic salmon has since then been reported in Canada (New Brunswick in 1996; Mullins et al., 1998), the United Kingdom (Scotland in 1998), the Faroe Islands (2000), the USA (Maine in 2001) and in Chile (2007) (Cottet et al., 2011; Rimstad et al., 2011). The presence of the HPR0 ISAV variant has been reported in all countries where infection with HPR-deleted ISAV has occurred, with the known exception of Iceland.

Intrafish reported in November 2019:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

ISA detected at SalMar-run salmon farm

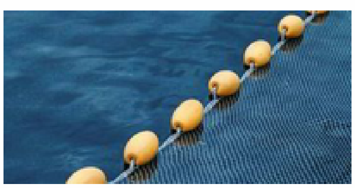
Salmar notified the authorities on Nov. 15 following a positive test, leading to restrictions on the movement of fish from the site.

22 November 2019 3:50 GMT *UPDATED 26 November 2019 16:18 GMT*

By **John Evans**

Traces of ISA disease have been detected at a salmon farm operated by Norwegian producer SalMar in conjunction with Havbrukstasjonen in Tromso, Norway, according to feed producer Skretting and Stim, the Norwegian Food Safety Authority.

SalMar notified the authority on Nov. 15 following a positive test.



ISA virus detected at Cermaq Chile site

Read more

Anyone traveling to the area and engaging in activities related to fish farming is now under orders to show due care and attention to avoid the spread of any disease.

Under restrictions, fish cannot be moved from the site without special permission.

Should ISA be confirmed, the Norwegian Food Safety Authority will order that all the fish at the site be slaughtered.

Due to its proximity, the neighboring Mollvika site will be subject to the same restrictions. If ISA is confirmed, a restricted area will be established to combat the spread of the disease.

[Note that Scottish Sea Farms are owned by SalMar and the Lerøy Seafood Group ASA of Norway via the company Norskott Havbruk]

Scottish Salmon Watch reported in May 2019:

Scottish Salmon Watch, 13 May 2019

Salmon Eggsclusive: Scotland's 'King of Fish' is Now Viking Not Scottish!

Campaigners are calling on the 'Scottish' salmon industry to come clean to consumers and label fish sourced from imported eggs (ova) as 'Made in Norway'.

New data disclosed by the Scottish Government via Freedom of Information in May 2019 reveals that over 90 million salmon ova have been imported for use on 'Scottish' salmon farms since 2018 with Norway accounting for over 80%. Since 2003, 'Scottish' salmon farmers have imported over 650 million ova mostly from Norway but also from Iceland and Ireland **[1]**.

According to the latest Scottish Fish Farm Production Survey (published in October 2018), 90% of the ova laid down to hatch in 2017 (59.2 million out of 65.7 million) were foreign ova. Whilst the Scottish Government is flooding lochs with foreign fish, the Norwegian Government has banned the import of salmon ova from Scotland citing unacceptable disease and genetic risks under the Norwegian Nature Diversity Act.

In February 2019, Scottish Salmon Watch revealed that AquaGen's import of 2.5 million salmon eggs to the Scottish Sea Farms hatchery at Barcaldine in November 2018 was delayed due to fears over the spread of Infectious Salmon Anaemia (ISA).

An inspection of AquaGen's Holywood Salmon Farm in November 2018 by the Scottish Government's Fisheries Health Inspectorate reported that: "The biosecurity measures plan for the site was inspected and found to be inadequately maintained".

In a stinging attack last year, the head of the only independent Scottish salmon egg producer (Landcatch - owned by Hendrix Genetics) accused the Scottish Government of taking a "massive risk" by allowing salmon eggs from Norway and Iceland to flood 'Scottish' salmon farms citing the danger of "transfer of ISA from infected countries such as Norway".

"This means that the Scottish salmon industry is now 100% reliant on imported eggs - both a massive risk in the event of borders closing for disease issues (and also the transfer of ISA from infected countries such as Norway), but also making a complete mockery of the brand 'Scottish Salmon'," stated an email to Fergus Ewing, Cabinet Secretary for the Rural Economy, dated 12 February 2018.

Notes to Editors:

[1] Data disclosed by the Scottish Government via FOI in November 2018 detailed a staggering 342 million ova imported from Norway between 2003 and 2015 (out of 462 million ova imported - i.e. Norway represented 74% of ova imports):

Country of origin	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Australia	550,000	1,860,000	0	2,400,000	0	0	0	0	0	0	0	0	0
Iceland	9,518,000	3,475,000	570,000	300,000	0	0	0	0	0	0	2,719,000	4,346,000	8,978,000
Norway	2,900,000	6,750,000	13,210,000	15,940,000	33,555,000	22,703,000	29,938,000	26,533,000	35,851,000	23,848,000	35,044,000	49,831,000	45,926,000
Rep of Ireland	7,820,000	4,450,000	2,610,000	11,575,000	10,511,000	5,600,000	5,460,000	2,150,000	3,400,000	10,134,000	10,700,000	5,218,000	4,815,000
USA	400,000	450,000	450,000	0	0	0	0	0	0	0	0	0	0

Numbers of salmon ova collated from health certificates

A scientific paper published in PLoS One in April 2019 reported:

› PLoS One. 2019 Apr 16;14(4):e0215478. doi: 10.1371/journal.pone.0215478. eCollection 2019.

Wild and farmed salmon (Salmo salar) as reservoirs for infectious salmon anaemia virus, and the importance of horizontal- and vertical transmission

Are Nylund [1], Jarle Brattespe [1], Heidrun Plarre [1], Martha Kambestad [1], Marius Karlsen [2]

Affiliations + expand

PMID: 30990853 PMCID: PMC6467415 DOI: 10.1371/journal.pone.0215478

Free PMC article

Abstract

The infectious salmon anaemia virus (ISAV) is an important pathogen on farmed salmon in Europe. The virus occurs as low- and high virulent variants where the former seem to be a continuous source of new high virulent ISAV. The latter are controlled in Norway by stamping out infected populations while the former are spreading uncontrolled among farmed salmon. Evidence of vertical transmission has been presented, but there is still an ongoing discussion of the importance of circulation of ISAV via salmon brood fish. The only known wild reservoirs are in trout (Salmo trutta) and salmon (Salmo salar). This study provides the first ISAV sequences from wild salmonids in Norway and evaluates the importance of this reservoir with respect to outbreaks of ISA among farmed salmon. Phylogenetic analyses of the surface protein hemagglutinin-esterase gene from nearly all available ISAV from Norway, Faeroe Islands, Scotland, Chile and wild salmonids in Norway show that they group into four major clades. Including virulent variants in the analysis show that they belong in the same four clades supporting the hypothesis that there is a high frequency of transition from low to high virulent variants in farmed populations of salmon. There is little support for a hypothesis suggesting that the wild salmonids feed the virus into farmed populations. This study give support to earlier studies that have documented local horizontal transmission of high virulent ISAV, but the importance of transition from low- to high virulent variants has been underestimated. Evidence of vertical transmission and long distance spreading of ISAV via movement of embryos and smolt is presented. We recommend that the industry focus on removing the low virulent ISAV from the brood fish and that ISAV-free brood fish salmon are kept in closed containment systems (CCS).

The Ferret reported in February 2019:

Government officials raised concerns over the import to Scotland of salmon eggs from a Norwegian company called AquaGen after the outbreak of a deadly virus.

Documents obtained under freedom of information law reveal that fears were expressed by both Scottish and UK government officials over Infectious Salmon Anaemia (ISA), a devastating viral disease transmitted through water affecting fish.

Under European Union (EU) legislation urgent action must be taken to contain an outbreak of ISA. There are no treatments for the virus and no licensed vaccines.

Hundreds of thousands of fish have previously been slaughtered in bids to control outbreaks of ISA, with affected fish farms disinfected and placed under close surveillance.

In recent years, millions of salmon ova have been imported from Norway to Scottish salmon farms and hatcheries, including 22.6 million in 2016 from AquaGen, one of the world's major suppliers.

Documents requested by Scottish Salmon Watch have now revealed there was an outbreak of ISA at an AquaGen site in Norway in July 2017.

The company confirmed to The Ferret there was an outbreak two years ago but said that its ISA free status was reinstated in November 2018, adding there was no risk to Scottish farms.

AquaGen said its ISA free status was "immediately suspended" in July 2017 following the discovery of the virus. But emails reveal that concerns were raised by the UK authorities in November 2018 when exports of salmon ova to Scotland were due to resume.

On 7 November 2018, a senior fish health inspector in the Scottish Government said they were "very wary of an export happening".

This was in relation to a plan by AquaGen to export salmon ova from Rimstad to Scotland on 21 November 2018 "2 days after their 60 day assessment period is completed for the reinstatement of their ISA free status".

The email continued: "I am aware that there have been some questions raised by the UK and other countries. Do you know if these have been resolved?"

On the 8 November 2018, an official at the UK government's Centre for Environment, Fisheries and Aquaculture Science (Cefas) also raised concerns in an email over the export of ova from Rimstad to a Scottish farm.

"Until our concerns have been adequately addressed by the Norwegian CA (Competent Authority) we would support Marine Scotland in refusing the import of salmon ova from this site," the official wrote.

On 14 November 2018, the UK government again raised objections to the import of ova to a Scottish fish farm from Norway scheduled for 21 November 2018.

The documents also show that in July 2017, Hendrix Genetics – owners of Landcatch, the only independent Scottish salmon egg producer – asked the Scottish Government, "if the ISA outbreaks in Norway, in particular AquaGen, would have any effect on their ability to export eggs into Scotland".

In a later email to Fergus Ewing, Cabinet Secretary for Rural Economy, in February 2018, Landcatch accused the Scottish Government of taking a "massive risk" by allowing salmon eggs from Norway and Iceland to flood Scottish salmon farms, citing the danger of "transfer of ISA from infected countries such as Norway".

A scientific paper published in PLoS One in January 2019 reported:

PLOS | ONE

RESEARCH ARTICLE

Sea lice, *Lepeophtheirus salmonis* (Krøyer 1837), infected Atlantic salmon (*Salmo salar* L.) are more susceptible to infectious salmon anemia virus

Sarah E. Barker[1¤], Ian R. Bricknell[1,2], Julia Covello[3], Sarah Purcell[3], Mark D. Fast[3], William Wolters[4], Deborah A. Bouchard[1*]

1 Aquaculture Research Institute, University of Maine, Hitchner Hall, Orono, Maine, United States of America, **2** School of Marine Sciences, The University of Maine, Hitchner Hall, Orono, Maine, United States of America, **3** Hoplite Lab, Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada, **4** USDA ARS National Cold Water Marine Aquaculture Center, Franklin, Maine, United States of America

¤ Current address: Benchmark Animal Health Ltd, Edinburgh Technopole, Milton Bridge, Penicuik, United Kingdom
* deborah.bouchard@maine.edu

Abstract

The role of parasitic sea lice (Siphonostomatoida; Caligidae), especially *Lepeophtheirus salmonis*, in the epidemiology of Infectious Salmon Anemia Virus (ISAv) has long been suspected. The epidemiological studies conducted during the 1998 major Infectious Salmon Anaemia (ISA) outbreak in Scotland demonstrated a strong correlation between sea lice presence and ISAv positive sites or subsequent clinical outbreaks of ISA. The question posed from this observation was "do sea lice infestations on Atlantic salmon make them more susceptible to viral infections?"

This study investigated the role that sea lice infestations have on the severity of ISAv infections and disease mortality in experimental populations of farmed Atlantic salmon (Salmo salar). A series of experiments was carried out that investigated the potential of sea lice to modify the outcome of an ISAv infection. Experimental populations of Atlantic salmon were established that had: no lice and no ISAv, a single infection with either ISAv or lice and a co-infection with lice then ISAV. The results were quite clear, the process of infestation by the parasite prior to ISAv exposure significantly increased the mortality and death rates of Atlantic salmon, when compared to uninfected controls and ISAv infected groups only. This was consistent over two source strains of Atlantic salmon (Pennobscot and Saint John River), but the severity and timing was altered. Immunological responses were also consistent in that pro-inflammatory genes were induced in lice only and co-infected fish, whereas the anti-viral response, Mx, MH class I β, Galectin 9 and TRIM 16, 25 genes were down-regulated by lice infection prior to and shortly after co-infection with ISAv. It is concluded that the sea lice settlement on Atlantic salmon and the parasite's subsequent manipulation of the host's immune system, which increases parasite settlement success, also increased susceptibility to ISAv.

A scientific paper published in Frontiers of Veterinary Science in December 2018 reported:

Front Vet Sci. 2018; 5: 308.
Published online 2018 Dec 6. doi: 10.3389/fvets.2018.00308

PMCID: PMC6292176
PMID: 30574509

Risk Factors Associated With Outbreaks of Infectious Salmon Anemia (ISA) With Unknown Source of Infection in Norway

Trude Marie Lyngstad, Lars Qviller, Hilde Sindre, Edgar Brun, and Anja B. Kristoffersen*

Abstract

Go to:

The occurrence of infectious salmon anemia (ISA) outbreaks in marine farmed Atlantic salmon constitutes a recurring challenge in Norway. Here, we aim to identify risk factors associated with ISA outbreaks with an unknown source of infection (referred to as primary ISA outbreaks). Primary ISA outbreaks are here defined by an earlier published transmission model. We explored a wide range of possible risk factors with logistic regression analysis, trying to explain occurrence of primary ISA with available data from all Norwegian farm sites from 2004 to June 2017. Explanatory variables included site latitude and a range of production and disease data. The mean annual risk of having a primary outbreak of ISA in Norway was 0.7% during this study period. We identified the occurrence of infectious pancreatic necrosis (IPN), having a stocking period longer than 2 months, having the site located at high latitude and high fish density (biomass per cage volume) in the first six months after transfer to sea site as significant risk factors ($p < 0.05$). We have identified factors related to management routines, other disease problems, and latitude that may help to understand the hitherto unidentified drivers behind the emergence of primary ISA outbreaks. Based on our findings, we also provide management advice that may reduce the incidence of primary ISA outbreaks.

Keywords: infectious salmon anemia, ISA, ISAV, HPR0, HPR-del, Atlantic salmon, risk factors

There are two main variants of ISAV, one variant is highly virulent and associated with ISA outbreaks, termed HPR-deleted ISAV (HPR-del ISAV). The other variant (termed HPR0 ISAV) is assumed to be non-virulent. HPR0 ISAV was proposed in 2002 to be an ancestral form of HPR-del ISAV (5). A direct link between HPR0 ISAV and HPR-del ISAV remains to be demonstrated, but a strong indication of the evolution from HPR0 ISAV to HPR-del ISAV was recently reported from a Faroese Atlantic salmon marine farm (6). HPR0 ISAV is prevalent and described as a transient infection (7–10). Interestingly, a high proportion of fish groups experience infection with HPR0 ISAV during the seawater production phase without evolving into the virulent HPR-del ISAV (9–11). This shows that HPR0 ISAV may represent a risk for the emergence of clinical ISA, even though the risk of transition from HPR0 ISAV to HPR-del ISAV appears to be low.

The transmission mechanisms behind primary ISA outbreaks depend on the introduction of either high virulent strains from unknown reservoirs or non-pathogenic strains of the ISA virus (HPR0 ISAV) that facilitate an evolution toward higher virulence. Therefore, an increased susceptibility of a salmon production site may introduce risk of a primary ISA outbreak. In addition, factors like viral reproduction and shedding may affect the rate of evolution in the virus. Suboptimal management, stocking and fallowing routines, disease events, production density (number of fish on farm), handling and treatment of fish are all factors that have been associated with an increased risk of ISA outbreaks (21–24). Good management and biosecurity as defined by the Office International des Epizooties (OIE) (25), are therefore in general considered important for the prevention and control of ISA, but these factors have not been structurally examined specifically for primary cases. We argue that a better understanding of factors associated with an increased risk of primary ISA outbreaks may elucidate unknown transmission mechanisms and may help to optimize preventive measures, facilitate early detection, and reduce the risk of local ISA epidemics.

Stocking

We identified that a long stocking period was associated with an increased risk of contracting a primary ISA outbreak (Table 2 OR = 3.885 and Figure 4). Stocking involves the movement of fish and repeated visits by well-boats; both these factors are associated with an increased risk of infection (23, 37). A likely consequence of a prolonged stocking period is that the well-boats have more intermediate visits to other farms, followed by an increased probability of indirect contact with cohorts infected with both HPR0 ISAV and HPR-del ISAV. A long stocking period may also involve fish from several smolt producers, different size and age groups, and thereby jeopardizes the biosecurity principle of separating the generations.

Biomass

We found that increasing maximum density during the first 6 months was a significant risk factor, illustrated along the x-axis in Figure 4. High density (biomass per cage volume) is associated with fish abundance, a factor known to affect the susceptibility to both pancreas disease (26) and ISA (12). Risk factors associated with poor management and stress have been associated with ISA outbreaks in early epidemiological studies (21, 22), but these studies did not differentiate between primary or secondary ISA outbreaks.

Intrafish reported in July 2018:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

ISA suspected at Marine Harvest sites in Norway

The Norwegian Food Safety Authority will conduct on-site analysis today to confirm the outbreak.

2 July 2018 12:11 GMT *UPDATED 4 July 2018 8:13 GMT*

By **IntraFish Media**

The Norwegian Food Safety Authority alerted of a possible outbreak of infectious salmon anemia (ISA) at Marine Harvest's Breivika and Breivika S sites June 28.

The Norwegian Food Safety Authority will conduct further analysis at the site on July 2 and will send samples to the Veterinary Institute to confirm the diagnosis.

"To prevent possible spread of infection, both sites are subject to restrictions, including prohibiting fish moving without a separate permit," said the authority.

"If the suspicion is confirmed, the Norwegian Food Safety Authority will order emptying of the sites, there is an ongoing eviction at the plants." (Copyright)

news Salmon Aquaculture Alert me about these topics Manage My Alerts

RELATED NEWS

ISA confirmed at Cooke farm in Newfoundland
Aquaculture
2 July 2018 12:10 GMT

Norway's Bremnes group reports record earnings
Finance
29 June 2018 7:19 GMT

Leroy suspects ISA outbreak at mid-Norway site
Aquaculture
27 June 2018 12:07 GMT

ISA suspected at Nova Sea site

Intrafish reported in January 2018:

IntraFish | Aquaculture | Fisheries | Processing | Markets | Feed | Opinion | Species

Leroy salmon farming sites hit with ISA

Company disposing of salmon in conjunction with authorities.

31 January 2018 12:07 GMT *UPDATED 31 January 2018 13:27 GMT*

By **IntraFish Media**

Two salmon farming sites at Leroy Seafood Group's Leroy Sjotroll division tested positive for infectious salmon anemia (ISA), the company said Tuesday.

The suspected outbreak was first reported on Jan. 26.

The harvesting of the site, in Buholmen near Austevoll, Norway, will be carried out as planned, the company said, in conjunction with the Norwegian Food Safety Authority.

A report - The surveillance program for infectious salmon anaemia (ISA) and bacterial kidney disease (BKD) in Norway 2017 – published by the Norwegian Veterinary Institute in 2018 reported:

Summary

Infectious salmon anaemia (ISA) (pathogenic ISAV HPR-del) and ***Renibacterium salmoninarum*** were not detected in conjunction with surveillance in ISA free zones or compartments in 2017.

According to the received reports from private laboratories on surveillance carried out in ISA control zones and compartments, ISAV HPR-del was detected on six sites.

Introduction

Infectious salmon anaemia (ISA) is a serious disease in salmon caused by ISA virus (ISAV), within the ***Orthomyxoviridae*** family. 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: USA, UK, Scotland, Canada, Faroe Islands, and Chile. In Norway, the number of outbreaks peaked in the early 1990s with more than 80 cases per year. In the late 80ies and early 90ies several measures were implemented by the Norwegian Food Safety Authority (NFSA) in order to combat and limit spread of the disease. Since 1993, the number of annual outbreaks has varied between 1 and 20, and ISA is still a recurring challenge to the salmon farming industry in Norway. There are two main variants of ISAV, one being associated with ISA outbreaks, virulent and highly pathogenic, termed HPR-deleted ISAV (ISAV HPR-del), and the other variant termed ISAV HPR0, assumed non-pathogenic.

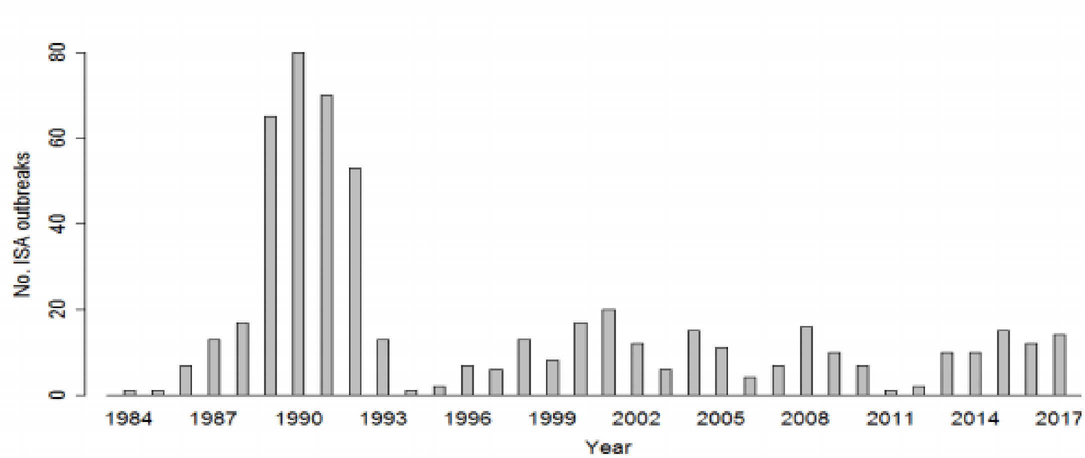


Figure 1. Annual numbers of registered ISA-outbreaks in Norway in the period of 1984 - 2017.

ISA is an OIE listed disease (1) and is notifiable (list 2) in Norway, and within the EU (Council Directive 2006/88). In Norway, there is a legal obligation to report suspicion of ISA to the NFSA. Following a suspicion, the NFSA performs fish sampling at the suspected site, and submits the samples to the national ISA reference laboratory (i.e. the Norwegian Veterinary Institute) to perform a diagnostic investigation. If this investigation confirms an ISA diagnosis, this is reported to the NFSA. The NFSA then makes the legal diagnosis regarding the site and makes decisions as to the implementation of control measurements. The latter includes establishment of a control zone, and restrictions on fish movement. All ISA diagnoses are reported to the OIE.

Positive PCR-tests for ISAV HPR0 have so far not been considered notifiable by the law on food production and food safety (https://lovdata.no/dokument/NL/lov/2003-12-19-124) in Norway.

Results and discussion

In total, 5286 samples from 33 farms with Atlantic salmon were investigated for ISAV in within **ISA free zones or compartments**. Geographical locations of the fish farms are shown in Figure 2. ISAV HPR-del was not detected, while ISAV HPR0 was detected in samples from three farms.

This summary includes only data from farms in established ISA free zones and segments, and not data from farms that have screened for ISAV in an attempt to obtain an ISA free status.

In conjunction with **ISA control zones**, 16726 samples from 182 farms with Atlantic salmon and 150 samples from eight farms with rainbow trout were investigated. Geographical locations of the fish farms are shown in Figure 3. ISAV HPR-del was detected in six Atlantic salmon farms. ISAV HPR0 was detected in samples from 21 farms with Atlantic salmon.

With regard to ***R. salmoninarum***, a total number of 3957 samples from 52 farms with Atlantic salmon and 198 samples from six farms with rainbow trout were investigated. Geographical locations of the fish farms are shown in figure 4. ***R. salmoninarum*** was not detected in any of the samples.

Design and evaluation of the surveillance programmes are not considered in this report.

Table 1: Number of tested farms in ISA free zones, and in ISA control zones.

Category	Number of farms tested	Number of ISAV HPR0+ farms	Number of ISAV HPR-del farms
ISA free zones	33	3	0
Control zones	190	21	6

References

1. Office International des Epizooties, 2016. Manual of Diagnostic tests for Aquatic Animals. Infectious salmon anaemia. http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/chapitre_isav.pdf.

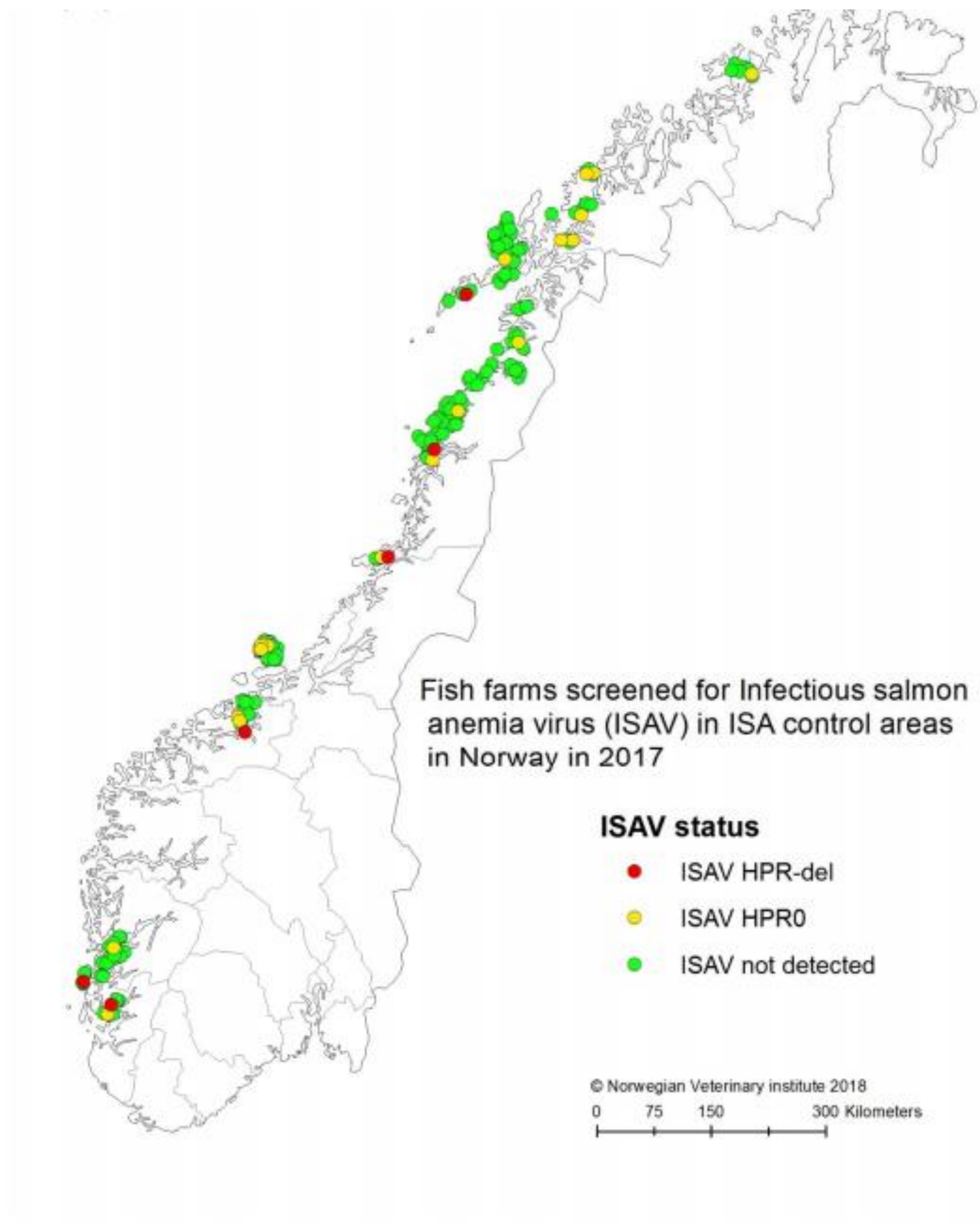


Figure 3: Geographic locations of fish farms tested for infectious salmon anaemia virus (ISAV) in conjunction with infectious salmon anaemia (ISA) control zones in 2017.

Intrafish reported in December 2017:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

ISA suspicions arise at Marine Harvest salmon site

Company notified authorities Thursday.

RELATED NEWS

Benchmark looking to develop ISA-resistant salmon
Aquaculture
7 December 2017 12:08 GMT

Norway Royal Salmon suspects ISA outbreak in Finnmark
Aquaculture
14 November 2017 13:02 GMT

Chilean salmon prices could continue to fall through 2019
Prices
8 November 2017 12:11 GMT

18 December 2017 12:08 GMT *UPDATED 18 December 2017 13:18 GMT*

By **IntraFish Media**

Norway's Food Safety Authority said Friday it suspected an ISA outbreak at Marine Harvest's salmon Ringja site in Rogaland.

ISA detected at NRS salmon farms
Read more

Marine Harvest notified the agency Thursday after it detected ISA and preliminary analysis indicates that detected virus is not an HPR0 variant, which increased suspicions. More samples are being tested to confirm.

The site is in an ISA control area after the disease was found in the region back in September.

The salmon already reached market size and were harvested to be processed, said Marine Harvest Communications Manager Ola Helge Hjetland.

"Now we are waiting to see if the diagnosis is verified and if it is, we will intensify processing and speed up the drainage of the plant." (Copyright)

A scientific paper published in the Journal of General Virology in April 2017 (the experiments were carried out in Aberdeen at Marine Scotland Science) reported that "ISAV-HPR0 represents a reservoir and risk factor for the emergence of ISA disease":

JOURNAL OF GENERAL VIROLOGY

RESEARCH ARTICLE
Christiansen *et al.*, *Journal of General Virology* 2017;98:595–606
DOI 10.1099/jgv.0.000741

MICROBIOLOGY SOCIETY

First field evidence of the evolution from a non-virulent HPR0 to a virulent HPR-deleted infectious salmon anaemia virus

Debes H. Christiansen,[1,*] Alastair J. A. McBeath,[2] Maria Aamelfot,[3] Iveta Matejusova,[2] Mickael Fourrier,[2] Patricia White,[2] Petra E. Petersen[1] and Knut Falk[3]

Abstract

The putatively non-virulent subtype of infectious salmon anaemia virus (ISAV), ISAV-HPR0, is proposed to act as a progenitor and reservoir for all virulent ISAVs and thus represent a potential risk factor for the emergence of infectious salmon anaemia (ISA) disease. Here, we provide the first evidence of genetic and functional evolution from an ISAV-HPR0 variant (FO/07/12) to a low-virulent ISAV virus (FO/121/14) in a Faroese Atlantic salmon marine farm. The FO/121/14 virus infection was not associated with specific clinical signs of ISA and was confined to a single net-pen, while various ISAV-HPR0 subtypes were found circulating in most epidemiologically linked marine and freshwater farms. Sequence analysis of all eight segments revealed that the FO/121/14 virus was identical, apart from a substitution in the fusion (F) gene ($Q_{266}L$) and a deletion in the haemagglutinin-esterase (HE) gene, to the FO/07/12 variant from a freshwater farm, which supplied smolts exclusively to the FO/121/14-positive net-pen. An immersion challenge with the FO/121/14 virus induced a systemic infection in Atlantic salmon associated with a low mortality and mild clinical signs confirming its low pathogenicity. Our results demonstrate that mutations in the F protein and deletions in the highly polymorphic region (HPR) of the HE protein represent a minimum requirement for ISAV to gain virulence and to switch cell tropism from a localized epithelial infection to a systemic endotheliotropic infection. This documents that ISAV-HPR0 represents a reservoir and risk factor for the emergence of ISA disease.

INTRODUCTION

Infectious salmon anaemia (ISA) is a disease of farmed Atlantic salmon, *Salmo salar* L., listed by the World Organization for Animal Health (OIE). Epidemics have occurred in all major Atlantic salmon producing countries including Canada, Chile, the Faroe Islands, Norway, Scotland and the USA, causing severe economic and production losses [1–7]. The causative agent, infectious salmon anaemia virus (ISAV), belongs to the *Orthomyxoviridae* family [8]. The viral genome is composed of eight single-stranded RNA segments of negative polarity, encoding at least 10 proteins, including two glycoproteins: the haemagglutinin-esterase (HE) on segment 6 and the fusion protein (F) on segment 5. Together, these two surface glycoproteins regulate receptor binding/destroying functions and fusion activity, respectively [9, 10].

Differences in virulence among ISAV strains have been described in several studies [11–14]. Putatively uncultivable non-virulent ISAV variants (ISAV-HPR0) are characterized by an HE protein carrying a full-length highly polymorphic region (HPR) composed of at least 35 amino acids just upstream of the transmembrane domain [15–19]. All currently described pathogenic ISAV isolates associated with ISA disease have deletions in the HPR with over 30 different HPR-deleted subtypes identified in Europe, North America and Chile [16–22]. Sequencing and functional characterization further suggest both HPR-deletion and a $Q_{266}L$ substitution, or insertion adjacent to the cleavage site in the F protein influences ISAV virulence [22], by promoting viral fusion and the activation of proteolytic cleavage [23, 24]. However, other viral functions, for example virus receptor binding [25], virus uptake, replication rate, shedding of new virions [26, 27], modulation of the host immune response [28, 29] and the ability to spread to new hosts [30], can also influence virulence.

One major challenge for the control of ISA disease is the lack of knowledge of the mechanisms associated with the re-emergence of pathogenic strains, and the drivers for the evolution to higher virulence [31]. The common hypothesis is that all virulent ISAV strains have evolved from a reservoir of ISAV-HPR0 progenitors through deletion events in the HE-HPR [17–19, 32–34]. While ISAV-HPR0 is present in most Atlantic salmon producing countries and causes frequent and transient infections [19, 33–35], whether this non-pathogenic variant acts as a progenitor and reservoir for virulent ISAV still remains unclear. Moreover, recent legislation adopted by the OIE made both HPR0 variants and HPR-deleted strains notifiable, which could have a far-reaching impact on salmon product exports. This further emphasizes the urgent need to understand the factors driving the evolution of a disease causing ISAV-HPR-deleted virus.

In January 2014, a new ISAV-HPR-deleted strain (FO/121/14) was identified through routine screening at an Atlantic salmon marine farm in the Faroe Islands. The purpose of this study was (i) by sequence analysis of the ORF of all eight segments to investigate whether the FO/121/14 virus originated from any of the HPR0 variants known to circulate in Faroese aquaculture at that time and (ii) to characterize the virulence of the FO/121/14 virus using an *in vivo* immersion challenge and *in vitro* experiments. We here demonstrate the genetic evolution of a non-virulent ISAV-HPR0 to a low-virulent ISAV-HPR-deleted virus and discuss the functional effect of key HE and F protein mutations on the shift in ISAV virulence and organ tropism.

DISCUSSION

In this study, we present the first evidence for the evolution of an ISAV-HPR0 to a low-virulent ISAV-HPR-deleted virus in Atlantic salmon reared at a marine farming site in the Faroe Islands. The genetic and functional evolution was documented through sequence analysis and experimental infection, and was supported by strong epidemiological links between the HPR0 variant and the HPR-deleted strain. Our results revealed that a deletion in the HPR of the HE gene combined with a mutation in the F gene is a minimum requirement for a shift in virulence and organ tropism. Indeed, while ISAV-HPR0 causes only a sub-clinical localized mucosal infection, the mutated low-virulent FO/121/14 virus was able to induce a systemic infection of the circulatory system. The observed mutations in FO/121/14 were however not sufficient for the evolution to a highly virulent

ISAV capable of inducing noticeable disease such as the Glesvær/2/90 virus.

The discovery of a new virulent ISAV in the Faroe Islands in 2014 was the first since 2005, despite extensive surveillance and frequent detection of ISAV-HPR0. Phylogenetic analysis showed that FO/121/14 was not a re-emergence of the viruses responsible for the Faroese ISA epidemic [19]. Based on field observations, FO/121/14 demonstrated a low virulence since it did not induce any mortality or conclusive clinical signs of ISA. This was confirmed in the experimentally infected fish, which also showed a significantly lower mortality with less pronounced and milder clinical signs compared to the highly virulent Glesvær/2/90 virus. However, the internal organs of all FO/121/14-infected fish were ISAV-positive on challenge termination and over half of the fish displayed at least one of the characteristic clinical and pathological signs of ISA disease, albeit to a milder extent than fish infected with Glesvær/2/90. These results, combined with the present sequence data suggest that virulence acquisition in ISAV may be a stepwise process where accumulation of specific mutations in the two surface glycoproteins is an important first step. This is consistent with the hypothesis that these two mutations, the HPR deletion in the HE gene and the $Q_{266}L$ substitution in the F gene, represent necessary mutational steps for the transition to virulence in ISAV [18, 22]. This is also in agreement with observations in avian influenza (AI) where highly pathogenic AI viruses (HPAIV) arise from low-pathogenic AI viruses (LPAIV) by mutations of specific virulence markers and where the acquisition and increase in virulence is a stepwise process [36–41]. Although the role of other proteins than HE and F in ISAV pathogenesis so far remains elusive, further mutations of other proteins are expected to increase virulence [11, 22, 42, 43] as demonstrated in other members of the orthomyxovirus family, e.g. influenza viruses [44–48].

A major finding in the present work was that mutations of the F and HE genes are key first steps in the within-host infection dynamics, i.e. the functional shift of cell and organ tropism from an epithelial to an endothelial infection, including generalized dissemination. This is in agreement with our previous findings that non-virulent ISAV-HPR0 causes only a localized epithelial infection of mucosal surfaces of the gills and skin [19, 49], whereas virulent ISAV-HPR-deleted shows a generalized endothelial infection of the circulatory system [50]. Furthermore, recent functional analysis demonstrated that deletions in the HE-HPR promote ISAV receptor fusion of viral and cellular membranes by which the virus delivers its genetic material into the host cell for subsequent replication [23]. Deletion of HPR facilitates the activation of the F protein post-receptor binding and subsequent proteolytic activation by a host cellular protease, while F protein mutations (including the $Q_{266}L$ substitution) directly influence proteolytic cleavage [24]. This suggests that the two mutations determine viral cell and organ tropism, and enables ISAV to fuse and replicate in a wider range of cells and organs [24].

Two key questions that remain to be answered, particularly related to the risk of the potential emergence of a pathogenic ISAV strain from HPR0 are: when did the evolution from the FO/07/12 variant to the low-virulent FO/121/14 occur, and what was driving this evolution? It could have taken place either during the freshwater phase or after transfer to seawater. RNA viruses are characterized by a high mutation rate and rapid evolutionary potential because of the intrinsic error-prone nature of virus RNA polymerases generating viral quasispecies to maximize diversity and adaptability [51, 52]. Recently, ISAV quasispecies were demonstrated in farmed Atlantic salmon [53]. The authors identified low-frequency HPR-deleted variants in HPR0-positive screening samples from healthy fish as well as low-frequency HPR0 variants in subsequent HPR-deleted samples from ISA diseased fish [53]. Similar findings of low-frequency HPAIV in LPAIV and *vice versa* have been documented in samples from a natural outbreak of AI [40]. Therefore, it cannot be excluded that the FO/121/14 variant existed as a low-frequency minority variant in FO/07/12-

Table 4. Number of fish (*n*=4) positive for ISAV nucleoprotein by IHC measured from 2 to 20 days p.i. shows the dissemination of the low-virulent FO/121/14 compared to the highly virulent Glesvær/2/90 in the gills, kidney, heart and liver of Atlantic salmon

	Days post infection										Isolate
Organs	2	3	4	5	6	7	8	10	15	20	
Gills	0	1	0	0	0	0	1	0	2	1	FO/121/14
	1	1	0	0	0	2	4	4	4	NA	Glesvær/2/90
Kidney	0	0	0	0	0	3	2	4	3	1	FO/121/14
	0	0	0	1	4	3	4	4	4	NA	Glesvær/2/90
Heart	0	0	0	0	0	0	0	2	3	3	FO/121/14
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Glesvær/2/90
Liver	0	0	0	0	0	0	0	2	3	1	FO/121/14
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Glesvær/2/90

NA, not applicable.

positive samples from smolt farm B. However, we consider this unlikely as the highly sensitive FO/121/14-specific assay, developed in this study, did not detect this strain in smolt farm B. The FO/121/14 strain was detected exclusively in samples from the affected net-pen at marine farm 4, 17 months post sea transfer. This late detection indicates, together with the fact that the FO/121/14 virus was at an early evolutionary stage and was spreading fast among fish in the affected net-pen, that the transition to virulence most likely took place post seawater transfer.

The shift in virulence from ISAV-HPR0 to a disease-causing ISAV has been suggested to be a stochastic event dependent on the replication rate of the virus and the time available for virulence mutations to occur and accumulate [21]. Studies in fish and mammals have demonstrated that stress can play a key role in facilitating an increased viral replication rate because of a weakened immune response [54, 55]. The specific factors responsible for the evolution of FO/121/14 are unknown. However, before first detection of the virus, the farm had experienced a prolonged period of extreme weather conditions with high sea waves combined with several treatments for sea lice (*Lepeophtheirus salmonis*). Similar stressful farming conditions, including bad weather, handling, infection with sea lice, treatments for sea lice and other environmental stressors have in several studies been associated with decreased resistance to viral pathogens and increased disease susceptibility, including ISA [54, 56–60].

Our study presents for the first time data substantiating the evolution from an ISAV-HPR0 to a virulent ISAV-HPR-deleted in a field situation. Furthermore, our study suggests that a deletion in the HPR of the HE-protein together with a specific amino acid substitution in the F-protein were sufficient to change the pathogenesis from a superficial epithelial infection for ISAV-HPR0 to a generalized infection of endothelial cells for FO/121/14. These findings have several important implications for ISAV control procedures. First, this clearly demonstrates that ISAV-HPR0 detected in the Atlantic salmon production compartments can act as a reservoir from which virulent ISAV strains evolve and therefore represents a non-negligible risk for the re-emergence of ISA disease. Second, while mutations in the two surface glycoproteins represent an important first step, other mutations in different parts of the genome most likely contribute to the acquisition of higher ISAV virulence in a stepwise process. The fact that the FO/121/14 virus was detected by chance suggests that other low-virulent ISAV-HPR-deleted strains could potentially arise and go unnoticed, because most surveillance programmes rely on an infection presenting itself with conspicuous clinical and pathological signs. This clearly emphasizes the importance of implementing biosecurity procedures, in particular strict year class separations (i.e. all-in/all-out systems) during production of Atlantic salmon in both the fresh- and seawater phases to prevent the potential build-up of virulence-enhancing mutations and emergence of fully virulent ISAVs.

Intrafish reported in August 2016:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

Suspicion of ISA lingers on at SalMar

No ISA has so far been detected on samples, but doubts remain.

10 August 2016 13:12 GMT *UPDATED 10 August 2016 12:31 GMT*

By **Anders Furuset**

No fish were found with infectious salmon anemia (ISA) in the samples taken from the SalMar sites of Kattholmen and Kattholmen II in Frøya Municipality, but an element of doubt remains.

On July 29, the Norwegian Food Safety Authority (NFSA) reported it had suspicions of ISA at the two sites after an alert had been received from Salmar Farming, which through its sampling routine suspected ISA could be present.

Kattholmen I and II lie in an area that contains many fish farms with a lot of fish in the sea. These fish were released into cages in the spring of 2015 and harvesting is planned for this fall.

"In order to prevent any spread of infection there have been restrictions placed on the farm sites, including a ban on taking fish in or out of the site," according to the NFSA release.

"Everyone passing through or operating in the area must exercise the necessary care so that any spread of disease is minimized."

NFSA senior inspector Aud Skrudland confirmed no fish had been discovered with ISA in the sample – but stressed that the sites are still under suspicion, and that new samples would be taken.

"The status is that the sites are still under suspicion," Skrudland told **IntraFish**. "There is no doubt that the ISA virus has been substantiated on two fish in a Salmar cage during one of their own sample routines, but we haven't managed to verify this yet."

Skrudland said the fact the infection had been discovered at a very early stage was good, as the only alternative otherwise would have been discovery after an actual outbreak. In her experience suspicions of ISA were usually confirmed.

The area where the sites are located is still in quarantine.

A scientific paper published by PLoS One in 2016 reported:

PUBLISH ABOUT BROWSE

PLOS ONE

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Localised Infection of Atlantic Salmon Epithelial Cells by HPR0 Infectious Salmon Anaemia Virus

Maria Aamelfot, Debes H. Christiansen, Ole Bendik Dale, Alastair McBeath, Sylvie L. Benestad, Knut Falk

Published: March 21, 2016 • https://doi.org/10.1371/journal.pone.0151723

Article | Authors | Metrics | Comments | Media Coverage

Abstract

Infectious salmon anaemia (ISA) is an important, systemic viral disease of farmed Atlantic salmon, *Salmo salar* L. Endothelial cells are the main target cells for highly virulent HPR-deleted ISA virus (ISAV) types. Here we examine the pathogenesis of non-virulent ISAV HPR0 infections, presenting evidence of an epithelial tropism for this virus type, including actual infection and replication in the epithelial cells. Whereas all HPR0 RT-qPCR positive gills prepared for cryosection tested positive by immunohistochemistry (IHC) and immunofluorescent labelling, only 21% of HPR0 RT-qPCR positive formalin-fixed paraffin-embedded gills were IHC positive, suggesting different methodological sensitivities. Only specific epithelial cell staining was observed and no staining was observed in endothelial cells of positive gills. Furthermore, using an ISAV segment 7 RT-PCR assay, we demonstrated splicing of HPR0, suggesting initial activation of the replication machinery in the epithelial gill cells. Immunological responses were investigated by the expression of interferon-related genes (*e.g.* Mx and γIP) and by ELISA for presence of anti-ISAV antibodies on samples taken sequentially over several months during an episode of transient HPR0 infection. All fish revealed a variable, but increased expression of the immunological markers in comparison to normal healthy fish. Taken together, we conclude that HPR0 causes a localized epithelial infection of Atlantic salmon.

Introduction

Infectious salmon anaemia virus (ISAV) is the causative agent of a systemic and lethal disease (ISA) in farmed Atlantic salmon, *Salmo salar* L. The virus belongs to the genus *Isavirus* of the family *Orthomyxoviridae*. Clinical signs suggest circulatory failure with severe anaemia, ascites, congestion and enlargement of the liver and spleen (reviewed in [1]). Endothelial cells are the main target cells for virulent virus replication [2], however epithelial cells may also be infected in early stages, especially by low virulent isolates [3, 4]. Immersion infection experiments suggest variations in replication dynamics in gill [5] and other mucosal surfaces [4] between ISAV strains of low and high virulence, with subsequent variations in disease severity and pathogenesis [6].

Putatively non-virulent genotypes of the virus (ISAV HPR0) have been detected in all major Atlantic salmon producing countries including Norway [7], Scotland [8, 9], Canada [10], Chile [11] and the Faroe Islands [12], but is not associated with clinical ISA disease. HPR0 is differentiated from the low and highly virulent types based on a 'full length' highly polymorphic region (HPR) located upstream of the trans-membrane region of genomic segment 6 encoding the HE protein [8, 13, 14]. Even though additional changes are likely required for the transformation, the main theory is largely accepted that virulent ISAV strains have arisen following various deletions within the HE gene of HPR0 types. This suggests the HPR0s are ancestors of virulent HPR-deleted (HPR-del) ISAV strains.

A scientific paper published in the Virology Journal in 2016 reported:

Virology Journal

Home About Articles Sections Submission Guidelines

Research | Open Access | Published: 06 January 2016

Discovery of variant infectious salmon anaemia virus (ISAV) of European genotype in British Columbia, Canada

Molly JT Kibenge, Tokinori Iwamoto, Yingwei Wang, Alexandra Morton, Richard Routledge & Frederick SB Kibenge

Virology Journal **13**, Article number: 3 (2016) | Cite this article

Abstract

Background

Infectious salmon anaemia (ISA) virus (ISAV) belongs to the genus *Isavirus*, family *Orthomyxoviridae*. ISAV occurs in two basic genotypes, North American and European. The European genotype is more widespread and shows greater genetic variation and greater virulence variation than the North American genotype. To date, all of the ISAV isolates from the clinical disease, ISA, have had deletions in the highly polymorphic region (HPR) on ISAV segment 6 (ISAV-HPRΔ) relative to ISAV-HPR0, named numerically from ISAV-HPR1 to over ISAV-HPR30. ISA outbreaks have only been reported in farmed Atlantic salmon, although ISAV has been detected by RT-PCR in wild fish. It is recognized that asymptomatically ISAV-infected fish exist. There is no universally accepted ISAV RT-qPCR TaqMan® assay. Most diagnostic laboratories use the primer-probe set targeting a 104 bp-fragment on ISAV segment 8. Some laboratories and researchers have found a primer-probe set targeting ISAV segment 7 to be more sensitive. Other researchers have published different ISAV segment 8 primer-probe sets that are highly sensitive.

Intrafish reported in January 2016:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

Questions surround new study claiming ISA found in BC salmon

Research purporting evidence of the virus published in new journal, but a familiar cast of anti-salmon farming activists behind the research raises questions of findings.

8 January 2016 14:10 GMT UPDATED 8 January 2016 13:28 GMT

By Drew Cherry

Newly published research claims the European strain of the infectious salmon anemia (ISA) virus is present in farmed salmon samples collected in British Columbia, but a host of questions are being raised by both industry and government officials about the findings.

The research, published in the *Virology Journal*, claims a genetic sequence of ISA was detected in 79 farmed salmon samples collected at retail outlets, as well as in a sea louse attached to a wild sockeye salmon.

Researchers, which include anti-salmon farming campaigner Alexandra Morton, Atlantic Veterinary College University of Prince Edward Island's Fredrick Kibenge and Simon Fraser University's Richard Routledge, claimed a genetic sequence of "non-negative" ISA was detected in 79 samples of the more than 1,000 wild and farmed salmon tested.

While none of the findings of ISA would be considered "positive" under the threshold trigger for federal action, the researchers noted, the presence of the European strain of the virus itself was cause for alarm.

"The potential that viruses such as ISAV are contributing to widespread decline in sockeye salmon populations cannot be taken lightly," Routledge wrote of the results. "The findings in this paper should lead to development of more sensitive screening for this specific virus. This opportunity needs to be pursued with vigor."

The study was not the first by the authors alleging the discovery of ISA. In 2011, research conducted by Morton, together with Simon Fraser University, claimed to have found ISA in wild salmon. However, subsequent research by the Canadian Food Inspection Agency (CFIA) could not find any positive results.

RELATED NEWS

BC's salmon industry publishes sustainability report
News
28 October 2015 13:04 GMT

More than 100,000 people sign petition to stop BC salmon expansion
News
28 May 2015 13:17 GMT

Grieg: Partnership, educated media improving salmon farming image in Canada
News
3 November 2015 14:09 GMT

Seafood's secret sustainability
News
18 November 2015 14:10 GMT

First Nations call for investigation into critically low salmon returns
News
13 November 2015 14:05 GMT

Picture gallery: On the farm at a Marine Harvest BC site
News
24 November 2015 14:10 GMT

Grieg offers BC fishermen $157,000 compensation
News
10 July 2015 13:08 GMT

A scientific paper published in PLoS One in 2015 reported:

PLOS ONE

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Dual Mutation Events in the Haemagglutinin-Esterase and Fusion Protein from an Infectious Salmon Anaemia Virus HPR0 Genotype Promote Viral Fusion and Activation by an Ubiquitous Host Protease

Mickael Fourrier, Katherine Lester, Turhan Markussen, Knut Falk, Christopher J. Secombes, Alastair McBeath, Bertrand Collet

Published: October 30, 2015 • https://doi.org/10.1371/journal.pone.0142020

Article | Authors | Metrics | Comments | Media Coverage

Abstract

In Infectious salmon anaemia virus (ISAV), deletions in the highly polymorphic region (HPR) in the near membrane domain of the haemagglutinin-esterase (HE) stalk, influence viral fusion. It is suspected that selected mutations in the associated Fusion (F) protein may also be important in regulating fusion activity. To better understand the underlying mechanisms involved in ISAV fusion, several mutated F proteins were generated from the Scottish Nevis and Norwegian SK779/06 HPR0. Co-transfection with constructs encoding HE and F were performed, fusion activity assessed by content mixing assay and the degree of proteolytic cleavage by western blot. Substitutions in Nevis F demonstrated that K276 was the most likely cleavage site in the protein. Furthermore, amino acid substitutions at three sites and two insertions, all slightly upstream of K276, increased fusion activity. Co-expression with HE harbouring a full-length HPR produced high fusion activities when trypsin and low pH were applied. In comparison, under normal culture conditions, groups containing a mutated HE with an HPR deletion were able to generate moderate fusion levels, while those with a full length HPR HE could not induce fusion. This suggested that HPR length may influence how the HE primes the F protein and promotes fusion activation by an ubiquitous host protease and/or facilitate subsequent post-cleavage refolding steps. Variations in fusion activity through accumulated mutations on surface glycoproteins have also been reported in other orthomyxoviruses and paramyxoviruses. This may in part contribute to the different virulence and tissue tropism reported for HPR0 and HPR deleted ISAV genotypes.

Intrafish reported in June 2015:

Sernapesca maps out Marine Harvest ISA outbreak area

Agency will supervise the infected area, which is considered as at risk.

29 June 2015 13:08 GMT UPDATED 9 May 2016 18:46 GMT

By IntraFish Media

The Chilean National Fisheries and Aquaculture Service (Sernapesca) confirmed early last week an outbreak of infectious salmon anemia (ISA) at a Marine Harvest site in Los Lagos.

Sernapesca established on Thursday the defined infected area as five nautical miles area around Marine Harvest's Punta Lalin center. Centers within the infected area are considered as at risk and will undergo sampling in order to monitor the virus.

Sernapesca said the cage, where the ISA variant HPR 14 virus was detected, was harvested last Tuesday.

"As in previous cases, Sernapesca will strengthen supervision through health campaigns by official veterinarians in order to assess the health status of the farms located in the area, in addition to permanent monitoring of the Punta Lalin center," said Sernapesca National Director José Miguel Burgos. (Copyright)

RELATED NEWS

Marine Harvest cutbacks will impact volumes in 2017
News
29 June 2015 13:10 GMT

Marine Harvest restructures Chilean operations amid weak prices, poor fish health
News
29 June 2015 13:09 GMT

Sernapesca confirms ISA at Marine Harvest Chile site
News
24 June 2015 17:10 GMT

Analysts: Marine Harvest, AquaChile break-up bad news for Chile
News

Intrafish reported in June 2015:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

Norwegian producer ordered to destroy ISA-infected stock

Total destruction of fish 'least risky solution' to prevent spread of disease in Hordaland, Norwegian Food Safety Authority says.

8 June 2015 13:07 GMT *UPDATED 8 May 2018 18:54 GMT*

By **IntraFish Media**

The Norwegian Food Safety Authority (NFSA) ordered producer Bolaks to destroy all of its fish at the farm site in Mid-Hordaland, after an outbreak of infectious salmon anemia (ISA) was confirmed.

"This must happen as soon as possible to prevent the ISA infection spreading to a greater area," said Hallgeir Herikstad, regional director at NFSA, in a press release.

The disease was detected in mid-May at the Nystolvagen site in Fusa, Hordaland County.

According to the newspaper *Bergens Tidende* the farm contains 23,000 farmed salmon averaging 12.7 kilos in weight. These are big fish, which makes the job of harvesting particularly demanding.

"ISA is a grim fish disease, and the danger of infection increases the longer these fish are left in the sea. Mid-Hordaland hasn't had ISA for many years, and that's the way we want to keep it. First and foremost to ensure sound fish health, but also to save the aquaculture industry major financial losses," said Herikstad.

"It's always an extremely serious situation when ISA crops up, and we are in contingency mode.

"In Chile the disease played havoc with the aquaculture industry and caused a total collapse. There is every reason to treat an outbreak of ISA with utmost care, especially in an area such as Hordaland with so much marine farming activity," he said.

NFSA considered several different alternatives for how the fish at the Bolaks site should be dealt with, before it finally decided it had to be destroyed.

A decision to slaughter the fish, instead of destruction, would mean they could be used as food for people.

RELATED NEWS

Researcher: Salmon industry will never conquer disease challenges
News
21 May 2015 13:08 GMT

ISA confirmed at western Norwegian site
News
21 May 2015 13:00 GMT

North Norwegian salmon farm hit by ISA
News
19 May 2015 13:07 GMT

ISA suspected at northern Norwegian farm
News
4 May 2015 13:18 GMT

Sernapesca: Chilean farmed salmon health improved in 2014
News
28 April 2015 13:05 GMT

A scientific paper published in the Virology Journal in 2013 reported:

> Virol J. 2013 Nov 23;10:344. doi: 10.1186/1743-422X-10-344.

Infectious salmon anaemia virus (ISAV) in Chilean Atlantic salmon (Salmo salar) aquaculture: emergence of low pathogenic ISAV-HPR0 and re-emergence of virulent ISAV-HPRΔ: HPR3 and HPR14

Marcos G Godoy, Molly J T Kibenge, Rudy Suarez, Eduardo Lazo, Alejandro Heisinger, Javier Aguinaga, Diego Bravo, Julio Mendoza, Katerina O Llegues, Rubén Avendaño-Herrera, Cristian Vera, Fernando Mardones, Frederick S B Kibenge [1]

Affiliations + expand

PMID: 24268071 PMCID: PMC4222741 DOI: 10.1186/1743-422X-10-344

Free PMC article

Abstract

Infectious salmon anaemia (ISA) is a serious disease of marine-farmed Atlantic salmon (Salmo salar) caused by ISA virus (ISAV), which belongs to the genus Isavirus, family Orthomyxoviridae. ISA is caused by virulent ISAV strains with deletions in a highly polymorphic region (HPR) of the hemagglutinin-esterase (HE) protein (designated virulent ISAV-HPRΔ). This study shows the historic dynamics of ISAV-HPRΔ and ISAV-HPR0 in Chile, the genetic relationship among ISAV-HPR0 reported worldwide and between ISAV-HPR0 and ISAV-HPRΔ in Chile, and reports the 2013 ISA outbreak in Chile. The first ISA outbreak in Chile occurred from mid-June 2007 to 2010 and involved the virulent ISAV-HPR7b, which was then replaced by a low pathogenic ISAV-HPR0 variant. We analyzed this variant in 66 laboratory-confirmed ISAV-HPR0 cases in Chile in comparison to virulent ISAV-HPRΔ that caused two new ISA outbreaks in April 2013. Multiple alignment and phylogenetic analysis of HE sequences from all ISAV-HPR0 viruses allowed us to identify three genomic clusters, which correlated with three residue patterns of ISAV-HPR0 (360PST362, 360PAN362 and 360PAT362) in HPR. The virus responsible for the 2013 ISAV-HPRΔ cases in Chile belonged to ISAV-HPR3 and ISAV-HPR14, and in phylogenetic analyses, both clustered with the ISAV-HPR0 found in Chile. The ISAV-HPR14 had the ISAV-HPR0 residue pattern 360PAT362, which is the only type of ISAV-HPR0 variant found in Chile. This suggested to us that the 2013 ISAV-HPRΔ re-emerged from ISAV-HPR0 that is enzootic in Chilean salmon aquaculture and were not new introductions of virulent ISAV-HPRΔ to Chile. The clinical presentations and diagnostic evidence of the 2013 ISA cases indicated a mixed infection of ISAV with the ectoparasite Caligus rogercresseyi and the bacterium Piscirickettsia salmonis, which underscores the need for active ISAV surveillance in areas where ISAV-HPR0 is enzootic, to ensure early detection and control of new ISA outbreaks, as it is considered a risk factor. This is the first report of ISA linked directly to the presence of ISAV-HPR0, and provides strong evidence supporting the contention that ISAV-HPR0 shows a strong relationship to virulent ISAV-HPRΔ viruses and the possibility that it could mutate to virulent ISAV-HPRΔ.

The European Food Safety Authority reported in 2012:

European Food Safety Authority

EFSA Journal 2012;10(11):2971

SCIENTIFIC OPINION

Scientific Opinion on infectious salmon anaemia (ISA)[1]

EFSA Panel on Animal Health and Welfare (AHAW)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Atlantic salmon is the only species in which the disease infectious salmon anaemia (ISA) has been observed naturally. Initial reports of findings of infectious salmon anaemia virus (ISAV) before 2002, did not distinguish between non virulent HPR0 and virulent HPRΔ viruses, thus making interpretation of older findings difficult in the light of current knowledge. Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the relationship between HPR0 and HPRΔ, the risk of HPRΔ ISAV emerging from HPR0 ISAV, and possible risk factors for such an emergence. HPR0 ISAV does not cause clinical disease in Atlantic salmon; however, it causes a transient subclinical infection and replicates mainly in gills. There is no evidence for HPR0 ISAV leading to natural infection and replication in fish species other than Atlantic salmon. Virulent ISAV have deletions in the HPR region of the HE gene and they have either an insertion or the Q266L mutation in the F gene. The most plausible hypothesis is that virulent ISAV (HPRΔ) is derived from HPR0 ISAV. This is further supported by the close association between the genetic relatedness and spatio-temporal distances of virus strains in solitary outbreaks. Epidemiological and historical data from solitary disease outbreaks indicates that the risk of HPRΔ ISAV emerging from HPR0 is low, but not negligible. The risk factors for HPRΔ emergence from HPR0 are unknown. Nevertheless, any factor that affects virus replication or host susceptibility could possibly influence the risk of emergence. More research is needed on the drivers for transition from HPR0 to HPRΔ and factors affecting host susceptibility and thereby emergence of clinical disease. A quantitative assessment of the different evolutionary forces for ISA would be useful, as well as the prevalence of ISAV HPR0 in farmed and wild Atlantic salmon.

The scientific opinion included:

> Pathogenic HPRΔ ISAV variants cause a systemic infection, infecting endothelial cells of the blood circulatory system whereas HPR0 ISAV does not cause clinical disease in Atlantic salmon but causes a transient subclinical infection and replicates mainly in gills.
>
> ISAV can be genetically differentiated on the basis of the sequence of a highly polymorphic region (HPR) of genomic segment 6 which encodes the Haemagglutinin-Esterase (HE) protein. A deletion within the HPR region (HPRΔ ISAV) is necessary for pathogenicity. ISAV without any deletions in the HPR region (Hereinafter: HPR0 ISAV) has been reported only in apparently healthy fish and has never been associated with clinical ISA disease.

ISAV isolates vary in virulence, as observed by differences in disease development and clinical signs in field outbreaks as well as in experimental trials. All ISAV isolates from ISA disease outbreaks have deletions in the HPR region with respect to the HPR0 variant. In addition, all virulent strains of ISAV have either an amino acid substitution or a short amino acid insertion immediately upstream or downstream of the putative arginine cleavage site in the fusion (F) protein.

The hypothesis that virulent HPRΔ ISAV is derived from HPR0 ISAV by deletions in the HPR of the HE molecule provides the best fit with current knowledge and epidemiological evidence. Epidemiological and historical data from solitary disease outbreaks indicate that the risk of emergence of virulent ISAV is low but not negligible.

Generic biosecurity measures such as segregation of generations, caution regarding contact points (water, equipment), sanitary handling of dead fish, cleaning and disinfection, and synchronous fallowing appear to have a good effect in terms of prevention and control of ISA.

Prior adaptive immunity provides some protection against subsequent infection with virulent ISAV. Little is known about antigenic variation in the haemagglutinin-esterase gene of ISAV and it is not possible to conclude whether this may impact population immunity. Likewise, it is not known if or to what extent prior infection with HPR0 ISAV may induce some degree of protective immunity.

The evolutionary relationship between virulent and low-virulent ISAV forms, where HPR0 mutates into a virulent form of the ISAV, appears plausible. However, no predisposing risk factors have been demonstrated or suggested to drive or increase such an evolutionary process.

Based on general virological knowledge, the risk of emergence of HPRΔ and subsequent development of disease can be expected to be related to the overall replication of HPR0 ISAV and the presence of susceptible hosts. Any factor that affects replication or host susceptibility would, therefore, also influence the risk of emergence of HPRΔ ISAV.

Table 1: Major outbreaks of infectious salmon anaemia

Year	Country	Reference
1984	Norway	Thorud and Djupvik, 1988
1996	Canada	Mullins et al., 1998
1998	Scotland, UK	Rodger et al., 1998
2000	Faroe Islands	Christiansen et al., 2011
2001	USA	Bouchard et al., 2001
2007	Chile	Godoy et al., 2008 Mardones et al., 2009
2009	Scotland, UK	Murray et al., 2010

1.2. History of ISAV diagnosis

The initial reports of findings of ISAV did not distinguish between HPR0 and HPRΔ, making interpretation of older studies more difficult in the light of current knowledge. The distinction between HPR0 and HPRΔ ISAV was established in 2002 (Mjaaland et al., 2002a; Nylund et al., 2003). Based on current evidence and subsequent sequencing of associated ISAV isolates, most of these older studies probably reflect infections with HPRΔ ISAV.

2.4. Geographical distribution of HPR0 ISAV

The first detection of HPR0 ISAV was done on gill tissue from a wild-caught Atlantic salmon in Scotland (Cunningham et al., 2002). In addition to Scotland, HPR0 ISAV has also been detected in farmed Atlantic salmon from the Faroe Islands, Norway, Canada, Chile and Denmark (N.J. Olesen, personal communication).

HPR0 has also been detected in wild Atlantic salmon in the Faroes and Norway. Three out of 88 confirmed wild Atlantic salmon caught by a Faroese research vessel at the feeding grounds in the North Atlantic were HPR0 positive (D.H. Christiansen, personal communication). Furthermore, 4 out of 305 Atlantic salmon caught in rivers in mid-Norway were found to be positive by PCR. Viral RNA from one of them was sequenced to HPR0, clustering phylogenetically with the Faroes cluster. The amount of RNA from the other three was too scarce for sequencing, but still empirically indicated the presence of HPR0. All salmon were caught in an area with on-going ISA outbreaks with virus subtypes associating with a cluster different from that identified as HPR0. The four positive ones were all from the same river and confirmed as "wild salmon" according to fish scale examination. (R. Grøntvedt and T. Lyngstad, personal communication).

The Faroe Islands documented findings of HPR0 in Atlantic salmon in their monitoring from 2005 to 2009. HPR0 was detected on gills 1–13 months post sea transfer (mean 7.7 months). The various cohorts (49) were sampled 5–12 times each year, and the presence of HPR0 on gills showed peaked transient infection profile with peak prevalence up to 100 % lasting for 4 months. Almost all of the cohorts were positive for HPR0. No clinical disease or histopathological consequences have been reported in association with this HPR0 infection in the Faroes (Christiansen et al., 2011).

In Chile, all ISAV strains detected in 2011 were identified as HPR0 (Kibenge et al., 2012). No outbreaks were observed and HPRΔ was not detected.

In a retrospective study in Norway (Lyngstad et al., 2012), ISAV was present in 23 % of 210 cohorts of marine farmed Atlantic salmon along the coast, with no suspicion of ISA. HPR0 ISAV was confirmed in 59 % of these ISAV-positive groups. The rest of the positive groups were not sequenced due to lack of RNA, but the low titres may indicate the presence of HPR0.

The groups were sampled once and at various points in time after sea transfer. In other screening studies, HPR0 has been detected in gill samples from juvenile salmon and in brood stock in the freshwater environment (M. Devold and D. H. Christiansen, personal communication). A low level of HPR0 has also been detected in ovarian fluid of farmed Atlantic salmon (D. H. Christiansen, personal communication).

4.2. HPRdelta emergence from endemic HPR0 infection

Fish susceptibility to virulent viruses may be modulated by acquired immunity. HE is a major antigen of ISAV and contributes to stimulating a protective immune response (see Section 2.4). Previous infections with ISAV, like vaccination with attenuated viruses, may induce a level of protection against subsequent infections with the same or other ISAV variants. The possibility that a subclinical infection with HPR0 might prevent or mitigate subsequent infections with virulent variants has not been investigated.

Industrialised farming of salmon is exposed to a range of environmental challenges, such as high density, low physical activity, water with periodically suboptimal oxygen levels and changing temperatures, intensive feeding and growth rate, and handling. Such exposures may be regarded as chronic or temporary stress inducers, harming their defence mechanisms and aggravating disease outbreak dynamics, as well as potentially influencing the evolution and replication ability of the infectious agents themselves. Moreover, high-density farming increases the number of possible infectious contacts whereby an infection and disease in a single salmon can become a communicable disease in an industrialised high-density setting.

Conclusions and recommendations

General conclusions

Atlantic salmon is the only species in which the disease ISA has been observed naturally.

ISAV can be genetically differentiated on the basis of the sequence of an HPR of genomic segment 6 which encodes the HE protein.

Initial reports of findings of ISAV before 2002 did not distinguish between HPR0 and HPRΔ, making interpretation of older findings more difficult in the light of current knowledge.

A single observation of HPR0 ISAV in wild Atlantic salmon indicates that the virus may exist outside the farmed salmon population.

ToR 1 The capability of HPR0 ISAV to cause clinical disease.

HPR0 ISAV does not cause clinical disease in Atlantic salmon.

It is known that HPR0 ISAV causes a transient subclinical infection and replicates mainly in gills.

There is currently no evidence indicating that HPR0 ISAV leads to natural infection and replication in fish species other than Atlantic salmon.

ToR 2 The risk of HPR-deleted ISA emerging from HPR0 ISA and, if relevant, indicating factors for such an emergence.

2a Risk of HPRΔ emerging from HPR0

All virulent ISAV have deletions in the HPR region of the HE gene and they have either an insertion or the Q266L mutation in the F gene. Transitional viruses having only one of the HE or F mutations have not been found.

The most plausible hypothesis is that virulent ISAV (HPRΔ) is derived from HPR0 ISAV.

The close association between genetic relatedness and the spatio-temporal distance of virus strains in solitary outbreaks further supports the hypothesis that virulent ISAV evolve from HPR0-ISAV.

Epidemiological and historical data from solitary disease outbreaks indicate that the risk of emergence of virulent ISAV is low but not negligible.

2b Risk factors

There is a lack of knowledge on risk factors for HPRΔ emergence from HPR0.

The risk of emergence of HPRΔ ISAV and subsequent development of disease can be expected to be related to the overall replication rate of HPR0 ISAV and the presence of susceptible hosts Any factor that affects replication or host susceptibility would therefore also influence the risk of emergence of HPRΔ ISAV.

Recommendation

Baseline monitoring is needed to estimate the prevalence of ISAV HPR0 in farmed Atlantic salmon and wild fish in proximity to farming facilities.

Recommendations for research

Research should be done to address the drivers for transition from HPR0 to HPRΔ and factors affecting host susceptibility and thereby emergence of clinical disease

To gain further insights into the process of virulence evolution, a quantitative assessment of the different evolutionary forces for ISA would be useful, in particular the relative contribution of mutation and reassortment processes.

A scientific paper published in Diseases of Aquatic Organisms in 2012 reported:

Vol. 101: 197–206, 2012
doi: 10.3354/dao02520

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published November 19

OPEN ACCESS

Low virulent infectious salmon anaemia virus (ISAV-HPR0) is prevalent and geographically structured in Norwegian salmon farming

Trude M. Lyngstad[1,*], Anja B. Kristoffersen[1,2], Monika J. Hjortaas[1], Magnus Devold[3], Vidar Aspehaug[3], Rolf B. Larssen[4], Peder A. Jansen[1]

[1]Norwegian Veterinary Institute, PO Box 750 Sentrum, 0106 Oslo, Norway
[2]University of Oslo, Department of Informatics, PO Box 1080, Blindern, 0316 Oslo, Norway
[3]PatoGen Analyse AS, PO Box 1527, 6025 Ålesund, Norway
[4]Norwegian School of Veterinary Science, PO Box 8146 Dep, 0033 Oslo, Norway

ABSTRACT: Infectious salmon anaemia (ISA) is a severe disease in farmed Atlantic salmon *Salmo salar* that has caused epidemic outbreaks in most salmon-producing countries worldwide. The disease is caused by virulent ISA virus (ISAV). Low virulent variants of the virus, characterised by a full-length sequence in the highly polymorphic region of segment 6 in the virus genome, have been reported with increasing frequencies. These variants of the virus, termed HPR0, have been proposed to be ancestors of virulent ISAV. We examined this idea through studies of the phylogeographic and environmental distribution of ISAV-HPR0, as well as phylogeographic associations between virulent ISAV and ISAV-HPR0. Samples from 232 fish groups were screened for ISAV. Real-time RT-PCR was used for detection of ISAV, and the ISAV haemagglutinin esterase (HE) gene was characterised for positive samples. A Mantel test was used to test phylogeographic associations between pairs of ISAV-HPR0 HE gene sequences. A rank test was used to test associations between HE gene sequences from virulent ISAV and ISAV-HPR0. ISAV-HPR0 was detected in fish groups both in freshwater and marine environments, and in juveniles, on-grown marine salmon and broodstock salmon. Genetic and geographic distances between pairs of ISAV-HPR0 HE gene sequences were positively correlated, suggesting that the population of ISAV-HPR0 is geographically structured. Finally, we found a spatial association between fish groups with virulent ISAV (n = 21) and fish groups with ISAV-HPR0 (n = 27), supporting the hypothesis that ISAV-HPR0 may undergo a transition to virulent ISAV.

INTRODUCTION

Infectious salmon anaemia (ISA) is a severe disease in farmed Atlantic salmon *Salmo salar* that has caused epidemic outbreaks in most salmon-producing countries worldwide (Office International des Epizooties 2009). The disease is caused by the ISA virus (ISAV) within the family *Orthomyxoviridae* (Kawaoka et al. 2005).

ISAV has a genome consisting of 8 segments (Mjaaland et al. 1997), of which segment 6 encodes the haemagglutinin esterase (HE) gene (Krossøy et al. 2001, Rimstad et al. 2001, Falk et al. 2004). The HE gene consists of an N-terminal part, a transmembrane domain and a C-terminal part. A small highly polymorphic region (HPR) is located upstream of the transmembrane region (Devold et al. 2001, Mjaaland et al. 2002). It has been proposed that the polymor-

*Email: trude.lyngstad@vetinst.no

phism in the HPR of virulent ISAV HE genes could be explained by a differential deletion model (Mjaaland et al. 2002). These authors suggested that a longer ancestral sequence, stretching over a 130 nucleotide long region (HPR0), represents a variant from which virulent ISAV with deletions in the HPR may arise. Shortly after, a variant with a corresponding long gene sequence was reported from an apparently healthy specimen of wild Atlantic salmon in Scotland (Cunningham et al. 2002). Detection of ISAV by RT-PCR from wild and farmed salmon without clinical signs of ISA (Raynard et al. 2001, Cook-Versloot et al. 2004, Plarre et al. 2005) further supported the notion that some ISAV strains may be of low virulence. The first reports on the putative ancestral low virulent ISAV (ISAV-HPR0) sequences were followed by numerous findings of ISAV-HPR0 variants from the Faroe Islands, Norway and Scotland, all from apparently healthy fish, supporting low virulence of ISAV-HPR0 (Nylund et al. 2007, McBeath et al. 2009, Christiansen et al. 2011, Lyngstad et al. 2011).

The driving forces behind transitions between virulent and low virulent variants of ISAV are not known. ISAV-HPR0 has not been isolated in cell culture, and a direct link between ISAV-HPR0 and virulent ISAV remains to be demonstrated. Nevertheless, deletion events are more likely to occur than inserts. The presence of different HPR deletion patterns in otherwise highly similar ISAV sequences strongly supports a model of several independent deletions, rather than insertion events (Mjaaland et al. 2002, Nylund et al. 2003).

The occurrence of ISA in Norwegian salmon farming in recent years has been described as partly spatio-temporal clusters of farms with disease outbreaks and partly outbreaks in farms isolated from apparent sources of infection. The local clusters have been explained by horizontal spread of infection between proximate farms, whereas the isolated outbreaks have had an unknown source of infection (Aldrin et al. 2011). It has been suggested that ISA may arise from spontaneous transitions of ISAV-HPR0 to virulent ISAV (Cunningham et al. 2002, Mjaaland et al. 2002, Nylund et al. 2003) and that this may cause either solitary outbreaks or local epidemics through local transmission (Lyngstad et al. 2011). Here we examined this idea through studies of the phylogeographic and environmental distribution of ISAV-HPR0, as well as phylogeographic associations between virulent ISAV and ISAV-HPR0. Since we provide data supporting the scenario of spontaneous transitions of ISAV-HPR0 to virulent ISAV causing disease outbreaks, we also suggest estimates of the annual risk of isolated ISA outbreaks arising from transitions of ISAV-HPR0.

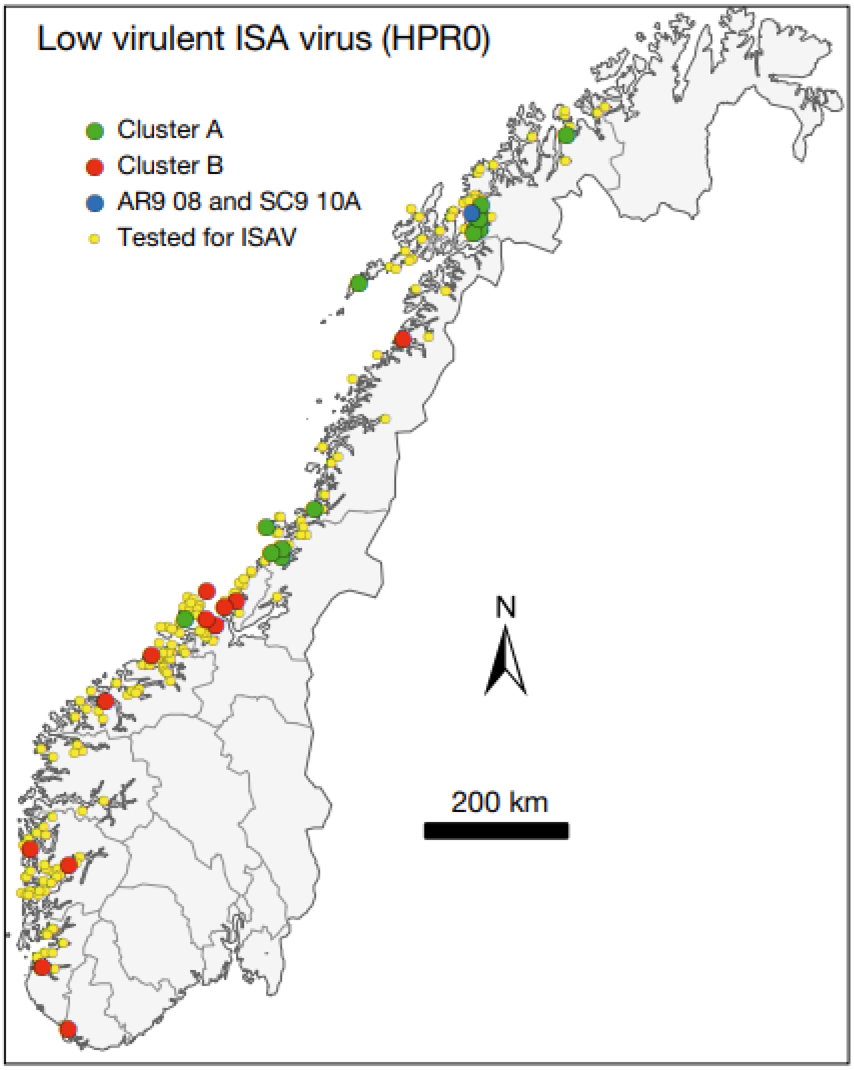


Fig. 1. Farm sites (yellow) in Norway hosting groups of Atlantic salmon that were tested for infectious salmon anaemia virus (ISAV) in the study period (2006 to 2010). The geographical locations for salmon groups with low virulent ISAV (ISAV-HPR0) are shown. Clusters A (green) and B (red) correspond to Clusters A and B in Fig. 2. AR9/08 and SC9/10A represent the sequences also shown in Fig. 2

DISCUSSION

The main results from this study were that ISAV-HPR0 was frequently present in farmed populations of Atlantic salmon in Norway and that it occurs both in marine and freshwater environments, supporting previous findings (Nylund et al. 2007). We found that genetic and geographic distances between pairs of ISAV-HPR0 HE genes were positively correlated, suggesting that the population of ISAV-HPR0 is geographically structured. Finally, we found a spatial association between virulent ISAV and ISAV-HPR0, supporting the hypothesis that ancestral ISAV-HPR0 may undergo a transition to virulent ISAV.

ISAV-HPR0 was detected in Atlantic salmon both in freshwater and marine environments, and in juveniles, on-grown marine salmon and broodstock salmon. In the screening data, all positive samples that were confirmed by sequencing were identified as ISAV-HPR0. Low virus levels in the remaining screening samples, and no ISA suspicion reported in these fish groups, suggests that these were also ISAV-HPR0.

ISAV-HPR0 was frequently detected in the fish groups. Ct-values from previous screenings indicated markedly higher amounts of virus in gill tissues than in kidney tissues or in tissues from a mix of organs (Lyngstad et al. 2011), implying that analysing different tissue material affects the diagnostic sensitivity. Nevertheless, we did find ISAV-HPR0 in a large proportion of the 232 fish groups, and these findings were widely distributed along the Norwegian coast. Hence, this material gave an informative cross-sectional status of the geographical distribution of different genotypes of ISAV-HPR0 in the population of farmed salmon in Norway.

Numerous findings of ISAV-HPR0 have also been reported from the Faroes, where ISAV-HPR0 was detected in nearly 100% of the production cycles (Christiansen et al. 2011). The Faroese results were based on 5 to 12 samplings from all sites each year and showed that after the initial detection of ISAV-HPR0 the level of detectable virus peaked after 2 mo in marine farms. There was no further detection of ISAV-HPR0 after another 2 mo (Christiansen et al. 2011). In Norway, on-grown salmon are kept at marine sites for approximately 18 mo (Kristoffersen et al. 2009). On account of the frequency with which ISAV-HPR0 was detected in groups of marine salmon sampled only once (Table 1), it would not be surprising if practically all groups of marine salmon in Norway are infected with ISAV-HPR0 at some point, i.e. comparable to the Faroese findings.

The statistical analysis showed that sequences of ISAV-HPR0 from fish groups located in relative proximity were more similar than those located farther apart. Also, the phylogenetic analysis grouped the ISAV-HPR0 gene sequences in 2 main clusters: one mainly located in the southern part of Norway and one mainly in the north. The geographically structured phylogenetic pattern for ISAV-HPR0 suggests that local transmission of the virus contributes to shape local virus populations. There was no indication that the geographic structure of ISAV-HPR0 sequences was influenced by the tissue material in the samples. For example, when the analysis was limited to gill-tissue samples only, the geographic structure was retained (Mantel correlation coefficients = 0.59; $p < 0.002$). The geographic structure of ISAV-HPR0 may alternatively agree with vertical pathways of transmission (Nylund et al. 2007), horizontal transmission within farmed populations of salmon (Christiansen et al. 2011) and/or involve a wild reservoir. It is not possible with the present data to distinguish among these alternatives. Nevertheless, salmon farms in Norway are usually stocked with fish from several smolt-producing sites (Lyngstad et al. 2008), and the ISAV-HPR0 detected in juvenile salmon may thus cause unintended spread of the virus when fish are transferred to marine sites (Murray et al. 2002).

If spontaneous transition of ISAV-HPR0 to virulent ISAV does occur, then the fact that the population of ISAV-HPR0 was found to be geographically structured raises the expectation that virulent ISAV should be genetically related to local ISAV-HPR0. The present rank test showed that isolated virulent ISAV-positive fish groups were located significantly closer in distance to the genetically closest ISAV-HPR0 than would be expected by chance. This result supports the hypothesis that ISA may arise from spontaneous transitions of ISAV-HPR0 to virulent ISAV (Cunningham et al. 2002, Mjaaland et al. 2002, Nylund et al. 2003). Assuming that ISAV-HPR0 occurs in 100% of the fish groups, we speculate that the probability of an isolated ISA outbreak occurring reflects the probability of transition from ISAV-HPR0 to virulent ISAV in the given fish group. The risk of such an event in a given group of fish appeared to be low. However, due to the large number of groups of farmed salmon stocked to marine farms annually, this pathway to ISA outbreaks could be an annually occurring phenomenon in Norwegian salmon farming.

With regard to associations between low virulent and virulent ISAV, some studies have suggested that possible transitions may be explained by insertion events, i.e. from virulent ISAV to ISAV-HPR0 (Kibenge et al. 2009, Castro-Nallar et al. 2011), rather than by deletion events (Mjaaland et al. 2002, Nylund et al. 2003). In the present analyses, the time period between sampling did not contribute to explain the association between ISAV-HPR0 and virulent ISAV, lending no support to either direction of hypothetical transition events. Nevertheless, deletion events are argued to be more likely to occur than inserts (Mjaaland et al. 2002, Nylund et al. 2003). It is also worth noting that all detections of ISAV in juvenile fish referred to in the present study were of the low virulent HPR0 type and, furthermore, that outbreaks of ISA have rarely been reported from juvenile salmon in the freshwater phase (Rimstad et al. 2011). Both of these lines of evidence support the hypothesis that ISAV-HPR0 may be a precursor variant from which virulent ISAV may evolve.

In conclusion, ISAV-HPR0 was detected in fish groups both in freshwater and marine environments, and in juvenile, on-grown marine salmon and broodstock salmon. Genetic and geographic distances between pairs of ISAV-HPR0 HE gene sequences were positively correlated, suggesting that the population of ISAV-HPR0 is geographically structured. The spatial association between fish groups with virulent and low virulent ISAV supports the hypothesis that ISAV-HPR0 may evolve to virulent ISAV, indicating that there is a risk associated with ISAV-HPR0 infection. If virulent ISAV evolves from ISAV-HPR0 in a given fish group, then neighbouring fish groups are at elevated risk (Aldrin et al. 2011, Lyngstad et al. 2011). Early detection of ISA outbreaks and implementation of measures to reduce the spread of virulent ISAV thus remain important strategies for controlling ISA.

Intrafish reported in June 2012:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

Smolt producer denies activist allegations

Stofnfiskur denies that its smolt was affected by disease issues, saying it stopped selling to Canada due to Chilean demand.

RELATED NEWS

Iceland accounts for fifth of Chilean smolt
News
8 June 2012 12:08 GMT

20 June 2012 7:15 GMT *UPDATED 8 May 2018 10:29 GMT*

By **Jógvan H. Gardar**

The Icelandic smolt producer Stofnfiskur has dismissed allegations by an environmental activist that its smolt was affected by disease.

In an email to **IntraFish** on June 8, the activist Alexandra Morton took issue to a claim by the World Organization for Animal Health that Iceland "has no viral diseases for fish."

The claim was cited in an article saying Stofnfiskur had supplied 24 million Atlantic salmon smolt to Chile in 2011.

According to Morton, this begs the question of why leading Canadian salmon producers Marine Harvest, Mainstream, and Grieg all "suddenly stopped importing eggs from Stofnfiskur in 2010."

"This was immediately after Marine Harvest began asking the province of British Columbia for over 30 infectious salmon anemia (ISA) virus tests in late 2009, early 2010," Morton wrote.

Morton said Stofnfiskur does not meet Canada's Fish Health Protection Regulations, "but when the Norwegian operators in BC pushed Canada to allow them to have the eggs, Canada agreed citing fear of trade sanctions."

"One of the first shipments had to be destroyed," she said.

"As you may know I am finding ISA virus as well as piscine reovirus in BC farm salmon, both diseases the industry denies exist here. Why have the Norwegian operators in BC suddenly stopped importing eggs from Iceland?"

Intrafish reported in October 2011:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

Alert me about News

Letter: 'Don't shoot the messenger, Marine Harvest'

Salmon farming critic Don Staniford says Marine Harvest should be held accountable for the spread of ISA to wild salmon in Canada.

RELATED NEWS

B.C. wild salmon catch ISA virus
News
18 October 2011 13:09 GMT

20 October 2011 12:59 GMT *UPDATED 20 October 2011 13:27 GMT*

By **IntraFish Media**

Marine Harvest's Jorgen Christiansen is blowing smoke when he doubts the reports of infectious salmon anemia (ISA) in British Columbia because the information came from Alexandra Morton.

Just because Marine Harvest does not like the disastrous news -- which will surely damage their share price and open the floodgates to expensive lawsuits from First Nations and fishermen -- does not make it untrue.

Don't shoot the messenger, Marine Harvest! The bearer of bad news was not so-called 'activist' Alexandra Morton but Fred Kibenge of the Atlantic Veterinary College at the University of Prince Edward Island. Kibenge reported ISA to the Canadian Food Inspection Agency on Oct. 15 in a formal report from the World Organization for Animal Health (OIE).

Marine Harvest knows and respect Kibenge's work all too well – Marine Harvest funded and co-authored a scientific paper on the spread of ISA from Norway to Chile in 2008 and Marine Harvest invited Kibenge down to Chile in 2007 when ISA was first reported. Kibenge's laboratory is an 'OIE Reference Laboratory' – that means it is approved and certified by the World Organization for Animal Health (OIE).

That Marine Harvest now choose to question the validity of Kibenge's results is absurd and smacks of desperation.

Marine Harvest and readers of **IntraFish** are encouraged to read the laboratory report for themselves – it is all here in black and white. The fact is that the European strain of ISA has now spread to British Columbia and is infecting wild Pacific salmon – far away from salmon farms and in young salmon smolts which had not even reached the open ocean.

The nearest farms – sites owned by Marine Harvest – are 100 kilometers away. That points to vertical transmission of ISA and the spread of this exotic disease over a wide geographical range.

We know from scientific evidence published by Cermaq employee Siri Vike and Professor Are Nylund from the University of Bergen that vertical transmission of ISA via infected eggs took place in the spread of ISA from Norway to Chile.

Aquagen - whose shareholders include Marine Harvest and Cermaq – was identified by genetic fingerprinting as the company responsible and this science was endorsed by Cermaq themselves.

Intrafish reported in August 2011:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

Norwegian companies dismiss Chilean ISA claims

Norwegian salmon companies are unequivocal. Economic compensation to Chile is not an option.

RELATED NEWS

Chilean MP wants Norway ISA compensation
News
1 August 2011 13:02 GMT

Cermaq backs study linking Norwegian eggs to Chilean ISA
News
28 July 2011 13:08 GMT

Cermaq backs study linking Norwegian eggs to Chilean ISA
News
28 July 2011 13:08 GMT

Cermaq backs study linking Norwegian eggs to Chilean ISA
News
28 July 2011 13:08 GMT

2 August 2011 13:03 GMT *UPDATED 8 May 2018 18:13 GMT*

By Bent-Are Jensen

On Monday, **IntraFish** reported that the Chilean MP Marisol Turres was re-opening a claim to demand a compensation from Norway over the spread of infectious salmon anemia in Chile.

"It is not an option for us to pay any compensation," Cermaq's spokesperson Lise Bergan told Dagens Naeringsliv, the financial Norwegian daily owned by the same company as **IntraFish**.

"We are big in Chile ourselves, and have lost a lot of money on the disease outbreak. In addition we have invested a lot to come back. So on that basis, we could be demanding compensation as well, but our company will not make such demands," Bergan said.

The background for Turres' claim is a study published in 2008 by the University of Bergen, western Norway. The study was led by professor Are Nylund along with Stian Nylund and Siri Vike. The latter is head of fish health at Mainstream, the salmon farming arm of Cermaq.

Pure Salmon, a U.S.-based non-governmental organization, has passed on the study's results to the Chilean right party, which has a strong base in Puerto Montt and surrounding areas -- the heart of Chile's salmon industry.

"We note the fact that one single Chilean politician wishes to use results from the Norwegian research environment that were published in 2008," Magne Roseth, who heads the salmon ova producer Aqua Gen, told the same newspaper.

Roseth pointed to the conclusions of research done by Chilean fishing authorities and the World Health Organization. "Their conclusion is that the ISA virus could not be traced back to one particular country or source of infection," Roseth said.

Bergan also added that the research's results are not new. "When the problems started in Chile, we did not think that ISA could be transmitted as we know today."

Intrafish reported in August 2011:

Chilean MP wants Norway ISA compensation

A Chilean deputy is demanding hefty compensation from Norway over the infectious salmon anemia (ISA) epidemic that hit Chilean salmon farms in 2007.

1 August 2011 13:02 GMT *UPDATED 8 May 2018 18:24 GMT*

By Bent-Are Jensen

Marisol Turres first announced in 2009 that she would use legal means to gain compensation from Norway for Chile's ISA crisis.

The case did not develop at the time, but now Turres – who is originally a lawyer -- is launching it anew.

Her decision follows the news, in the *New York Times*, that Cermaq has thrown its support behind a study showing that ova from Norway's salmon producers could have been a cause for bringing ISA to Chile.

"I wish to take up this case again based on the new information that has come to light," Turres told a Chilean newspaper, *El Llanquihue*.

Since 2005, Turres represents Chile's electoral department 57. This is at the heart of the country's salmon industry, encompassing the communes of Puerto Montt, Calbuco, Cochamó og Maullín.

Several scientists believe ISA could have been brought to Chile from Norway. This could have happened via the import of salmon ova, a study by the Norwegian University of Bergen – and backed by Cermaq – concluded in 2008.

One person backing the claim is Don Staniford, the anti-aquaculture activist who heads the Global Alliance Against Industrial Aquaculture. Staniford is also the spokesperson for the Pure Salmon Campaign.

Recently Staniford sent a letter to Chile's fisheries department, Subpesca, where he asked if authorities had followed up Turres' original demand of a police investigation into the ISA crisis.

RELATED NEWS

Cermaq backs study linking Norwegian eggs to Chilean ISA
News
28 July 2011 13:08 GMT

UPDATED: 'Put that in your pipe and smoke it!'
News
25 March 2011 7:08 GMT

Mainstream sues NGO
News
25 March 2011 7:04 GMT

Norwegian companies dismiss Chilean ISA claims
News
2 August 2011 13:03 GMT

Cermaq backs study linking Norwegian eggs to Chilean ISA
News
28 July 2011 13:08 GMT

UPDATED: 'Put that in your pipe and smoke it!'
News
25 March 2011 7:08 GMT

Cermaq backs study linking Norwegian eggs to Chilean ISA
News
28 July 2011 13:08 GMT

Chile imported 216 million salmon eggs between January and November 2007. Some 99 million of those came from Norway, while 42 million came from Denmark. In addition to costing some 20,000 jobs, the ISA crisis cost the industry some $900 million (€625.6 million) – without counting the debt to the banks, El Llanquihue said.

The New York Times reported in July 2011:

The New York Times

Norwegians Concede a Role in Chilean Salmon Virus

By Alexei Barrionuevo

July 27, 2011

The scientific study at the University of Bergen linking the virus to eggs was commissioned by Cermaq and first published in 2008 in the Archives of Virology. But in early 2009, shortly after publication, a Norwegian company that breeds fish eggs, Aqua Gen — which is partly owned by both Cermaq and Marine Harvest — filed a formal complaint about the study with Norway's National Commission for the Investigation of Scientific Misconduct, arguing that the science was flawed.

Patrick Dempster, general manager of Aqua Gen in Chile, said that Aqua Gen complained about the study because in 2006 they became the principal exporter of salmon eggs to Chile and were worried about losing business over concern about any vertical transmission connection with Norway.

The commission ruled on April 6 that there had been no scientific misconduct, clearing the three authors from the University of Bergen. Mr. Dempster said Aqua Gen stood by a study from the University of Prince Edward Island that concluded that the virus most likely entered Chile in 1996, when Aqua Gen was not exporting fish eggs to Chile. He noted that between 1996 and 2007 "a multitude" of Chilean and Norwegian companies sent eggs from Norway to Chile.

"We initiated that research because we wanted to understand how I.S.A. was transmitted," Ms. Bergan said. "Before that, the scientific consensus" was that the virus "could not be transmitted by eggs."

Intrafish reported in July 2011:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

Cermaq backs study linking Norwegian eggs to Chilean ISA

Cermaq has endorsed findings that Norwegian salmon eggs are a "likely reason" for the 2007 outbreak of infectious salmon anemia (ISA) in Chile, the New York Times reported.

28 July 2011 13:08 GMT *UPDATED 9 May 2016 18:31 GMT*

By **IntraFish Media**

The findings were the results of a study commissioned by Cermaq and first published in 2008, the newspaper said on July 28.

In the study, carried out by the University of Bergen and published in the Archives of Virology, the scientists concluded that the import of Norwegian eggs into Chile were a likely cause of ISA's subsequent spread in the Latin American country.

The first outbreak of ISA in Chile was reported in a Marine Harvest farm.

Cermaq commissioned the study because it wanted to find out how ISA was transmitted, Cermaq spokesperson Lise Bergan told the *NYT*. Prior to the study, Bergan said, the consensus was that ISA could not be spread by eggs, adding that the study did not identify any company as the culprit.

However, the findings were quickly contested by Aqua Gen, the salmonid egg breeding company which is partly owned by Cermaq and Marine Harvest. In 2009, Aqua Gen filed a complaint about the study with Norway's National Commission for the Investigation of Scientific Misconduct, "arguing that the science was flawed," the *NYT* said.

In April 2011, however, Aqua Gen's complaint was overturned and the commission ruled that no scientific misconduct had taken place.

Speaking to the *NYT*, an Aqua Gen spokesperson said the company stood by a separate study, by the University of Prince Edward Island.

That study, conducted by Frederick Kibeng and commissioned by Marine Harvest, concluded that the virus most likely entered Chile in 1996 – at a time when Aqua Gen was not exporting eggs to Chile -- and showed that some ISA virus strains in Chile diverged from Norwegian strains around 1996.

Like the Bergen study, however, the Prince Edward study leaves open the possibility that the virus spread via eggs.

Aqua Gen are not the only ones not fully endorsing the Bergen study. Chile's national fishing organization Semapesca told the NYT that it stood by findings by the World Organization for Animal Health, which claims that there is insufficient evidence that ISA can be transmitted through eggs.

Click here to read the full story in the *New York Times*. (Copyright)

RELATED NEWS

Chilean MP wants Norway ISA compensation
News
1 August 2011 13:02 GMT

Norwegian companies dismiss Chilean ISA claims
News
2 August 2011 13:03 GMT

CLARIFICATION: Chile reports 13 possible ISA-infected farms
News
5 July 2011 13:03 GMT

Cermaq confident about Chile
News
18 November 2010 8:08 GMT

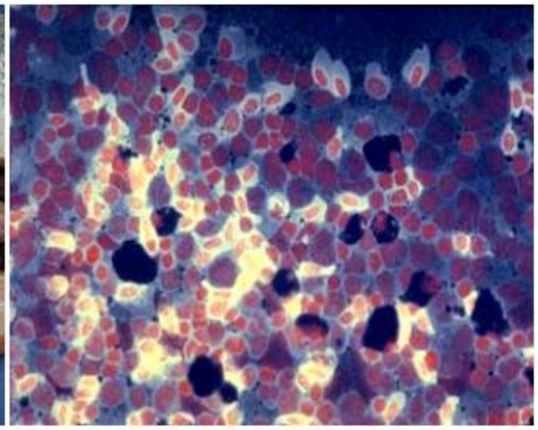
Media under fire from Ewos
News
7 January 2011 8:07 GMT

Chile, Norway butt heads over AquaChile pullout
News
8 September 2010 13:09 GMT

A report – "Fish Farmageddon: the Infectious Salmon Aquacalypse" – published by the Global Alliance Against Industrial Aquaculture in 2011 included:

Infectious Salmon Anaemia (ISA)

The global spread of ISA from Norway in 1984 is a critical case study in everything that is wrong with salmon farming. According to the Norwegian Veterinary Institute: "ISA is caused by a virus which infects and damages blood cells and cells lining the wall of blood vessels. This often results in haemorrhage in inner organs and the fish develops anaemia."



Photos: ISA symptoms and virus

A scientific paper in the *Virology Journal* in 2007 reported that: "Infectious salmon anemia (ISA) virus (ISAV) is a fish orthomyxovirus that has recently been assigned to the new genus *Isavirus* within the family *Orthomyxoviridae*. The virus causes a fatal clinical disease in Atlantic salmon with signs of exophthalmia, pale gills, ascites, congestion of gut, enlargement of liver and spleen, petechial hemorrhages in the visceral organs, and severe anemia."

"Consider ISA the hoof and mouth disease of the global salmon farming industry," reported Stephen Hume in *The Vancouver Sun* in 2008. "According to an information leaflet from the U.S. government's National Fish Health Research Laboratory, infected blood, feces, urine and mucus, animal wastes, contaminated slaughter facilities, transport vessels and workers all easily transmit it from fish to fish and from site to site. As with hoof and mouth, the standard treatment is to kill all infected or exposed stocks within designated containment zones, disinfect all equipment and facilities and then keep fingers crossed."

In Norway, ISA is an ongoing problem nearly thirty years after the first reported outbreak in 1984. A report in 2007 by the Norwegian Veterinary Institute stated that: "A total of 438 outbreaks have been reported in Norway during the time period 1984 and 2005. The yearly number of outbreaks peaked in 1990 with a total of 80 cases." A report – "The Health Situation in Norwegian Aquaculture 2009" – published by the Norwegian Veterinary Institute in 2010 stated that: "Infectious salmon anaemia (ISA) has also been particularly problematical in the South- and mid-Troms area during 2009." Cases of ISA in Norway in 2009 were 10 – down from 17 in 2008 and 23 in 2000 but up from 8 in 2003 and 4 in 2006.

The OIE's 'World Animal Health Information Database' reported three new ISA outbreaks in Norway between June and December 2009 with 1,397,056 'susceptible' fish and 1,087,927 'slaughtered'. In July 2010, there were two new outbreaks reported in Nordland with 987,806 'susceptible' fish and 904,557 'slaughtered'. In June 2011, one new outbreak in Finnmark was reported with 535,921 'susceptible' fish and 19,050 'slaughtered'.

The ISA problem is morphing as new variants of the virus appear. A 'Farmed Fish Health Report 2008' published by the Norwegian Veterinary Institute stated that: "A variant of ISA virus known as HPR0, which is considered to be of low virulence, has been detected in several salmon producing countries."

A scientific paper published in 2011 in the *Journal of General Virology* also detailed a "low-pathogenic variant of infectious salmon anemia virus (ISAV-HPR0)" in the Faroe Islands. The researchers reported that: "ISAV-HPR0 causes a subclinical respiratory infection more like seasonal influenza, as opposed to the systemic infection and serious disease caused by highly pathogenic ISAV" and that "ISAV-HPR0 might represent an ancestor of pathogenic variants and thus be a potential risk factor in the emergence of new strains of disease-causing ISAV".

In Canada, ISA is also still a problem 15 years after the first recorded case in 1996 in New Brunswick. According to a report by the Conservation Council of New Brunswick: "Between 1997 and 1999 nearly 4.5 million fish were slaughtered on 65 sites". The cost to the industry was $50 million.

According to data supplied by the New Brunswick Department of Agriculture Fisheries and Aquaculture, 9.6 million farmed salmon were culled due to ISA between 2000 and 2006: In 2000, nine fish farms were infected and 222,000 fish destroyed. Over the next two years, the numbers increased to 15 sites with 1.1 million fish destroyed in 2001. In 2002, 16 sites were infected with a record 2.4 million fish destroyed. In 2003, 10 sites destroyed 406,000 fish and in 2004 only one infected site was reported. From September 2005 to July 2006, 14 sites were positive for ISA and 950,000 fish were destroyed.

In Chile, ISA was first officially reported in Atlantic farmed salmon in July 2007 with Norwegian-owned Marine Harvest identified as the first company infected. In January 2008, *Fisheries Information Service* reported that 79% of ISA virus outbreaks reported by the Chilean Government and 80% of suspected cases solely involved Norwegian salmon farming company Marine Harvest. In February 2008, Marine Harvest's Q4 2007 financial results reported "a huge write-down of NOK 466 million (EUR 58.8 million)" with *Fisheries Information Service* reporting "A Half Billion Written Off".

In March 2008, *The New York Times* reported: "The new virus is spreading, but it has primarily affected the fish of Marine Harvest, a Norwegian company that is the world's biggest producer of farm-raised salmon...Since discovering the virus in Chile last July, Marine Harvest has closed 14 of its 60 centers and announced it would lay off 1,200 workers, or one-quarter of its Chilean operation." In April 2008, a report – "ISA in Chile" – by SalmonChile concluded: "ISA is an agent the industry will have to keep living with".

In June 2008, an article – "El gran secreto del salmon" (The Big Secret of Salmon) – in the Chilean newspaper *La Nacion* reported that a presentation by Professor Are Nylund from the University of Bergen identified a Norwegian company who brought ISA to Chile from Norway via infected eggs. "It is said that the ISA virus started with the company that imported eggs," said Ricardo Casas. "It is no coincidence that 60% of infected sites are from them". *NRK* in Norway reported in an article – "Sjuk Norsk Rogn til Chile?" ('Sick Norwegian Eggs to Chile') - that: "Norwegian salmon is the origin of sick salmon in Chile, says professor of fish health". Professor Are Nylund of the University of Bergen said: "There is no doubt. Norwegian roe is sold to Chile, and it has been the ISA virus. We have seen a number of ISA virus in Chile. When we go in and characterize the genetic material in them, according to the Norwegian author".

In November 2008, a scientific paper – "ISA virus in Chile: evidence of vertical transmission" - published in the *Archives of Virology* reported that: "Norway exports large amounts of Atlantic salmon embryos every year to Chile; hence, the best explanation for the Norwegian ISA virus in Chile is transmission via these embryos, i.e. vertical or transgenerational transmission.....The brood stock population belongs to a Norwegian brood stock company exporting large numbers of Atlantic salmon embryos to Chile".

A scientific paper – "Epidemiologic investigation of the re-emergence of infectious salmon anemia virus in Chile" - published in 2009 in the journal *Diseases of Aquatic Organisms* reported: "During the 64 wk study period, 76 ISAV-infected salmon farms, representing 17 companies, were reported in 65% of the management geographic zones of the 10th region in southern Chile. Approximately 20% of the farms at risk became infected, with the incidence rate increasing slightly over time. Results from epidemic analyses and observed spatial and spatiotemporal patterns suggested an initial dispersal and subsequent clustering of cases around the index case (IC) in a propagated epidemic mode."

Photo: The ISA crisis in Chile

In Scotland, ISA returned like a nightmare in 2009 a decade after the first recorded cases in 1998-9. "ISA back to haunt fish farmers" reported *The Shetland Times* in January 2009. *The Fish Site* reported that: "According to figures from the World Animal Health Information Database, over the course of 2008 the ISA virus claimed an outbreak in Canada, 20 outbreaks in Chile and 14 outbreaks across Norway. Now, with the inclusion of Scotland, all the big salmon farming countries have recently suffered".

Intrafish reported in an article – "Scottish Sea Farms confirms it owns ISA-infected site" - that Norwegian-owned company "Scottish Sea Farms confirmed it owns the salmon farm site in Scotland where ISA was discovered". Norwegian-owned Grieg Seafood reported the presence of ISA at one of their sites in Scotland and admitted that: "There was always a high risk that the ISAV would spread to neighbouring sites". In May 2009, *The Shetland Times* reported that: "The problems with infectious salmon anaemia (ISA) in Shetland cost the Norwegian multinational Grieg Seafoods over £810,000 in the first three months of the year".

The OIE's 'World Animal Health Information Database' reported in May 2009, for example, two new outbreaks of ISA (5 in total) with 179,008 fish listed as 'susceptible' and 6,658 'deaths' and 169,501 'slaughtered'. In January 2009, one new ISA outbreak was reported with 491,829 'susceptible' fish.

A Scottish report published in March, detailed the following in 2009 for a salmon farm at Langa Isle East in Shetland: "Infectious salmon anaemia. Total number removed from site since confirmation 174,480. Site had an unaccountable loss of 109,289."

ISA was reported in the Faroe Islands (one case) with 5 outbreaks in 2001 and 2002; 10 in 2003; 11 in 2004 and 1 in 2005. The Scottish Government reported that "The Faroe Islands' salmon farming industry was destroyed by ISA in 2000".

According to the Marine Institute in Ireland: "Ireland had its first case of Infectious Salmon Anaemia (ISA) in a trout farm in Mayo". The 2002 annual report stated that ISA Virus was "isolated from sub-clinically infected rainbow trout located on two sites in the west of Ireland".

ISA was first reported in the United States in Maine in 2001. In December 2001, the US Government's Department of Agriculture announced a "Declaration of Emergency Because of Infectious Salmon Anaemia" and estimated losses at $11 million. According to the Conservation Council for New Brunswick all 17 salmon farms in Cobscook Bay were either infected or exposed to the disease and one farm in Passamaquoddy Bay was infected. Two million farmed salmon were slaughtered. Two sites were infected in 2003 and six in 2004.

In Norway and Scotland, escaped farmed salmon infected with ISA have been reported. The Norwegian Veterinary Institute reported in their 'Farmed Fish Health Report 2008':

"Escaped farmed salmon are caught in several rivers in the region of south- and mid–Troms, and ISA-virus has been detected in some of these fish. This is an extremely unfortunate situation which has resulted in a significant degree of media attention. In addition to the risk of transmission of ISA to wild salmon, escaped salmon pose a risk of infection to salmon farms over an area larger than that possible by water-borne infection".

A scientific paper – "Disease interaction and pathogens exchange between wild and farmed fish populations with special reference to Norway" – published in the journal *Aquaculture* in May reported: "Recently, there were two instances of reported escapes of Atlantic salmon from two different sites with ISA in Troms County, North Norway. Escaped salmon were later caught in two different local rivers, and ISAV was isolated from these fish (Johansen et al., 2009b). These instances clearly demonstrate the potential for pathogen exchange from escaped farmed fish to wild fish."

A scientific paper published in the Journal of General Virology in 2011 reported:

› J Gen Virol. 2011 Apr;92(Pt 4):909-18. doi: 10.1099/vir.0.027094-0. Epub 2010 Dec 9.

A low-pathogenic variant of infectious salmon anemia virus (ISAV-HPR0) is highly prevalent and causes a non-clinical transient infection in farmed Atlantic salmon (Salmo salar L.) in the Faroe Islands

Debes H Christiansen [1], Peter S Østergaard, Michael Snow, Ole Bendik Dale, Knut Falk

Affiliations + expand

PMID: 21148272 DOI: 10.1099/vir.0.027094-0

Abstract

Infectious salmon anemia virus (ISAV) is an orthomyxovirus responsible for a significant disease of farmed Atlantic salmon. Fallowing and re-establishment of the Atlantic salmon farming industry in the Faroes following a recent devastating infectious salmon anaemia (ISA) disease epidemic provided a unique opportunity to study the risk of re-emergence of disease. Over 53 months, 2787 of 34 573 (8.1%) apparently healthy Atlantic salmon analysed tested positive for ISAV by RT-PCR. Sequence analysis revealed the putative low-pathogenic ISAV-HPR0 subtype in all cases. Results demonstrated that ISAV-HPR0 appeared as a seasonal and transient infection without detectable ISA mortality or pathology. This finding, coupled to an apparent gill tropism of ISAV-HPR0, suggests ISAV-HPR0 causes a subclinical respiratory infection more like seasonal influenza, as opposed to the systemic infection and serious disease caused by highly pathogenic ISAV. The mean time before marine sites became infected was 7.7 months after transfer to seawater of the fish, suggesting a potentially unknown marine reservoir of infection. Sequence analysis identified two main subtypes of ISAV-HPR0 sequences, one of which showed close genetic association with ISAV isolates responsible for the disease outbreak in the Faroes. Thus ISAV-HPR0 might represent an ancestor of pathogenic variants and thus be a potential risk factor in the emergence of new strains of disease-causing ISAV. Our data, however, suggest that the risk of emergence of pathogenic ISAV variants from a reservoir of ISAV-HPR0 is low. This risk is probably being further reduced by practical management strategies adopted in the Faroes and aimed at reducing the potential for maintenance and adaptation of ISAV-HPR0.

A risk factor proposed to explain new occurrences of ISA is the maintenance of putative low-pathogenic strains of infectious salmon anemia virus (ISAV) within both wild and farmed fish populations. Such strains, which have now been recognized in Norway (Nylund *et al.*, 2007), Scotland (Cunningham *et al.*, 2002; Anonymous, 2005; McBeath *et al.*, 2009), Canada (Cook-Versloot *et al.*, 2004) and Chile (Kibenge *et al.*, 2009), are differentiated on the basis of the sequence of a highly polymorphic region (HPR) in the *HE* gene just upstream of the transmembrane coding region (Cunningham *et al.*, 2002; Mjaaland *et al.*, 2002; Nylund *et al.*, 2003). Early comparative studies led to the suggestion that HPR subtypes associated with ISA outbreaks may have arisen within aquaculture following deletions within this gene with respect to a putative full-length ancestral progenitor, designated ISAV-HPR0 (Mjaaland *et al.*, 2002). The subsequent first identification of such a sequence in wild fish in Scotland (Cunningham *et al.*, 2002), in an area remote from aquaculture activity, lent credence to the hypothesis that ISAV-HPR0 may represent a wild-type form of ISAV which has been introduced and subsequently adapted to virulence as a result of selective pressures associated with intensive fish farming. In support of this requirement for adaptive change is the fact that ISAV-HPR0 viruses differ phenotypically from those responsible for disease outbreaks in that they have not been directly associated with ISA disease, have mainly been detected in gills and remain non-culturable on conventional ISAV-permissive cell lines (Nylund *et al.*, 2007).

From analysis of ISAV sequences originating from Norway, it has been suggested that the shift in virulence from ISAV-HPR0 to a disease-causing ISAV is a stochastic event that is dependent on the replication frequency of the virus and the time available for changes in the highly polymorphic region of the *HE* gene to occur (Nylund *et al.*, 2007). Reducing the capacity for maintenance of ISAV-HPR0 infection within individual farming areas thus offers a potential strategy to minimize the risk of re-emergence of pathogenic variants of ISA and the likelihood of disease emergence.

During the five year period from spring 2000, a total of 33 ISA outbreaks were recorded in the Faroes, with all but two of the 25 licensed salmon-farming areas being affected. Synchronized fallowing of all but one Faroese Atlantic salmon farming site, eradication of ISA and subsequent re-establishment of the industry has provided a unique opportunity to better understand and manage the risks of re-emergence of ISA disease within this area. Practical measures employed following restocking have included tightening of biosecurity procedures within the industry in order to safeguard against the recognized risk of import of ISA from other affected territories, reduction in production intensity, year class separation and scheduled fallowing, vaccination and comprehensive screening programme for ISAV. During a 53 month period, all farms have been regularly monitored for clinical findings and screened for the presence of ISAV using molecular detection and characterization methods. Here we present results from this comprehensive screening, demonstrating that ISAV-HPR0 is widespread, exhibits a different tissue tropism to pathogenic variants of ISAV, shows a transient and seasonal appearance and does not result in clinical ISA.

RESULTS

ISAV-HPR0 is prevalent in Atlantic salmon in the Faroes

Throughout the 53 months (August 2005–December 2009) of the study period a total of 34 573 Atlantic salmon were tested for the presence of ISAV. In total 2787 of the 34 573 (8.1 %) fish tested ISAV positive by RT-PCR (used August 2005–March 2007) or real-time RT-PCR (used April 2007–December 2009) (Table 1). The annual prevalence ranged from 0 % ISAV positive in 2005 to 15.1 % ISAV positive in 2007. The markedly lower detection of prevalence in kidneys obtained prior to 2007 (0.9 % for 2005 and 2006) compared with those obtained in gills post 2007 (10.9 % for 2008 and 2009) reflect changes in the sampling and

Table 1. ISAV-HPR0 prevalence in all seawater farms with production of Atlantic salmon

Total number (*n*) of Atlantic salmon kidneys and gills screened for ISAV and number and percentage (%) tested ISAV positive by RT-PCR (August 2005 to March 2007) or real-time RT-PCR (April 2007 to December 2009) throughout the study period from August 2005 to December 2009.

Year	Kidneys			Gills		
	Total	ISAV positive		Total	ISAV positive	
	n	*n*	%	*n*	*n*	%
2005	2 998	0	0	–	–	–
2006	7 157	10	0.1	–	–	–
2007	6 505*	142	2.2	5 387*	811	15.1
2008	–	–	–	9 066	1 100	12.1
2009	–	–	–	8 847	852	9.6
Total	16 660	152	0.9	23 300	2 763	11.9

*In January and February 2007 kidneys only were collected from 1118 fish.

detection methodology rather than a genuine increase in the prevalence of ISAV-HPR0 (Table 1). During the study period, a total of 49 production cycles were initiated of which 36 (73.5 %) tested ISAV positive. Considering completed production cycles only, an overall total of 27 of 31 (87 %) tested positive for ISAV (data not shown). Of production cycles completed in 2009, for which the more sensitive method of gill testing using real-time RT-PCR was employed throughout, 100 % tested ISAV-HPR0 positive, whereas completed production cycles which commenced prior to the implementation of this improved screening method did not all test positive (data not shown). Thus, the actual prevalence of ISAV-HPR0 in Atlantic salmon production cycles completed prior to 2009 has been underestimated.

Sequencing of the HPR of the *HE* gene of 1–20 selected samples from all 36 production cycles testing positive for ISAV RNA revealed the presence of the assumed low-pathogenic ISAV-HPR0 subtype in all cases.

ISAV-HPR0 exhibits a different tissue tropism to classical ISAV

Parallel testing of kidney and gill tissues for the presence of ISAV-HPR0 from samplings where both gills and kidneys tested positive by real-time RT-PCR showed a significantly higher overall detection in gill tissue compared with kidney (601 gills ISAV HPR0 positive versus 141 positive kidneys, $P<0.0001$ Fisher's exact test (two-sided); Table 2). Only 14 fish tested kidney positive and gill negative, and the highest median C_t values were observed among these samples (median 35.9; range 31.4–36.5), whereas the lowest median C_t values were among the gills of the 127 samples testing both kidney and gill positive (median 28.1; range 19.0–36.7) (Table 2). From one of the sea-site samplings in 2007, individual gill, kidney and heart samples from 80 Atlantic salmon were tested in parallel for the presence of ISAV RNA by real-time RT-PCR yielding HPR0 detection prevalences of 75, 7.5 and 5 %, respectively (data not shown), thus emphasizing the apparently different tissue tropism and higher detection rate in gill tissue.

ISAV-HPR0 infection is not associated with clinical or pathological signs of ISA disease

Despite the high prevalence of ISAV-HPR0 in Atlantic salmon at marine grow-out farms, intensive surveillance revealed no clinical or gross pathological signs consistent with ISA disease. In fact, mortality in the marine grow-out sites with Atlantic salmon proved historically low throughout the study period, suggesting that the presence of ISAV-HPR0 does not result in classical ISA disease or other signs of disease. Furthermore, histological examination of 111 gills, 78 hearts and 105 kidneys from three selected cases with very low C_t values showed no lesions indicative of ISA disease. Although the gills often had focal, moderate hypertrophy and hyperplasia of the lamellar epithelium, no parasites or bacteria, and no major cellular inflammatory cell response were observed. Histopathological findings thus also support the fact that the presence of ISAV-HPR0 itself does not lead to the development of clinical or pathological signs consistent with ISA disease.

ISAV-HPR0 infections are transient and highly contagious

After the initial infection, the spread of ISAV-HPR0 throughout the population followed one of two typical patterns. In the first pattern only one infection peak was observed throughout the production cycle (Fig. 1a). A second pattern was as shown in Fig. 1(b) with two infection peaks. Looking at only the 18 completed production cycles where gills were examined throughout production, 10 (56 %) showed one peak with a mean peak prevalence of 74 % (range 47–100 %) and six (33 %) showed two peaks with a mean first peak prevalence of 56 % (range 30–73 %) and a second peak prevalence of 64 % (range 29–85 %). The mean time lag between the two peaks was 7.5 months

Table 2. 2×2 table showing ISAV-HPR0 detection frequencies for 16 population samplings where at least one gill sample and one kidney sample was positive

Samplings were conducted in 2007 and only include fish ($n=1095$) where both gills and kidneys were tested by real-time RT-PCR. Numbers in bold type are the median C_t values and are followed by the range in square brackets. The threshold was set to 0.005.

		Kidneys		
		Negative	Positive	Total
Gills	Negative	480	14 **35.9** [31.4–36.5]	494
	Positive	474 **31.4** [18.6–37.9]	127 Gills=**28.1** [19.0–36.7] Kidneys=**35.7** [27.1–38.3]	601
	Total	954	141	1095

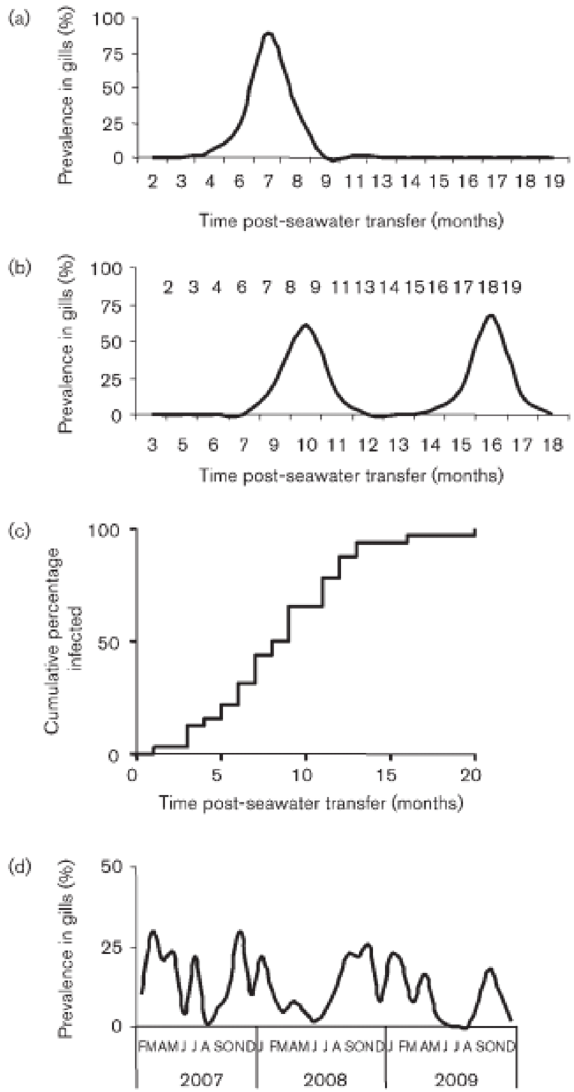


Fig. 1. ISAV-HPR0 infection pattern in Faroese Atlantic salmon marine grow-out sites. Examples of two infection patterns throughout the production cycle are outlined in (a) and (b). (a) Example of a farm with only one ISAV-HPR0 infection peak, at 7 months post-seawater transfer. (b) Example of another site with two infection peaks, the first peak 10 months and the second peak 16 months post-seawater transfer. (c) Outline of the time from post-seawater transfer to initial detection of ISAV-HPR0 shown as cumulative percentage infected for all 36 ISAV-HPR0-positive production cycles. The mean time of infection was 8.5 months post-seawater transfer (range 1–20 months). (d) Overall monthly prevalence of ISAV-HPR0 infection at all sea sites producing Atlantic salmon from 2007 to 2009. Prevalence appeared to peak during the winter. The mean number of fish examined per month for ISAV was 626 (range 151–1003).

(range 4–12 months). Only two production cycles (11 %) showed a peak prevalence <20 %. The lower peak prevalence of the cases with two peaks suggests that the second peak may indicate reinfection. Following these patterns of peaks in detection prevalence no further detections were made at the individual cage level, suggesting a transient appearance of ISAV-HPR0 in all cases with subsequent clearance of virus from the gills. Samplings with a 100 % ISAV prevalence showed a mean C_t value of 28 (range 17–36) whereas samplings with a mean prevalence <20 % gave a mean C_t value >34 (range 30–37) (data not shown).

Marine production sites become infected with ISAV-HPR0 following seawater transfer

The time period from seawater transfer of Atlantic salmon to first detection of ISAV-HPR0 infection is indicated in Fig. 1(c). ISAV-HPR0 was first detected in the marine production sites a mean of 8.5 months after seawater transfer (range 1–20 months). Considering sites where only gills were examined throughout the entire production cycle (2007–2009), the mean time to first detection was 7.7 months (range 1–13 months). Following the initial detection of ISAV-HPR0, the level of detectable virus peaked after a mean of only 2 months (range 1–5 months) with no further detection after a mean of another 2 months (range 1–4 months; data not shown). This strongly suggests a transient ISAV-HPR0 infection in most if not all production cycles of Atlantic salmon in the Faroe Islands.

Infection with ISAV-HPR0 is seasonal

The monthly prevalence of ISAV-HPR0 detections between 2007 and 2009 based on gill screening is indicated in Fig. 1(d). Over 35 months, prevalence of detection varied between 0 and 30 % of the total number of fish tested per month. Detection of ISAV-HPR0 in gill tissue appeared to follow a seasonal pattern with the highest and lowest prevalences being recorded in the winter and summer periods, respectively.

Different genotypes of ISAV-HPR0 are co-circulating in the Faroes

The phylogenetic relationships between Faroese ISAV *HE* sequences derived from ISAV-HPR0 detection, disease outbreaks in the Faroes and those representing each of the established European subtypes (Nylund *et al.* 2007) are depicted in Fig. 2. These results indicate the presence of two distinct classes of ISAV-HPR0 in the Faroe Islands, classified as EU-G2-like and EU-NA-like isolates, respectively. The first group of ISAV-HPR0 Faroese sequences share a close genetic relationship with those derived from the ISAV isolates responsible for the Faroese ISA epidemic, whereas the second group is related to European-like ISAV isolated in North America.

A Scottish Government presentation in 2010 reported:

Scotland update: the current situation in relation to ISA and BKD
Eann Munro
Marine Scotland Science, Marine Laboratory, Aberdeen

The Scottish Government

Introduction: Infectious salmon anaemia (ISA)

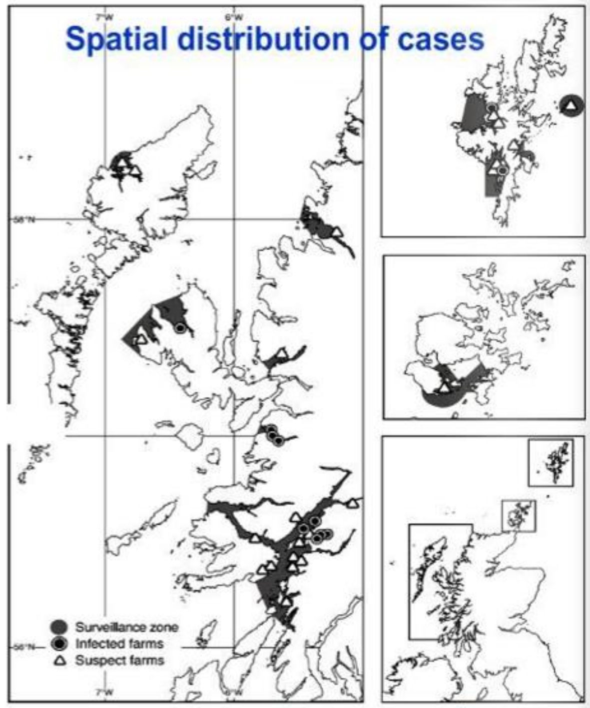
- Multisystemic contagious disease of farmed Atlantic salmon caused by the orthomyxovirus ISAV
- Worldwide distribution
- ISAV capable of mutation
- Genetic analysis indicates independent origins in Europe and North America

EU Legislation governing ISA

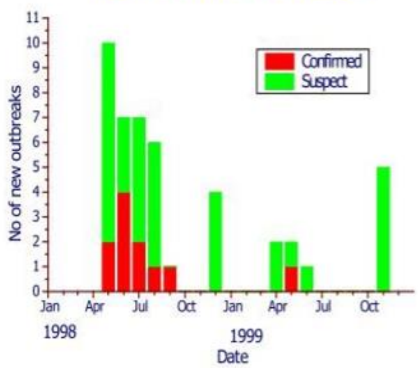
- Council Directive 2006/88
  - Lists ISA as a non-exotic disease
  - Details the control measures to be applied
- Commission Decision 2003/466
  - Diagnosis and criteria for suspicion and confirmation of ISA
  - Explains the criteria for zoning and surveillance

The 1998 ISA outbreak in Scotland

- 11 cases
- May 1998 - May 1999
- Cost to industry >Euro100M



Incidence of cases



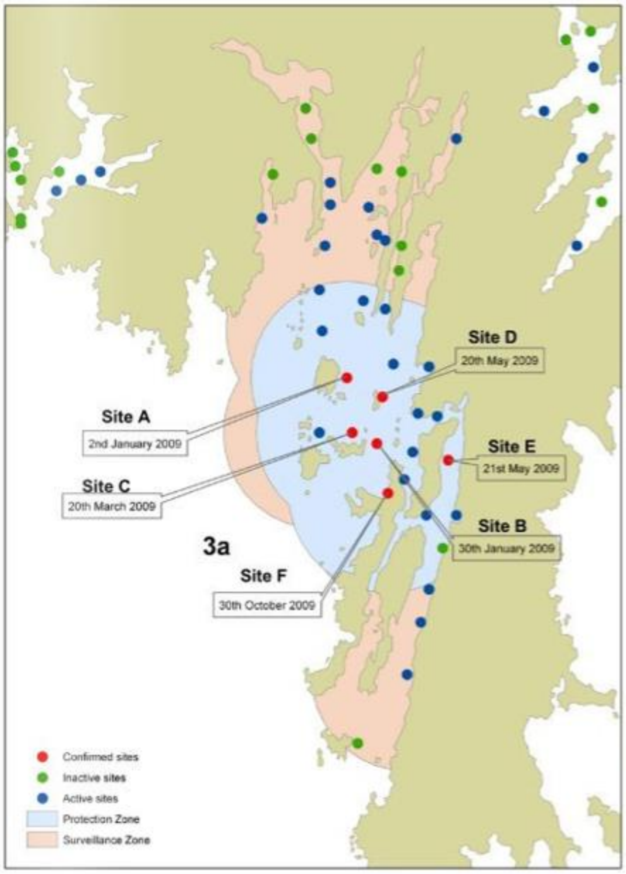
marinescotland

Summary of ISA positive sites (2009) Shetland Isles

Date	Confirmatory Diagnosis	Mortality rates	Atlantic salmon
Site A 2 Jan	PCR, virus isolation.	*Moderate mortality. 10% in one month	42,000 (4.5Kg) Harvesting
Site B 30 Jan	PCR, virus isolation.	*Very low mortality. 0.3% per week	324,000 (1.5Kg)
Site C 20 March	Clinical, gross signs, PCR. (VI)	*Moderate mortality. 7%	263,000 (1.5Kg)
Site D 18 May	Clinical, gross signs, liver pathology, PCR, IFAT. (VI)	*Low mortality. >1% per week	284,000 (2Kg)
Site E 21 May	Clinical, gross signs, PCR, IFAT. (VI)	*High mortality. 50% in one cage	4,000 (1Kg)
Site F 30 Oct	Clinical, liver pathology, PCR. (VI)	Low mortality. 0.7% per cage per week	145,000 (3.9Kg)

* Other diseases/agents present

marinescotland

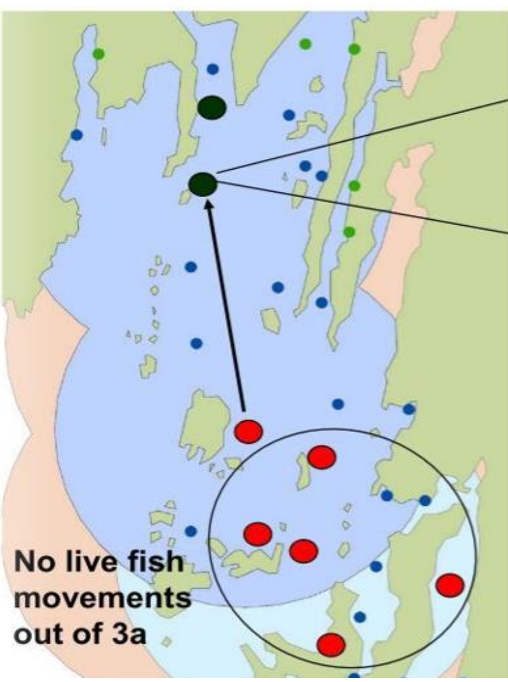


SG policy – eradicate ISA by removal of infected populations.

1 – 7 weeks to remove fish from ISA confirmed sites.
Mean = 26 days.

marinescotland

Epizootic Investigation



Fish moved on 27 June 2008
Last movement from first Infected site

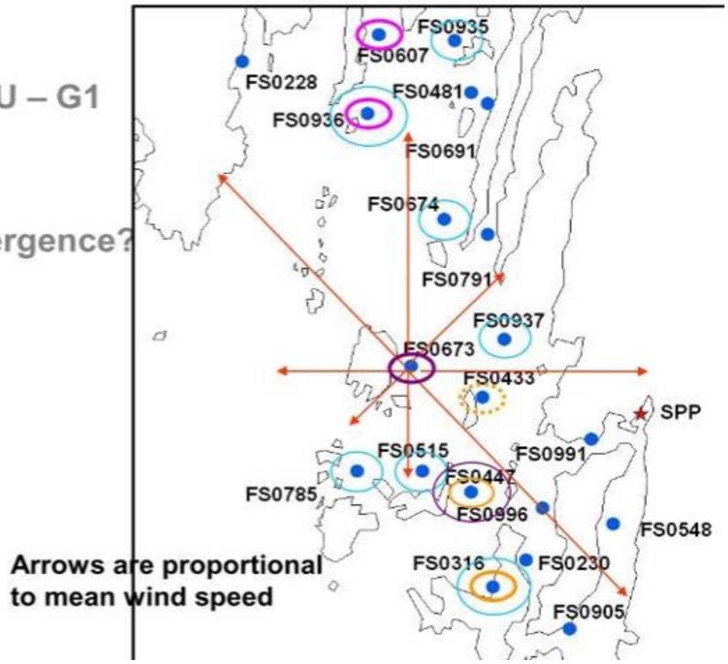
Farm tests negative January 2009

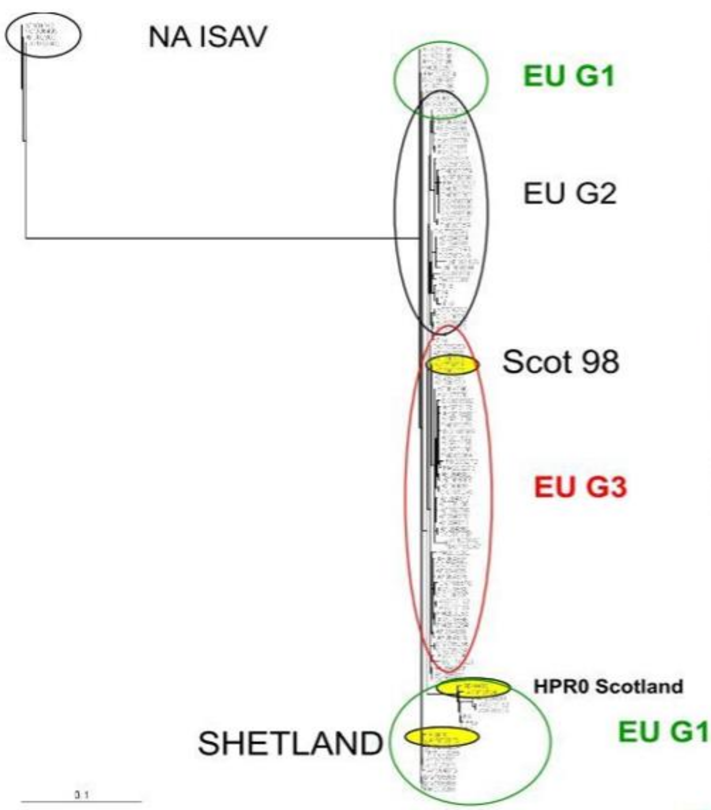
Conclusion
Direct movements did not spread infection therefore infection was not present before 27 June 2008

marinescotland

Likely source and spread

- Infection restricted to area 3a
  - Appears to have occurred after June and spread hydrodynamically
- Unknown source
  - Sequence analysis EU – G1
  - Novel HPR type
  - Unique – 99.4% nt
    - Possible new emergence?
    - Possible Import?





- •ISAV in Shetland (2009) differed to Scotland 1998
- • Neither were similar to Scottish HPR0 (avirulent)
- •Probably other HPR0s in the environment

marinescotland

In 2010, a scientific paper authored by Marine Scotland Science included:

Vol. 91: 189–200, 2010 doi: 10.3354/dao02262	DISEASES OF AQUATIC ORGANISMS Dis Aquat Org	Published September 17

Epidemiological investigation into the re-emergence and control of an outbreak of infectious salmon anaemia in the Shetland Islands, Scotland

Alexander G. Murray*, Lorna A. Munro, I. Stuart Wallace, Barbara Berx, Daniel Pendrey, David Fraser, Rob S. Raynard

Marine Laboratory, Marine Scotland Science, 375 Victoria Road, Aberdeen, AB11 9DB, UK

Vertical transmission: There is disagreement as to whether ISAV is vertically transmitted (Lyngstad et al. 2008, Vike et al. 2009). If vertical transmission did occur then this could be significant both as a potential source of the Shetland outbreak, since imported ova are used (although these are certified ISAV-free), and as a potential route of spread within Scotland and beyond via Shetland-reared broodstock. It is also possible that ISAV could be transmitted on the outside of poorly disinfected eggs (pseudovertical transmission). Therefore, the sources of input of smolts to Shetland and the potential exposure of ova that were produced from broodstock within Shetland are assessed.

A Scottish Government report in relation to the ISA outbreak in Shetland in 2009 (involving Scottish Sea Farms and Grieg Seafood) published in 2010 included: “Local evolution from an avirulent strain of ISAV; importation of ova; or association with movement of equipment could have caused the outbreak”.

Scottish Marine and Freshwater Science Reports

Formal report series, containing results of research and monitoring carried out by Marine Scotland Science

License

UK Open Government Licence (OGL)

Other Access

The information on this page (the dataset metadata) is also available in these formats.

JSON RDF

via the DKAN API

Social

- Twitter
- LinkedIn
- Reddit
- Google+
- Facebook

Report into the Epidemiology and Control of an Outbreak of Infectious Salmon Anaemia in the Shetland Islands, Scotland

Scottish Marine and Freshwater Science Vol 1 No 4

An outbreak of infectious salmon anaemia (ISA) in the Scottish Shetland Islands during 2008/9 is described during which six sites were confirmed ISAV positive. Spread of the virus via movement of fish between marine sites, harvest vessels, movements of smolts and wild fish appear to have been of little or no importance. The spread is associated with hydrodynamic currents, although local intra-company activity may have caused some spread. The application of a statutory control strategy by Marine Scotland, based on the use of its established model (Anon 2000) has apparently limited the occurrence and economic impact of ISA to management area 3a; however spread within this area has been extensive. This localised waterborne spread is in contradiction to a previous outbreak in 1998/9 which was spread over a wide geographic area by transport of fish and harvest vessels. The development of industry codes of practice and good biosecurity procedures, following the 1998/9 outbreak, that limited marine site-to-site movement of live fish and improved disinfection of vessels and processing plant waste, may explain why the 2008/9 spread of infection was localised. Depopulation of confirmed sites is a key element of eradication and this was achieved within 7 weeks of confirmation, although the last confirmed case suggests subclinical infection may persist undetected for months. The potential sources of ISAV infection that were investigated did not determine the origin of the 2008/9 outbreak. Local evolution from an avirulent strain of ISAV; importation of ova; or association with movement of equipment could have caused the outbreak. The virus responsible for the 2008/9 outbreak belongs to a different genogroup (group 1) to the 1998/9 virus (group 3). The intensive cultivation of salmon farming, close proximity of sites and historic absence of synchronous fallowing of management areas is considered to have increased the risk of disease outbreaks and their re-emergence, such as ISA in the Shetland Islands. A policy of synchronous fallowing and stocking of sites within management area 3a is being considered by industry in consultation with Marine Scotland to address this risk. Where movement of fish occurs between sites in 1 different management areas, this represents the greatest risk of regional-scale spread of diseases such as ISA. Controls appear to have been effective in minimising that risk.

doi:
10.7489/1505-1
Citation:
A G Murray, L A Munro, I S Wallace, M Hall, D Pendrey, D, I Fraser, B Berx, E S Munro, C E T Allan, M Snow, R McIntosh, D W Bruno, P A Noguera, D Smail & R S Raynard. 2010. Report into the Epidemiology and Control of an Outbreak of Infectious Salmon Anaemia in the Shetland Islands, Scotland. Scottish Marine and Freshwater Science Vol 1 No 4. Edinburgh: Scottish Government, 44pp.

Data and Resources

PDF **Scottish Marine and Freshwater Science Vol 1 No 4**

Go to resource

The Scottish Government report included:

Table 2

Chronology of the ISA outbreak illustrating the date of a positive sample or last negative sample before harvest. Only qPCR and virology results (in pools of 5 fish) are listed, other results by IHC, IFAT, and histology and diagnostic signs were also available for some cases. Sites harvested before testing began are not listed in table. The number of fish on site is that at the end of 2008, except site A for which this is the number before harvesting began.

Site	Owner	Species	Last Date Sampled	Date Confirmed	Test Result	Date Depopulated	Fish on site
A	C1	Salmon	16/12/2008	02/01/2009	PCR 8/30 V 7/30	20/12/2008	87,000*
B	C2	Salmon	12/01/2009	30/01/2009	PCR 4/30 V8/30	06/03/2009	316,000
C	C2	Salmon	18/03/2009	20/03/2009	PCR 16/30 V15/30	08/04/2009	263,000
D	C2	Salmon	14/05/2009	18/05/2009	PCR 1/1 V1/1	02/07/2009	284,000
E	C3	Salmon	19/05/2009	21/05/2009	PCR 25/30 V26/30	27/05/2009	6,000
F	C4	Salmon	13/01/2009	30/10/2009	PCR 1/1 V 1/1	26/11/2009	286,000
g	C1	Salmon	06/01/2009	N/A	PCR 0/30 V 0/30	07/01/2009	297,000
h	C1	Salmon	08/01/2009	N/A	PCR 0/2	14/01/2009	178,000
i	C2	Salmon	14/01/2009	N/A	PCR 0/30	16/01/2009	301,000
j	C1	Salmon	17/01/2009	N/A	PCR 0/30	20/01/2009	249,000
k	C4	Salmon	16/01/2009	N/A	PCR 0/30	28/01/2009	60,000
l	C2	Salmon	13/01/2009	N/A	PCR 0/30	25/02/2009	250,000
m	C5	Trout	29/01/2009	N/A	PCR 0/30	02/04/2009	142,000
n	C6	Halibut	N/A	N/A	N/A	27/04/2009	6,000
o	C3	Wrasse	N/A	N/A	N/A	N/A	380

3.6.3. Practices Contributing to the Potential Future Re-Emergence of ISA

Virulent ISAV might re-emerge from local avirulent HPR0 or be imported (Murray and Peeler 2005). The HPR0 strain was found on 3 of 30 anonymously surveyed Scottish salmon farms (McBeath et al. 2009); however its presence is not associated with clinical ISA disease. Furthermore ISAV has been detected from wild salmonid fish in Scotland (Raynard et al. 2001; Cunningham et al. 2002). It is therefore unlikely that the eradication of HPR0 from Scottish salmon farms can be cost effective although co-ordinated fallowing will help keep prevalence low. However, as long as HPR0 is present there remains the potential for ISA disease to re-emerge (Cunningham et al. 2002). Utilising local well boats for local farm activities such as harvesting is a good practice and should continue. However, it is possible that ISAV could be imported with other vessels or even escaped or wild fish. It is also possible there is a risk of ISA emergence due to use of imported ova from Norway, however opinion on vertical transmission is divided. Since eradication of HPR0 is not practicable and contact risk for import of foreign virus is low, there is little that can be done to further reduce exposure risk, unless the risk of vertical transmission is significant. Since there is no evidence that the 1998/9 or the 2008/9 ISA outbreaks were linked to vertical transmission, this means that in the future there is a high likelihood that ISA will recur even if the import of ova ceased (although the time taken for this to occur might be increased).

In the event that ISAV is introduced, the emergence of disease is encouraged if the virus can circulate in populations of fish at high levels for long time periods. This circulation is broken by fallowing. Fallowing is currently carried out at the farm level but for it to be more effective in breaking local infection cycles, MAs should be synchronously fallowed. The operators of the fish farms within MA 3a were progressing towards a synchronous fallowing strategy however this had not yet been implemented. Due to the fact that ISAV seems to have persisted undetected on site F for many months, in spite of frequent visits by FHI, anything other than synchronous fallowing could allow such hidden infection to transmit to newly input smolts on neighbouring sites. The emergence of multiple disease problems at the same time (sea lice, SAV and BKD) in addition to ISA further illustrates the need for synchronisation. The 1998/9 outbreak began in a site containing multiple generations of fish (Stagg 2003) underlining the need for fallowing.

If ISA were to re-emerge, it is imperative that there is a quick response by industry and the regulatory authorities; this requires rapidly detecting the presence of ISAV. Neither the 1998/9 nor the 2008/9 index cases were detected by active surveillance. There simply can never be enough FHIs to visit sites frequently enough to detect ISAV within a short time from an outbreak beginning, in the absence of information to target particular sites. However, passive surveillance has also been disappointing with ISA only reported in 1998 once high levels of mortality had begun and the virus was widely dispersed (Murray et al. 2002) and in 2008 mortality was ascribed to high sea lice burdens. In 2008 inspectors did target the putative index site following up reports of mortality and took samples for diagnostic screening, even although there was an alternative explanation for the mortality. This use of intelligence-led sampling indicates that reporting of unusual mortality even if it is 'explained' could be a valuable tool to more effectively targeting Marine Scotland's resources. There is provision for an element of intelligence led surveillance in Scotland's current risk based surveillance programme.

Once ISAV was detected on the putative index site active surveillance of the remaining sites in MA 3a, and sites in other MAs identified as having potential contact with MA 3a, was effective in detecting further ISA cases. This is because the risk-based targeting of surveillance effort allowed these sites to be frequently visited. Site E was detected by passive surveillance, with suspicion of ISA being reported by a veterinarian.

4. Conclusions

An outbreak of ISA has occurred in Scotland. The outbreak is of unknown origin, but was not related to the ISAV that was responsible for the 1998/9 outbreak. Whether ISA evolved from a local avirulent HPR0 virus or was imported via an unknown route, such a re-emergence may be repeated and so, even on eradication, complacency cannot be allowed in aquaculture biosecurity. Policies such as co-ordinated fallowing of areas and risk based surveillance may deny emergent or introduced virus a chance to establish.

A rapid response to the outbreak by Marine Scotland was possible, because of rapid detection of infection. Detection at site A occurred as a result of intelligence from an unconfirmed source in relation to alleged high sea lice mortality in Shetland and inspectors following up general health problems, not specifically targeting ISA. This observation justifies the need for Marine Scotland to investigate reports of mortality, even if ascribed to a non-notifiable cause, and indicates the limits of passive surveillance for notifiable diseases in the face of multiple potential causes of mortality. Intelligence led surveillance plays an important role in Marine Scotland's current risk based surveillance programme. Active surveillance is of limited effectiveness unless the limited available effort can be targeted in a risk-based manner. Even then, subclinical infection may go undetected and so there may be a case for regular diagnostic sampling (instead of just the current one off sample) in the absence of signs of ISA if sites are likely to have been exposed to infection.

Fallowing is important in eliminating ISAV and other diseases and in preventing their emergence in the first place. Currently there is no requirement to fallow whole MAs synchronously, only control zones and this practice may allow subclinical infection to persist. Surveillance zones within a protected area do fallow for 6 weeks asynchronously, which will have a positive effect on minimising re-emergence of disease. Fortunately, all sites in MA 3a were fallowed for the whole winter of 2009/10.

The outbreak was confined to a relatively small area, although the virus spread easily within that area. The strategic breaking of the industry into biosecurity-determined management areas by Marine Scotland Science for the purpose of disease control appears to have been effective at controlling ISAV. Isolation has been maintained because of an absence of seawater-to-seawater movements of fish in the critical period and the disinfection of processing plant waste and well boats. Depopulation of confirmed sites prevents these from acting as sources of infection.

The industry has played a key role in the design of these effective disease control measures (Anon 2000; CoGPWG 2006); this has been done in collaboration with the Scottish Government; at significant financial cost to industry and the government.

There have been serious failings in these controls, seawater-to-seawater movements were quite extensive in the first six months of 2008, and if an outbreak had occurred at that time, ISAV could have spread much more widely, at least throughout Shetland. Similarly, harvest vessels practised ‘bus-stop’ harvests, also potentially risking spread at the regional scale. Some depopulation has been delayed owing to technical or other reasons, allowing large numbers of diseased fish to remain in the environment for considerably longer than the mean 3-4 week period. These detected failings suggest industry practice could be improved to further reduce the risk from any future ISA outbreaks, especially before infection was detected, and also reduce the probability of outbreaks occurring in the first place. These failings aside, industry behaviour combined with the speedy response of Marine Scotland, has played a key role in the confinement of ISAV relative to spread in other countries, or indeed Scotland in 1998/9.

The disease control mechanisms that appear effective at controlling ISAV in Scotland help to control other diseases. However, the contact structure involving freshwater sites spreads throughout the UK, compared to the networks of marine site-to-site movement (local and limited) and of harvest (regional). As such, diseases with freshwater spread are likely to be more difficult to control. Similarly more robust pathogens might transmit over greater distances and so be more likely to transmit between MAs. With suitable modifications, the system of local MAs with associated good biosecurity measures that appears to have been successful in the control of ISA, and refined in the light of epidemiological information, may be applied to the control of a range of diseases.

A scientific paper published in Diseases of Aquatic Organisms in 2009 reported:

Vol. 87: 161–169, 2009
doi: 10.3354/dao02128

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published December 3

Surveillance for infectious salmon anaemia virus HPR0 in marine Atlantic salmon farms across Scotland

Alastair J. A. McBeath, Nicola Bain, Michael Snow*

Marine Scotland Marine Laboratory, PO Box 101, Victoria Road, Aberdeen, AB11 9DB, UK

ABSTRACT: Infectious salmon anaemia virus (ISAV) is a serious and commercially important pathogen of Atlantic salmon. Multiple viruses have been defined based on a highly polymorphic region (HPR) of the haemagglutinin-esterase (HE) protein encoded by genomic segment 6. The viruses causing disease outbreaks in farms to date all have deletions in this region with respect to a putative ancestral variant with a longer HPR (HPR0). The presence of HPR0 nucleic acid has been detected in many countries including Scotland, where it has mostly been associated with healthy wild and farmed fish. Pathogenic ISAVs appear to have been derived from HPR0 ancestors on multiple independent occasions, which suggests that the presence of HPR0 could represent a risk factor in the re-emergence of infectious salmon anaemia (ISA) disease. In order to better understand this potential risk factor, anonymous samples of gill and heart tissues from marine Atlantic salmon farms throughout Scotland were collected and screened for the presence of ISAV RNA. Since it has not been possible to isolate HPR0 in conventional ISA-permissive cell cultures, a sensitive real-time RT-PCR method was employed for the detection of viral RNA. DNA sequencing was carried out on the positive samples to determine their HPR sequence. ISAV RNA was detected in 6 samples originating from 4 different locations and sequence analysis indicated the viruses were of the HPR0 type. Full length segment 6 sequence analysis of 1 positive sample indicated that it was most similar to a European genotype sequence previously obtained from North America.

KEY WORDS: Infectious salmon anaemia virus · HPR0 · Scotland · Real-time PCR · Segment 6 · Phylogeny · *Salmo salar*

INTRODUCTION

Infectious salmon anaemia (ISA) is a commercially important disease which has exerted a significant impact on Atlantic salmon *Salmo salar* aquaculture worldwide. It has been reported in Norway (Thorud & Djupvik 1988), Canada (Mullins et al. 1998, Bouchard et al. 1999, Lovely et al. 1999), the USA (Bouchard et al. 2001), Scotland (Rodger et al. 1998), Chile (Kibenge et al. 2001a, Godoy et al. 2008) and the Faroe Islands (Anonymous 2000). The aetiological agent, infectious salmon anaemia virus (ISAV), belongs to the genus *Isavirus* of the family *Orthomyxoviridae* and possesses a genome comprised of 8 single-stranded RNA segments (Mjaaland et al. 1997). The sixth largest gene segment encodes a haemagglutinin-esterase (HE) protein which mediates both receptor binding (Krossøy et al. 2001, Rimstad et al. 2001) and receptor destroying activities (Falk et al. 2004) and may also promote fusion activity (Aspehaug et al. 2005).

A highly polymorphic region (HPR) limited to a 35 amino acid stretch has been identified within the ISAV HE protein (Devold et al. 2001, Kibenge et al. 2001b, Krossøy et al. 2001, Rimstad et al. 2001, Mjaaland et al. 2002, Falk et al. 2004). Based on this region, 24 differing predicted protein sequences have been identified in Europe and 4 in North America (Nylund et al. 2007). The observed differences in this polymorphic region can all be explained as deletions (Mjaaland et al. 2002) from a putative ancestral sequence (HPR0) which was

*Corresponding author. Email: m.snow@marlab.ac.uk

first identified in asymptomatic wild fish in Scotland (Cunningham et al. 2002). All currently characterised isolates from ISA disease outbreaks have deletions in their HPR with respect to the HPR0, which strongly indicates that this region is important in ISAV pathogenecity in farmed Atlantic salmon (Mjaaland et al. 2005). An alternative theory involving insertions into the HPR resulting in viral attenuation has also been proposed (Kibenge et al. 2007).

Slightly different HPR0 sequences have been detected in all the major Atlantic salmon growing regions of the Northern Hemisphere including Norway, Scotland, the Faroe Islands and Canada (Cunningham et al. 2002, Cook-Versloot et al. 2004, Christiansen & Østergård 2007, Nylund et al. 2007). While the HPR0 subtype has been reported in both wild and farmed Atlantic salmon, it has not been associated with clinical disease characteristic of ISA. Its presence has generally been detected in asymptomatic fish, with the exception of association with proliferative gill inflammation in some instances (FRS 2005, Nylund et al. 2007, Markussen et al. 2008).

Although the presence of ISAV HPR0 may not cause disease, its presence is of concern with respect to the potential re-emergence of ISA disease-causing variants in Scotland. The number and diversity of HPR0 variants circulating within the aquaculture industry indicates that emergence of pathogenic variants within aquaculture has likely occurred on numerous and independent occasions. Furthermore, the selective environment of aquaculture has previously been shown to promote a rapid evolution of RNA viruses (Einer-Jensen et al. 2004).

Following the eradication of ISA disease from the 1998–99 outbreak in Scotland, some evidence for the continued presence of viral RNA in Scottish waters has been detected (Cunningham et al. 2002, FRS 2005), although targeted surveillance aimed at detection of ISAV HPR0 had not been conducted. Nylund et al. (2007) identified evidence suggesting that nearly all marine production sites in Norway contained salmon that were positive for ISAV infection, despite only around 15 sites developing ISA disease annually. Furthermore, extensive evidence for viral presence, suggested to be HPR0, has been demonstrated in freshwater (Plarre et al. 2005, Nylund et al. 2007). Given such evidence, the status of Atlantic salmon farms in Scotland with respect to ISAV is clearly an important issue to address. An assessment of the distribution of ISAV HPR0 across Scotland will help understand and manage the risk posed by its presence. Whether the presence of HPR0 was a contributing factor to the recent isolation of ISAV in the Burra region of the Shetland Islands remains to be determined (FRS 2009).

Since ISAV HPR0 has not been cultured to date, real-time PCR of segment 8 and subsequent sequencing of segment 6 from positive samples was applied to the detection of ISAV variants within the Scottish Atlantic salmon marine aquaculture industry. Surveillance was conducted anonymously and organs known to harbour HPR0 ISAV (gill and heart) were assayed. The present study details the results of surveillance for ISAV within Atlantic salmon aquaculture and discusses the implication of findings with respect to the potential for re-emergence of ISA disease in Scotland.

RESULTS

Surveillance of Scottish marine Atlantic salmon farms for ISAV

Of 36 Atlantic salmon marine aquaculture sites screened for the presence of ISAV RNA using the segment 8 real-time PCR assay, 4 sites proved positive (11.1 %) (Table 2). All positive samples originated from the Scottish mainland, with 3 in NWM and 1 in SWM. No positives were identified in samples originating in OS or WI. A sample was only deemed positive for ISAV following detection of viral RNA in all 3 of the triplicate quantitative PCR (qPCR) reactions. All positives resulted from gill tissue (ELF mean ± SD cycle threshold [Ct] value = 17.24 ± 0.45). Of the 4 sites, 1 collected in May 2007 resulted in all 3 gill tissue pools positive (mean Ct values: NWM10 p1, 36.59 ± 0.95; p2, 29.69 ± 0.55; p3, 32.50 ± 1.5) and the remaining 3 sites had only 1 positive pool in each (NWM7 p1, 35.56 ± 0.36; NWM2 p1, 35.93 ± 1.2; SWM17 p3, 36.53 ± 1.18, collected in November 2006, April 2007 and November 2007, respectively); thus 5.6 % (6 out of 108) of the total gill tissue pools were positive.

Table 2. Number of sites from which gill and heart samples were received and tested for infectious salmon anaemia virus using real-time PCR targeting segment 8. Positives were detected in gill samples only. SWM: southwest mainland; NWM: northwest mainland; WI: Western Isles; OS: Orkney & Shetland Isles. Mean ± SD cycle threshold (Ct) values shown (n = 3)

Area	No. of sites tested	Results	Ct values (area code)
SWM	6	1 positive site	36.53 ± 1.18 (SWM17)
NWM	13	3 positive sites	35.93 ± 1.2 (NWM2) 35.56 ± 0.36 (NWM7) 36.59 ± 0.95, 29.69 ± 0.55, 32.50 ± 1.5 (NWM10)[a]
WI	6	Negative	–
OS	11	Negative	–
Total	36	–	–

[a]This site positive in all 3 tissue pools

DISCUSSION

The present study presents evidence for the presence of detectable levels of ISAV HPR0 RNA in approximately 10% of the Scottish marine Atlantic salmon production sites sampled. This extends the known distribution of HPR0, the presence of which had previously been described in wild and farmed fish in Scotland (Cunningham et al. 2002, FRS 2005), Norway (Plarre et al. 2005, Nylund et al. 2007), North America (Cook-Versloot et al. 2004) and the Faroe Islands (Christiansen & Østergård 2007).

The results obtained indicate the presence of very low levels of viral RNA which were detectable only in gill and not in heart tissues. Previous studies have supported high levels of viral detection in gills (FRS 2005), which were selected for sampling on this basis. Pooling of samples was employed for practical purposes, which could have resulted in reduced sensitivity. Replicated results from highly controlled assays coupled to subsequent genetic characterisation of the HE gene in some cases, however, support the reported findings.

To date, the isolation of the HPR0 ISAV variant in ISAV permissive cell lines has not been reported and its presence has only been demonstrated in apparently healthy fish using PCR-based methods. This evidence, coupled to a different tissue tropism with HPR0 largely being detected in gill tissues, suggests that HPR0 differs in its fundamental biological properties to variants which are responsible for outbreaks of disease in Atlantic salmon. It is tempting to speculate that these biological differences may be directly linked to HPR0 alteration of the ISAV HE protein stalk region, although this remains to be definitively demonstrated. Similar alterations in paramyxoviruses can lead to differences in important virulence-related factors such as receptor specificity and cellular tropism (de Leeuw et al. 2005) and, in influenza neuraminidase, stalk length has been correlated with replication efficiency (Castrucci & Kawaoka 1993).

While ISAV HPR0 may not directly cause disease, increasing evidence suggests that disease-causing variants of ISAV may have evolved from HPR0-like ancestors on multiple independent occasions. HPR0 sequences are present in each of the 3 main European subgroups described by Nylund et al. (2007) and shown in the wider analysis of the present study (data not shown). According to Nylund et al. (2007) the emergence of a pathogenic variant from an endemic reservoir of ISAV HPR0 may be based on a stochastic event that is dependent on the replication frequency of the virus and the time available for changes in the HPR of the HE gene to occur. The number of HPR variants characterised to date is testament to the adaptability of ISAV and indicative of such changes occurring relatively frequently. The maintenance of an HPR0 reservoir on marine aquaculture farms thus represents a likely risk factor in the emergence of ISA disease.

The localisation of positive detections to mainland Scotland, in particular the northwest, was not statistically significant and likely reflected the non-random nature of the sampling strategy. The anonymous nature of the sampling also makes epidemiological follow-up difficult.

At least 2 geographical reservoirs of ISAV, a North American subtype (NA-ISAV) and a European subtype (EU-ISAV), have previously been suggested (Nylund et al. 2007). The 2 broad groupings were supported by the present study which classified the ISAV HPR0 subtype detected here within the European clade. The HPR0 sequence type identified here has not been reported previously in Europe but is very similar to a type originating in Maine, USA (AY973194), which was not associated with ISA-induced mortality (Nylund et al. 2007) and also in asymptomatic fish from New Brunswick, Canada (AY646060–AY646061) (Cook-Versloot et al. 2004). Interestingly, the other 3 sequences within this small cluster (EU-NS) — AF294881 (Kibenge et al. 2001b), AY973182 (Nylund et al. 2007) and AY601904 (Devold et al. 2006) — are from HPR3-type viruses which were associated with ISA disease (Nylund et al. 2007). The origin of these viruses might thus be traced to an HPR0 ancestor similar to that characterized in the present study. The relative efficiency of horizontal transmission of virulent ISA viruses, however, makes it difficult to determine whether this cluster represents multiple independent emergences or a single emergence followed by horizontal transmission of the virulent virus.

Molecular epidemiological analysis suggests that viruses in subgroup EU-NS, despite being isolated from salmon farms in North America, most likely resulted from transfer of a European-type virus to this region, possibly as a result of anthropogenic activity (Nylund et al. 2007). The present study further supports this hypothesis by identifying closely related sequences currently in circulation within Europe. Despite the fact that 1.8 million Atlantic salmon ova (A. Warwick, Marine Scotland Fish Health Inspectorate, pers. comm.) have been imported to Scotland from the US in recent years, no evidence of transfer of North American subtypes of ISAV was identified in the present study. It remains feasible, however, that European viruses in circulation in North America could have been returned to Scotland through such a mechanism.

Vertical or transgenerational transmission of avirulent ISAV isolates has been proposed as a mechanism for maintenance of ISAV HPR0 within Atlantic salmon populations in Norway (Nylund et al. 2007). Evidence of vertical transmission of ISAV in Chile has also recently been published (Vike et al. 2009). Another proposed transmission route is that of infection from wild marine and freshwater sources. To date, ISAV has been detected in healthy wild salmonid species in Scotland (Raynard et al. 2001, Cunningham et al. 2002) and Norway (Plarre et al. 2005). Whether marine farmed Atlantic salmon pick up HPR0 infection from wild reservoirs that have been shown to harbour ISAV HPR0 or whether infection is sustained in farmed fish populations is unclear.

It is also unclear where the previously reported HPR0s from Scotland would fit into Fig. 1, as in both cases only partial sequencing of the HPR was performed (Cunningham et al. 2002, FRS 2005). However, the results do suggest that the cause of the Scottish outbreak in 1998, Nevis AJ276859 (Rimstad et al. 2001), probably did not arise directly from an NWM10 HPR0-like ancestor. The origin of the disease outbreak was never explained with certainty, but 2 hypotheses were put forward: accidental transportation from an infected area or emergence from a wild reservoir. In the latter case, emergence may have occurred from an as-yet uncharacterised HPR0 in Scotland.

Evidence for the presence of a putatively avirulent ISAV HPR0 virus in Scottish Atlantic salmon farms is of concern in relation to the risk of reemergence of ISA disease in Scotland. The fast evolution rate of viruses and suggested hypothesis for deletions or reassortment giving rise to pathogenic strains underlines the requirement for maintenance of a vigilant surveillance regime for ISAV in Scotland. It also highlights the role of good husbandry practice in limiting the long-term maintenance of HPR0 within populations and thus reducing the opportunity for evolution and emergence of disease-causing ISAV variants. Further research, including understanding the distribution of the agent in freshwater production, is fundamental to understanding the likely origin, maintenance and risk posed by ISAV HPR0 to Scottish Atlantic salmon aquaculture.

Acknowledgements. The involvement of the anonymous farms within the industry who allowed the provision of samples is greatly appreciated. Thanks also to the Marine Scotland Fish Health Inspectorate for carrying out the sampling.

The World Organization for Animal Health (OIE) reported in 2009:

CHAPTER 2.3.5.

INFECTIOUS SALMON ANAEMIA

1. Scope

Infectious salmon anaemia (ISA) is an orthomyxovirus infection of sea-farmed Atlantic salmon (*Salmo salar*) (28) inducing a systemic and lethal condition characterised by severe anaemia and variable haemorrhages and necrosis in several organs. The disease course is prolonged with low daily mortality (0.05–0.1%) typically only in a few cages, but cumulative mortality may become very high. For the purpose of this chapter, ISA is considered to be infection with salmon anaemia virus (ISAV) (12).

2.3. Disease pattern

2.3.1. Transmission mechanisms

The disease is spread horizontally by water-borne transmission as shown by experimental infection studies. There is no strong evidence for vertical transmission through infected gonadal products. It has been suggested that ISAV is spread over long distances by transportation of smolt, either infected prior to shipping or by well boats contaminated with ISAV. Contamination of well boats may be due either to previous transport of infected fish or through intake water from areas with farms harbouring diseased fish.

Epidemiological studies have shown that the risk of ISA transmission is closely linked to husbandry practices in aquaculture and horizontal transmission. Geographical or hydrological (via prevailing currents) proximity (<5 km) to farms with ISA outbreaks or slaughterhouses/processing plants releasing contaminated water, numerous smolt deliveries and the use of well boats, and sharing staff and equipment are all considered significant risk factors (1, 11, 15, 25).

Other horizontal pathways have also been suggested, such as transmission through sea lice, infected wild fish and various harvesting methods (14, 24). According to Nylund *et al.* (22) vertical or transgenerational transmission may occur. Carryover or stocking of multiple year-classes on a given site, or within a region connected hydrologically, may also influence occurrence of ISA (11).

Manual of Diagnostic Tests for Aquatic Animals 2009

The Fish Site reported in November 2009:

New ISA Case Found in Shetland Control Zone

HEALTH

by The Fish Site
2 November 2009, at 12:00am

SCOTLAND, UK - A sixth case of the highly contagious salmon virus infectious salmon anaemia (ISA) has been found off the south-west coast of Shetland, almost one year after the disease was first identified.

Shetland News reports that on 30 October, fish inspectors announced that they had discovered ISA at a site owned by Skelda Salmon, within a control zone imposed in January 2009 when the disease was first identified in the area.

Skelda Salmon, a family business owned by crofter Robert Nicolson, of Twatt, Bixter, had been the only company to remain unaffected by the disease, which has cost the industry in Shetland millions of pounds this year.

"It's very unfortunate because we thought we had done everything we could to get that fish as healthy as possible, and I think it is a credit to us that we have gone so long without ISA," Mr Nicolson said.

The latest discovery throws a question mark over plans by fish farmers in the area to restock their cages next March under a new area management agreement, as there has to be a fallow period of six months after the disease is identified.

ISA is a notifiable disease, but though it can prove fatal to fish it does not pose any risk to human health.

According to *Shetland News*, Mr Nicolson had been allowed to keep growing the 250,000 salmon he had in eight cages, and had harvested half of his stock by the time scientists found a 'slow moving fish' on 28 October. They subsequently tested six fish and identified ISA.

He is being allowed to cull the rest of his stock, which he is growing on behalf of Norwegian firm Lakeland, under strict supervision.

ISA was first discovered in the area on 2 January at a salmon farm owned by Norwegian multinational Scottish Sea Farms, after government scientists from Marine Scotland carried out random samples following reports of large infestations of sea lice.

A control zone was immediately imposed containing 11 fish farming sites, with a surveillance zone extending out to include 31 sites.

A second case of ISA was found a week later on a farm owned by another Norwegian multinational, Hjaltland Seafarms. Two more Hjaltland sites were found to be infected in March and May, with experimental cages operated by NAFC Marine Centre scientists testing positive in May also.

BBC News [reported in March 2009]:

NEWS | LIVE BBC NEWS CHANNEL

- News Front Page
- World
- UK
- England
- Northern Ireland
- Scotland
- Scotland politics
- Wales
- Business
- Politics
- Health
- Education
- Science & Environment
- Technology
- Entertainment
- Also in the news
- Video and Audio
- Have Your Say
- Magazine
- In Pictures
- Country Profiles
- Special Reports

Related BBC sites

Page last updated at 17:41 GMT, Friday, 20 March 2009

E-mail this to a friend | Printable version

Third case of salmon infection

A disease which can kill salmon has been confirmed at a third fish farm in Shetland.

A previous outbreak of ISA in 1998 cost the industry £100m

Infectious Salmon Anaemia (ISA) was found at a fish farm close to two others where the disease was first detected in January.

All fish at the site, which is within a controlled zone, will now be killed as soon as possible.

The disease does not affect humans but can cause serious damage to stocks of farmed Atlantic salmon in seawater.

The latest outbreak was discovered at a farm run by Hjaltland Seafarms.

Protection measures will remain in place to try to avoid any further spread.

An outbreak of ISA in Shetland, the Western Isles and Orkney in 1998 and 1999 was estimated to have cost the industry £100m and led to the loss of 200 jobs.

SEE ALSO
- Second case of salmon infection 30 Jan 09 | North East/N Isles
- Deadly salmon infection detected 04 Jan 09 | North East/N Isles
- Report details disease links 23 Mar 09 | Scotland
- Lethal fish infection spreads 04 Nov 99 | Scotland
- Salmon farmers win compensation 06 Sep 99 | Scotland
- Salmon farming restrictions lifted 19 Aug 99 | Scotland

RELATED INTERNET LINKS
- Fisheries Research Service
- Scottish Government

The BBC is not responsible for the content of internet sites

TOP NORTH EAST/N ISLES STORIES
- Teenage pair shot dead at house

The Fish Site [reported in March 2009]:

10,000 Tonnes of Shetland Salmon Affected by ISA

ECONOMICS | POST-HARVEST | WELFARE | HUSBANDRY | BIOSECURITY

by The Fish Site
20 March 2009, at 12:00am

SHETLAND, UK - More than 10,000 tonnes of salmon, around one fifth of Shetlands annual production, will not go into the sea this year as a result of health problems on the west side of the islands.

The industry has warned that jobs could be affected with the loss of almost 3 million salmon smolts, worth around £30 million when harvested, which would have otherwise gone into cages in the Scalloway area, writes Pete Bevington in the Shetland Marine News.

According to the local report, this year no new fish will be grown in the control zone established by the Scottish government in January, following the discovery of infectious salmon anaemia (ISA) in January.

Three companies which are unable to stock fish this year are seeking compensation from the government for loss of business.

However by leaving the area fallow until spring next year the industry will be able to introduce a joint management regime, allowing them to more effectively control the major disease problem of sea lice, which caused huge losses during the last few months of 2008.

Intrafish reported in February 2009:

ISA virus returns to Scotland

The Scottish salmon industry is hoping to avoid the same devastation the infectious salmon anemia (ISA) virus caused a decade ago.

9 February 2009 8:18 GMT *UPDATED 25 July 2012 8:55 GMT*

By **null Raoheal**

ISA was discovered at a Scottish Sea Farms-owned site on the Shetland Islands last month, when government inspectors were called to investigate an outbreak of sea lice.

The salmon farm in question was harvested Dec. 21, 2008, Scottish Sea Farms said, adding it was cooperating fully with government investigators. The company is owned by Norway-based companies Leroy and SalMar.

Two further farms were suspected as containing the virus, but on Jan. 15 Scottish government scientists told the industry they traced no new ISA cases.

It will take at least until the end of February for the remaining samples to be analyzed and the all-clear to be given.

Even if the entire zone is declared ISA-free, all 27 farms within the control zone must be harvested and lay fallow for six weeks.

The infected site will be out of action for at least six months. Restrictions on the movement of fish were in place over a large area, affecting 43 fish farms.

The Scottish government said it has learned lessons from how it handled an ISA outbreak in 1998 and 1999 that cost the industry an estimated £100 million ($151 million/€ 112.7 million) and led to 200 workers losing their jobs.

"We will be applying those lessons vigorously with the clear aim of containing and then eradicating the disease from the current affected site," Environment Minister Mike Russell told BBC News.

"The presence of the virus does not mean that clinical disease is present, and indeed, fish tested from the farm site did not show any clinical symptoms of disease," Scott Landsburgh, CEO of the Scottish Salmon Producers Organization said.

"Of course this news is a concern, but we are confident that we can manage the situation quickly and effectively alongside the Fisheries Research Services inspectors," he said.

The Scottish government must look into whether salmon farming companies are meeting standards for preventing disease, the Pure Salmon Campaign said.

"What is the risk of infection to Shetland sites certified organic by the U.K. Soil Association and the Organic Food Federation, and to other companies in other regions across the Highlands and Islands of Scotland?" asked Don Staniford, the campaign's European representative.

"It remains to be seen if the lessons of the 1998-1999 ISA outbreak are heeded this time around." (Copyright)

Intrafish reported in January 2009:

Minister criticizes industry over ISA

Environment Minister Michael Russell blamed poor industry practice for the detection of a highly infectious disease at a Shetland salmon farm, amid moves to establish an independent inquiry into the outbreak.

28 January 2009 14:18 GMT UPDATED 8 May 2018 11:14 GMT

By **IntraFish Media**

Russell made the announcement during a visit to the Shetlands on Monday to examine the outbreak of infectious salmon anemia (ISA) virus earlier this month, saying the industry must improve its standards of husbandry.

"We have a code of good practice, and quite frankly, if they had followed it to the letter we wouldn't have this problem. They need to realize their practice must improve," he told *Shetland News*.

Government scientists discovered ISA at a site owned by Scottish Seafarms, east of Hildasay, near the village of Scalloway, when investigating high mortalities from the sea lice parasite.

All movements of fish were banned between fish farms within a 2.8-kilometer radius, a total of 42 salmon cages.

Tests were made on two suspected sites around the infected farm, but scientists announced Jan. 15 no further outbreaks of the disease were detected.

On Jan. 20, **IntraFish** reported the legacy of the IISA virus detected in the Shetland Islands would persist for at least seven months and force 27 farms to lay fallow.

All 27 fish farms located within the control zone must be synchronously laid fallow for a minimum of six weeks after the last fish is cleared, according to Scottish government regulations.

'Situation contained'

Russell said the ISA problem appeared to have been contained, and if that was the case, the next step is to eradicate it.

The entire incident will be thoroughly investigated by a review panel, which he intended to appoint shortly.

"We will undertake a full independent assessment of the outbreak and how it came about, and I hope it will show us some lessons for the future," he said.

"One of the lessons of ISA is it tends to occur when the fish are weakened following other diseases and by practices which quite frankly don't conform to best practice," he said, referring to the incidences of sea lice that lead to the detection of ISA.

"If that's the case in this instance, then I think that will come out in the independent review, but companies need to take responsibility. They need to make sure future practices are good and if their practices are anywhere less than good they need to improve those rapidly." (Copyright)

The Shetland Times reported in January 2009:

The Shetland Times
Established 1872. Online since 1996.

News | Weather | Jobs | Properties | Advertise | Bookshop

ISA back to haunt fish farmers

John Robertson | January 9, 2009

SHARE ON:

By JOHN ROBERTSON

THE THREE salmon farm sites caught up in an infectious salmon anaemia (ISA) scare off Scalloway have been cleared of fish, raising hopes the disease will not wreak the havoc suffered in Shetland 10 years ago. However, there are 10 more sites off the south-west Mainland with fish in them which may have been vulnerable to the virus.

There has been some concern about the length of time taken to react to a potential disaster for the industry, given that inspectors were first alerted in November to an unusually high number of fish deaths at the Scottish Sea Farms site east of Hildasay. Testing started on 9th December yet it took until Friday, 2nd January for action to begin after confirmation of ISA on the site, owned by the Norwegian company Scottish Sea Farms.

Two more of the company's sites to the north, at Fore Holm and Brei Geo in the entrance to Sandsound Voe, have been placed under suspicion of harbouring the virus, although one had already been empty of salmon for six weeks.

According to Shetland Aquaculture general manager David Sandison the only reason the two were made suspects was because they contained fish from the same source as those in the Hildasay cages. The last of the fish from the sites was harvested on Tuesday. Six more sites in the control zone still contain fish and four within the wider surveillance zone also have stock.

Government scientists from Fisheries Research Services (FRS) have been busy sending away fish samples from a number of the neighbouring farms and may have some results by the end of next week to show whether the virus has spread in an area which has the most densely located salmon farm sites in Shetland. A full set of results could take up to six weeks due to the processes they have to go through in the lab. In the meantime investigations will focus on how the virus got to the Hildasay site.

Mr Sandison said it was disappointing that ISA had popped up again after 10 years but there was a good opportunity to control the outbreak and he would have been much more worried if there had already been a clinical outbreak of disease.

"This is a situation which we shouldn't over-exaggerate," he said on Wednesday. "Already, because the fish are out, we've significantly lessened any risks that remain.

"It's now about making sure the inspectors get on with the job and get the sampling done that they need to really satisfy us that there is not a problem. The fact that only one site has been confirmed is very good indeed. But it is early and I'm not going to be complacent about it."

The government clampdown which swung into place has banned unauthorised movement of fish or fish farm traffic within a control zone which, along with Sea Farms, affects two other companies – the other Shetland salmon farming giant, Hjaltland Seafarms, and independent Shetland fish farmer Skelda Salmon, which has one site in the zone at Spoose Holm, between the Cheynies and Papa.

The farms in the wider surveillance zone are being monitored. Between the two zones there are more than 40 licensed farm sites, most of them disused or lying fallow.

The fish from the site at Hildasay had already been harvested and packed at Sea Farms' factory in Scalloway before confirmation of the virus surfaced. The last fish came out on 21st December. In fact, according to Mr Sandison, it was during harvesting in late November that company workers first noticed increased numbers of dead or dying fish, prompting a request to the FRS lab in Aberdeen to investigate.

A scientific paper published in Archives of Virology in 2009 reported:

› Arch Virol. 2009;154(1):1-8. doi: 10.1007/s00705-008-0251-2. Epub 2008 Nov 26.

ISA virus in Chile: evidence of vertical transmission

Siri Vike [1], Stian Nylund, Are Nylund

Affiliations + expand

PMID: 19034606 DOI: 10.1007/s00705-008-0251-2

Abstract

Infectious salmon anaemia virus (ISAV), genus Isavirus (family Orthomyxoviridae), is present in all large salmon (Salmo salar)-producing countries around the North Atlantic. The target species for this virus are members of the genus Salmo, but the virus may also replicate in other salmonids introduced to the North Atlantic (Oncorhychus spp.). Existing ISA virus isolates can be divided into two major genotypes, a North American (NA) and a European (EU) genotype, based on phylogenetic analysis of the genome. The EU genotype can be subdivided into several highly supported clades based on analysis of segments 5 (fusion protein gene) and 6 (hemagglutinin-esterase gene). In 1999 an ISA virus belonging to the NA genotype was isolated from Coho salmon in Chile, and in 2007 the first outbreaks of ISA in farmed Atlantic salmon was observed. Several salmon farms in Chile were affected by the disease in 2007, and even more farms in 2008. In this study, ISA virus has been isolated from salmon in a marine farm suffering an outbreak of the disease in 2008 and from smolts with no signs of ISA in a fresh water lake. Sequencing of the partial genome of these ISA viruses, followed by phylogenetic analysis including genome sequences from members of the NA and EU genotypes, showed that the Chilean ISA virus belongs to the EU genotype. The Chilean ISA virus groups in a clade with exclusively Norwegian ISA viruses, where one of these isolates was obtained from a Norwegian brood stock population. All salmonid species in the southern hemisphere have been introduced from Europe and North America. The absence of natural hosts for ISA viruses in Chile excludes the possibility of natural reservoirs in this country, and the close relationship between contemporary ISA virus strains from farmed Atlantic salmon in Chile and Norway suggest a recent transmission from Norway to Chile. Norway export large amounts of Atlantic salmon embryos every year to Chile; hence, the best explanation for the Norwegian ISA virus in Chile is transmission via these embryos, i.e. vertical or transgenerational transmission. This supports other studies showing that the ISA virus can be transmitted vertically.

A scientific paper published in Archives of Virology in 2007 reported:

Arch Virol. 2007 Jan;152(1):151-79. doi: 10.1007/s00705-006-0825-9. Epub 2006 Aug 28.

Transmission of infectious salmon anaemia virus (ISAV) in farmed populations of Atlantic salmon (Salmo salar)

A Nylund [1], H Plarre, M Karlsen, F Fridell, K F Ottem, A Bratland, P A Saether

Affiliations + expand

PMID: 16941061 DOI: 10.1007/s00705-006-0825-9

Abstract

In the present study, 24 smolt production sites were screened for the presence of infectious salmon anaemia virus (ISAV) with the help of a specific real-time RT PCR assay, and 22 of these sites had smolts that were positive. If these smolt production sites are representative for the prevalence of ISAV in Norwegian smolts, then most marine production sites must be considered to be positive for ISAV. In addition, 92 European ISAV isolates have been genotyped based on the hemagglutinin-esterase gene (HE), and their distribution pattern was analysed. This pattern has been coupled to information about the origin of smolt, eggs, and broodfish in those cases where it has been possible to obtain such information, and with information about ISAV in neighbouring farms. The pattern suggests that an important transmission route for the ISAV could be that the salmon farming industry in Norway is circulating some of the isolates in the production cycle, i.e. some sort of vertical or transgenerational transmission may occur. It has also been shown that avirluent ISAV isolates are fairly common in Norwegian farmed salmon. Based on this, it is hypothesized that the change from avirulent to virulent ISAV isolates is a stochastic event that is dependent on the replication frequency of the virus and the time available for changes in a highly polymorphic region (HPR) of the HE gene to occur. This, and the possibility that only avirluent ISAV isolates are vertically transmitted, may explain why ISA most often occurs at marine sites and why no more than about 15 farms get ISA every year in Norway.

Fish Farmer reported in 2004:

Fish Farmer

For all the latest industry news, markets and jobs in aquaculture

News Archive

Suspected ISA outbreak in Scotland – Fishupdate.com

By systemwyvex - 22nd September 2014

Suspected ISA outbreak in Scotland Published: 22 November, 2004

THE Scottish Executive has announced that the presence of infectious salmon anaemia (ISA) is suspected at Marine Harvest's Loch Sheilavaig salmon farm in South Uist.

FRS Fish Health Inspectors are currently investigating the affected farm. Statutory restrictions are in place controlling the movement into and out of the farm of all fish.

Marine Harvest are working with SEERAD to minimise any risk and ensure the health of their fish.

ISA is notifiable under European and domestic fish health legislation, which requires measures to eradicate rather than control the disease. An outbreak was confirmed in Scotland in 1998 and eradication measures including clearance of infected sites and controls on movements of fish in affected areas has succeeded in preventing further outbreaks. The EC Scientific Committee on Animal Health and Animal Welfare has concluded that there is no evidence of risk to human health from ISA.

Signs of ISA include high mortality rates, darkening of the liver, severe anaemia and internal haemorrhage.

Infectious salmon anaemia virus is an orthomyxovirus-like virus, similar to the influenza viruses of humans, mammals and birds. It is the only recorded orthomyxovirus disease of fish.

The ISA virus appears to cause disease only in Atlantic salmon, however other wild fish are also susceptible to infection, including brown trout, rainbow trout and herring.

ISA was first discovered in Norway in 1984. Since then, ISA has been found in New Brunswick, Canada in 1996, Nova Scotia in 1998, Scotland in 1998, Chile in 1999, Faroe Islands in 2000 and the United States in 2001.

A scientific paper published in Diseases of Aquatic Organisms in 2003 reported:

Comparative Study › Dis Aquat Organ. 2003 Aug 15;56(1):11-24. doi: 10.3354/dao056011.

Emergence and maintenance of infectious salmon anaemia virus (ISAV) in Europe: a new hypothesis

A Nylund [1], M Devold, H Plarre, E Isdal, M Aarseth

Affiliations + expand

PMID: 14524497 DOI: 10.3354/dao056011

Free article

Abstract

The present study describes the use of molecular methods in studying infectious salmon anaemia virus (ISAV), an important pathogen of farmed salmon in Norway, Scotland, the Faeroe Islands, Canada, USA and Chile. The nucleotide sequences of the haemagglutinin gene (HA) from 70 ISAV isolates have been analysed for phylogenetic relationship and the average mutation rate of nucleotide substitutions calculated. The isolates constitute 2 major groups, 1 European and 1 North American group. The isolate from Chile is closely related to the North American isolates. The European isolates can be further divided into 3 separate groups reflecting geographical distribution, time of collection, and transmission connected with farming activity. Based on existing information about infectious salmon anaemia (ISA) and new information emerging from the present study, it is hypothesised that: (1) ISAV is maintained in wild populations of trout and salmon in Europe; (2) it is transmitted between wild hosts mainly during their freshwater spawning phase in rivers; (3) wild salmonids, mainly trout, possibly carry benign wild-type ISAV isolates; (4) a change (mutation) in virulence probably results from deletions of amino acid segments from the highly polymorphic region (HPR) of benign wild-type isolates; (5) ISA emerges in farmed Atlantic salmon when mutated isolates are transmitted from wild salmonids or, following mutation of benign isolates, in farmed salmon after transmission from wild salmonids; (6) farming activity is an important factor in transmission of ISAV between farming sites in addition to transmission of ISAV from wild salmonids to farmed salmon; (7) transmission of ISAV from farmed to wild salmonids probably occurs less frequently than transmission from wild to farmed fish due to lower frequency of susceptible wild individuals; (8) the frequency of new outbreaks of ISA in farmed salmon probably reflects natural variation in the prevalence of ISAV in wild populations of salmonids.

A report published by the Royal Society of Edinburgh in 2002 included:

The Royal Society of Edinburgh

THE SCIENTIFIC ISSUES SURROUNDING THE CONTROL OF INFECTIOUS SALMON ANAEMIA (ISA) IN SCOTLAND

A Report of the Royal Society of Edinburgh Working Party on Infectious Salmon Anaemia

There has been anecdotal and unconfirmed evidence that the ISA virus is prevalent in open waters of Scotland as distinct from in and around fish farms, and limited and fragmentary evidence that it occurs also in other species of fish (paragraph 13, 14, 52). **We conclude that it is impossible to establish on the basis of presently available evidence whether this is the case or whether the virus is exotic to EU waters**. We note that the Fisheries Research Services (FRS) survey of the presence of ISA virus in wild salmon and other fish has been stopped. **We recommend that the survey for ISA virus in wild fish be re-established** (paragraph 52). **We further recommend that there should be extended surveillance of Scottish salmon farms for the ISA virus to determine whether, in the absence of the disease, the virus is still present**.

It is important that continued effort be sustained on surveillance for ISA in Scotland because there is always the possibility that ISA may recur. **We believe that the current eradication policy through withdrawal be continued in confirmed cases of ISA** (paragraphs 50-51). **We also recommend that the scientific criteria for the category "suspicion" of ISA should be re-examined and clearly defined** (paragraphs 53, 59). In the event of suspicion, the recommendations of the Joint Government/Industry Working Group on ISA provides a good basis for management of a potential outbreak (paragraphs 51, 52).

We are fearful that current policy disadvantages Scotland's salmon broodstock industry. The procedures advocated by the Joint Government/Industry Working Group on ISA do not well address the broodstock industry's needs and could result, in certain circumstances, in serious loss of its unique gene pool and resulting serious financial damage. **We recommend that the regulators and the broodstock industry together address the changes necessary to the current regulations to take account of the needs of the Scottish broodstock industry** (paragraph 59).

Intrafish reported in June 2001:

Scottish ISA strain closer to Norwegian origin

While the government report on the origins of the ISA outbreak in Scotland failed to find any definitive answers, it found that the ISA viral strain, which emerged from the Hydro Seafood GSP Loch Nevis site, is more closely related to the Norwegian strain than the Canadian virus.

13 June 2001 5:03 GMT *UPDATED 10 July 2012 10:17 GMT*

The scientific report, which attempted to identify the source of the ISA outbreak in Scotland, has ruled out Canada but has not fully implicated Norway as the original source of the outbreak.

"It is clear from our studies that Scottish ISAV is more closely related to the Norwegian strain of the virus than the Canadian New Brunswick strain. These results indicate considerable divergence between the North American and European isolates and would appear to rule out Canada as the source of the Scottish outbreak," the report reads.

The ISA virus was isolated from all of the areas with confirmed cases and genetic analysis indicates that the virus is identical between all confirmed sites. However, sequence data on the RNA segments (biological code for the virus) characterised from different ISA virus (ISAV) isolates from Scotland and Norway do not corroborate the origin of the virus, as minor differences are evident.

Insufficient data The report states: "The data are insufficient at present to distinguish specific Norwegian or Scottish forms of the virus, since the relatedness of isolates shows as much variation within the two countries as between them."

Some segments of the sequence of the Scottish and Norwegian isolates only differed by three nucleotides (units that make up sequence data), while more than 45 variations were found when the same segment was compared with Canadian isolates.

One site found with different ISA strain The report also found that one sample of ISAV from a suspect site in Scotland revealed a different sequence to that found in isolates from confirmed sites and represented a different source of the virus.

This sequence was found to be very closely related to that of an isolate obtained in Norway in the early 1990s and, according to the report, "could be consistent with the existence of a reservoir of ISAV in the marine environment." The report added that further research may ascertain whether different strains exist with different affinities for Atlantic salmon as a susceptible host for the disease.

The Scottish Government reported in April 2001:

FRS Marine Laboratory Report No 13/01

EPIZOOTIOLOGICAL INVESTIGATIONS INTO AN OUTBREAK OF INFECTIOUS SALMON ANAEMIA (ISA) IN SCOTLAND

R M Stagg, D W Bruno, C O Cunningham, R S Raynard, P D Munro, A G Murray, C E T Allan, D A Smail, A H McVicar and T S Hastings

April 2001

EXECUTIVE SUMMARY

FRS Marine Laboratory has completed an epizootic investigation into the source and spread of infectious salmon anaemia (ISA) in farmed Atlantic salmon in Scotland. This report covers the epizootic occurring between the first confirmation of ISA in May 1998 through to the last suspected outbreak in November 1999. Eleven cases have been confirmed. Records show significant stock transfer from the primary infected site prior to the detection of the outbreak. The site was characterised by overlapping generations and the absence of fallowing. The pattern of spread of ISA from the initial outbreak was discontinuous. This reflected the activities of fish farmers who inadvertently broadcast the disease primarily through fish movements in well boats and other site to site contacts. ISA virus has been isolated from all of the areas with confirmed cases and genetic analysis carried out to date indicates that the virus is identical. ISA in Scotland, therefore, would appear to have emerged from a single case and spread through work practices previously operated within the fish farming industry. Following the outbreak a Joint Government/Industry Working Group on Infectious Salmon Anaemia (ISA) was established which made recommendations to change working practices which might have contributed to the spread of ISA.

An investigation into the original source of the virus did not find evidence of illegal import of live fish, nor the movement of dead, imported, eviscerated fish to processing facilities discharging to adjacent coastal waters. There is no evidence that the disease arose because of infection transferred from freshwater hatcheries supplying the primary site or through vertical transmission from parents to offspring. Sequence data on the RNA segments characterised from different ISA virus (ISAV) isolates from Scotland and Norway show minor differences. The data are insufficient at present to distinguish specific Norwegian or Scottish forms of the virus, since the relatedness of isolates shows as much variation within the two countries as between them. Surveys of wild marine and freshwater fish did not reveal clinically diseased fish and ISAV was rarely isolated and only in areas close to infected farms. RT-PCR analysis demonstrated positive diagnostic tests in sea

trout, and salmon parr and brown trout in freshwater river systems. Sequence data for those parts of the RNA segments examined indicate they are reporting ISAV similar to that isolated from confirmed farms. Calculation of accurate prevalence levels from the low numbers of positive samples is difficult. However, modelling of the data indicates a decline in prevalence of ISAV corresponding to the decline recorded in farm stock. A survey of farmed fish using RT-PCR corroborates the finding that positive samples are located mainly in areas where outbreaks have occurred.

Hypotheses for the emergence of ISA in Scotland are discussed. It is unlikely that the true origins of ISA will ever be known with any certainty but two hypotheses provide plausible explanations for the emergence of the disease. The first of these is that it was introduced accidentally on a well boat from infected areas in Norway. The second is that, due to the conditions on the farm, where the primary outbreak occurred, a particularly virulent or pathogenic form of ISA emerged from a wild reservoir. It is unlikely that such conditions are unique in Scottish aquaculture. Since all Scottish outbreaks are linked to the primary infected site then an important epidemiological feature of the latter hypothesis is that it must have been an extremely rare event.

1. INTRODUCTION

Infectious salmon anaemia (ISA) is a contagious and significant viral disease of farmed Atlantic salmon which first emerged in Norway in 1984 (Thorud and Djupvik, 1988). Haemorrhagic kidney syndrome (HKS) was reported in Canada in 1996 and by 1998 the aetiology of HKS was subsequently established as ISA (Bouchard *et al.*, 1998; Lovely *et al.*, 1998; Mullins *et al.*, 1998; Simko *et al.*, 2000). In May 1998 ISA was officially confirmed in Scotland (Rodger *et al.*, 1998; Stagg *et al.*, 1999) and also in the Faroe Islands in May 2000.

The Scottish outbreak represented the first report of ISA within the European Union. Prior to this, ISA had not been recorded during official surveillance and testing of farmed or wild fish in the United Kingdom despite the disease being notifiable since 1990 (Hill, 1996). The outbreak has resulted in the confirmation of ISA on 11 farms in Scotland with the last case being diagnosed in May 1999. Within the European Union, ISA is classified as a List I disease. The legislation requires the withdrawal (compulsory slaughter) of fish on infected farms, the cleaning, disinfection and fallowing of the site following removal of all of the fish and an epizootic investigation into the source and spread of the disease. This report is an account of the results of these investigations and provides some insight into the mechanisms of disease transfer and the origins of ISA in Scotland. It is notable that this report represents the situation as it existed prior to and during the ISA outbreak. It is expected that industry practices will have changed following the recommendations in the Final Report of the Joint Government/Industry Working Group on Infectious Salmon Anaemia (Anon, 2000).

The Scottish Government report included:

Table 3.1

Evidence of ISA at sites confirmed as infected in Scotland during 1998 and 1999

Site no	Clinical signs	Histopathology (individual fish)	Evidence of anaemia	Virus isolation (pools of up to 5)	PCR (pools of up to 5)	IFAT (individuals)
ISA 98/01	Dark liver, pale gills, ascites, haemorrhage, abnormal behaviour	21/40	Haematocrit <20 in 18/40 fish	6/8	1/1	8/52
ISA 98/02	Dark liver, pale gills, ascites, haemorrhage	27/50	Pale hearts and gills	27/46	4/6	8/22
ISA 98/03	Dark liver, ascites, fish hanging vertically in the water, haemorrhage	4/40	Pale hearts	Negative	1/6	3/12
ISA 98/04	Dark liver, ascites	1/40	Pale hearts	Negative	6/6	10/36
ISA 98/05	Harvest station.			1/8	2/6	1/12
ISA 98/06	Dark and pale liver, pale gills, ascites	3/94	Haematocrit <20 in 17/23 fish	Negative	1/22	15/61
ISA 98/08	Dark and pale liver, pale gills, ascites	4/147	Haematocrit <20 in 34/50 fish	Negative	2/51	6/57
ISA 98/09	Dark liver, ascites, pale gills, enlarged spleen	2/23	Haematocrit <20 in 1/8 fish	Negative	Negative	4/24
ISA 98/10	Dark liver, pale gills, ascites, petechial haemorrage on viscera	1/25		Negative	1/4	4/24
ISA 98/18	Dark liver, pale gills, ascites, haemorrhage	4/22	Pale hearts	3/7	4/6	5/38
ISA 99/02	Dark liver, pale gills, ascites, haemorrhage	2/46	Haematocrit <20 in 3/14 fish	Negative	4/5	3/24

3.2 Chronology of ISA Outbreaks

On 5 May 1998, the FRS Marine Laboratory Aberdeen (MLA) received notification that an outbreak of ISA was suspected on a fish farm site (ISA 98/01) belonging to Hydro Seafoods GSP in Loch Nevis. This proved to be the start of an ISA epizootic that lasted for nearly two years. In the course of this event, 36 farms were suspected of being infected (Table 3.2) and of these, 11 were subsequently confirmed. In legal terms, one of the suspect infected farms could not be declared officially suspect because at the time of diagnosis all fish had been harvested from the site. Nevertheless and with the cooperation of the operators, the farm was disinfected and fallowed in the same way as the other suspect sites. The chronology of the event is shown in Figure 3.1 and the locations of all suspect and confirmed sites are shown in Figure 3.2.

3.2.1 The Primary Outbreak

The primary infected site (ISA 98/01) was originally stocked with over two million smolts from three different freshwater sources between October and December 1997 (Fig. 3.3). After some initial losses, mortalities on site remained relatively constant at 0.25-0.5% per week until they started to rise dramatically during April 1998 (Fig. 3.4). One of the seven cages was particularly badly affected with almost 30% mortalities per week at the end of April 1998. The remaining fish were culled in accordance with the requirements of EC and UK legislation and the site was emptied by 2 June 1998. The dead fish were ensiled in propionic acid (pH <4.0) and exported to Norway for rendering.

Salmon had been moved from Loch Nevis to a harvest station in Loch Creran (ISA 98/05) in January 1998 and smolts were sold in February 1998 to another company with a site in Loch Snizort (ISA 98/02). As a precaution, on 6 May 1998, statutory movement restrictions were placed on eight farms, three in Loch Nevis, one in Loch Snizort and four in Loch Creran (Table 3.2). Hydro Seafoods GSP owned six of the above sites, the others were owned by two other companies. The examination of fish movement records at the Nevis sites indicated that there had been significant stock transfer (Fig. 3.3).

There were three salmon farm sites operating in Loch Nevis in May 1998 and the stocking, harvesting and broodstock regimes since late 1995 are indicated in Figure 3.3. Notable features of the stocking regimes are:

- the transfer of production fish between the sites as the sites reached maximum carrying capacity due to fish growth;
- the transfer of fish from seawater sites from other geographic areas;
- the use of multiple sources of smolts;
- the selection and maintenance of brood fish on the production sites;
- the transfer of brood fish between the sites; and
- the presence of overlapping generations and absence of fallowing.

The immediate health records, reported by the company, on all three sites in Loch Nevis indicate a chronic disease history. These include suspect cardiomyopathy syndrome (CMS) in November 1997 at site ISA 98/03 (Rodger and Turnbull, 2000); a chronic history of winter ulcer disease, partially treatable with antibiotics, prior to the outbreak of ISA at site ISA 98/01 and an outbreak of furunculosis at site ISA 98/08 in February 1998. Mortality records from ISA 98/01 are shown in Figure 3.4 and corroborate these reports. It is noteworthy that aspects of the clinical signs and liver histopathology presented by CMS are very similar to ISA (OIE, 2000). The Official Service isolated both *Aeromonas salmonicida* and *Vibrio* sp. from the third site in May 1998.

Figure 3.1 The chronology of ISA suspect and confirmed cases in Scotland 1998-2000.

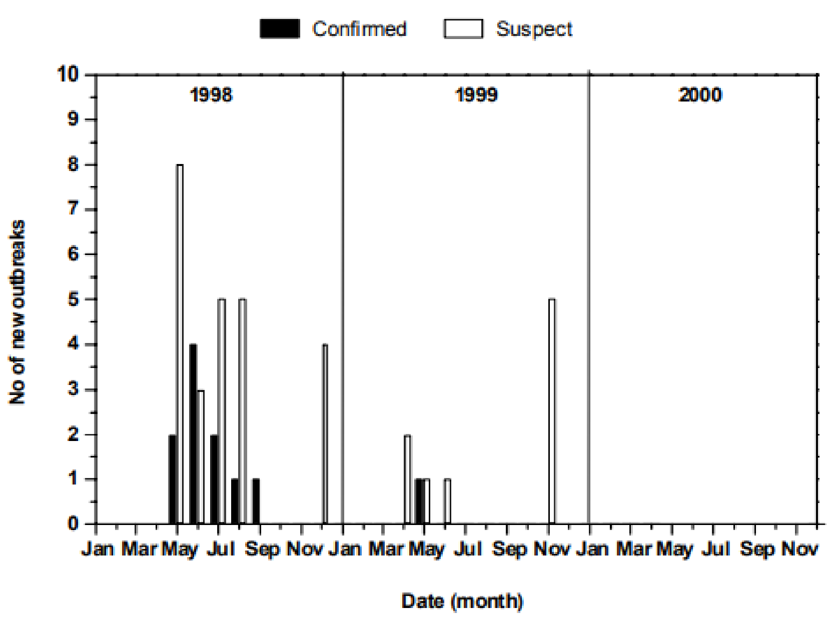
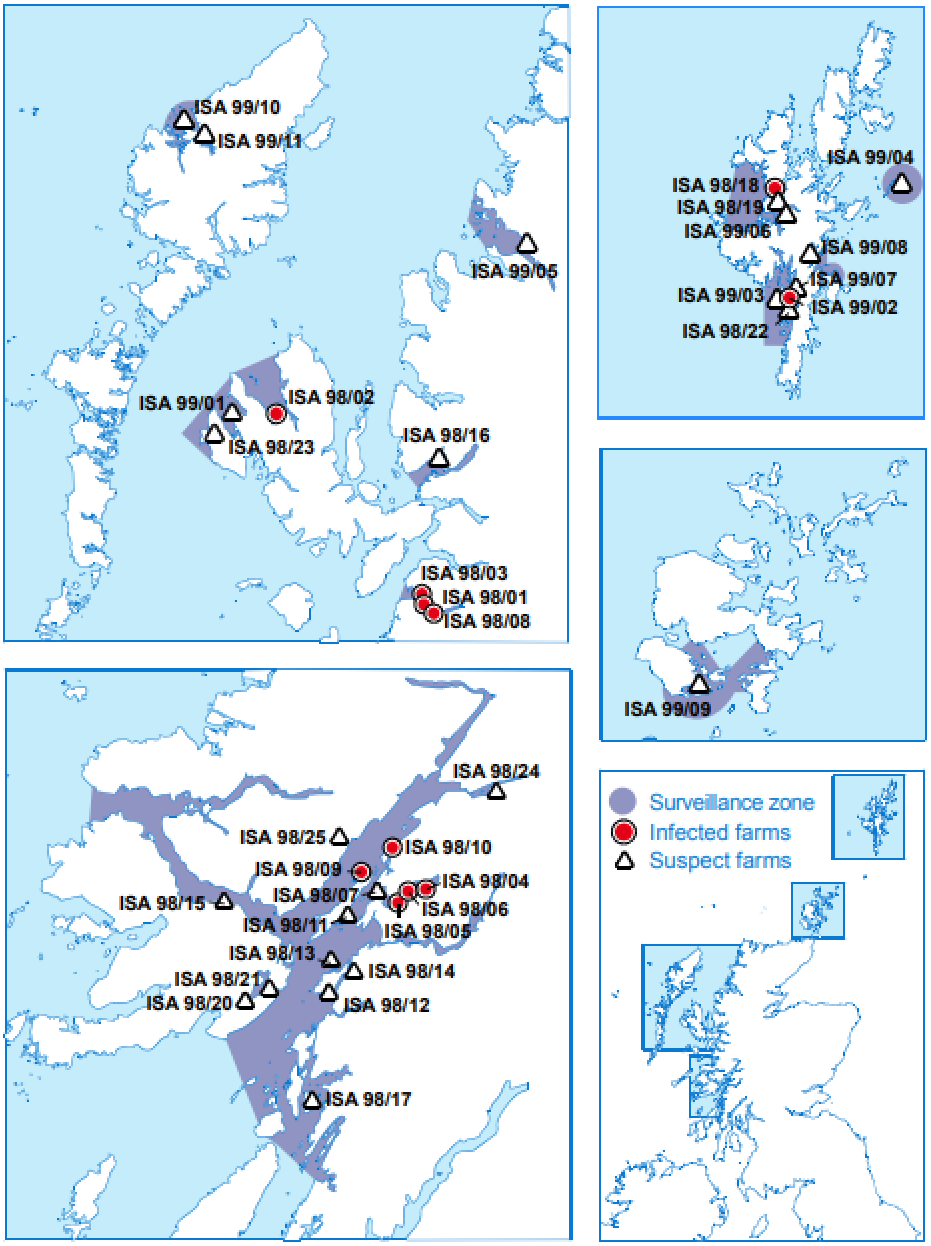


Figure 3.2 The location of confirmed and suspect cases of ISA in Scotland 1998-1999.

Note: Site ISA 99/01 was not officially suspect because no fish were present on the farm at the time the tests were complete.



3.2.3 Secondary Outbreaks

ISA was confirmed at Loch Snizort on Skye on 16 May 1998. The site (ISA 98/02) was stocked with approximately 450,000 smolts originating from six different sources, including Loch Nevis (ISA 98/01), from February to April 1998. The number of mortalities per week rose steadily, the last recorded number available was 7,699 during the first week in June 1998. The supervised removal of approximately 370,000 fish with an estimated biomass of 100 tonnes commenced immediately after the first cull in Loch Nevis was complete and the fish were disposed of in the same manner employed at Loch Nevis. On 9 June 1998, ISA was confirmed on three more sites, two in Loch Creran (ISA 98/04, ISA 98/05) and a second site in Loch Nevis (ISA 98/03). The second confirmed site in Loch Nevis was anticipated to contain some 70 tonnes of fish although only 10 to 15 tonnes were harvested. Inaccurate counting of fish into the cages, and greater than anticipated losses associated with a mortality episode in late 1997 were given as reasons for the discrepancy. A third site (ISA 98/06) in Loch Creran was confirmed on the 17 June 1998. The stocks in one of the confirmed sites in Loch Creran (ISA 98/04) were harvested. A second (ISA 98/05) operated as a harvest station where fish were held in cages prior to slaughter and processing in an adjacent factory. Prior to the outbreak, this factory discharged biological treated blood water effluent without disinfection directly into the sea in the vicinity of the farms. A third site (ISA 98/06) was stocked with some 70 tonnes of fish which were not of a marketable size and these were therefore destroyed. A fourth site in Loch Creran (ISA 98/07), which was ISA suspect and belonged to a small independent company, was harvested early after towing the single cage to the harvest station in the same loch at the end of June 1998. Interestingly, the clinical signs in these stocks became more apparent after the movement which may illustrate the role of stress factors in the manifestation of disease.

As a result of surveillance by the Official Service a further three farms belonging to the same company which owned the sites in Loch Nevis and Loch Creran, were declared suspect on 24 June 1998. Two of these sites (ISA 98/09, ISA 98/11) were located near Lismore Island and one (ISA 98/10) near Shuna Island in Loch Linnhe. Two of these sites were subsequently confirmed on 24 July 1998 at which time another five sites all belonging to, or operated by, the same company were declared suspect. Three of these sites were located around the Isle of Kerrera (ISA 98/12, ISA 98/13 and ISA 98/14), one in the Sound of Mull (ISA 98/15) and one in Loch Kishorn (ISA 98/16). The Official Service had been informed by the owners that there was a suspicion of ISA at site ISA 98/15 on 3 July 1998. The farmers suspected that the disease might have been introduced to the site by the movement of a barge containing grading equipment from Loch Creran. The two confirmed sites at Lismore Island and Shuna Island held a total biomass of over 2,500 tonnes which was harvested over a period of six weeks. Mortalities at Shuna Island peaked at just over 1,000 fish per week but mortalities at Lismore Island reached much higher levels, with almost 22,000 fish per week recorded during the third week in August 1998.

On 14 August 1998 another site (ISA 98/17) in Shuna Sound, near the island of Luing, was declared ISA suspect. This site had received fish from one of the suspect sites (ISA 98/14) at Kerrera Island during March and April 1998. In November of the same year all the stock on site, up to 17,000 harvest size fish, escaped when the nets became damaged. Also on 14 August, the Official Service were informed by the company which owned the infected site in Loch Snizort that they suspected a farm (ISA 98/18) in St Magnus Bay, Shetland, which they operated, was also infected. This was the first suspicion of ISA in Shetland and the disease was officially confirmed on 28 August 1998. On the same day a neighbouring site (ISA 98/19) in St Magnus Bay, belonging to an independent company, was also declared ISA suspect as a result of surveillance by the Official Service. Two more sites (ISA 98/20 and ISA 98/21) in Loch Spelve on Mull, belonging to the company associated with the primary outbreak, were also declared suspect on 28 August 1998.

Movement records and company information provided to the Official Service suggests that ISA was spread to Shetland directly, *via* a well boat delivering a part load of smolts to site ISA 98/02 in Loch Snizort in May 1998 and subsequently delivering smolts to St Magnus Bay. The biomass in 12 cages at the confirmed site (ISA 98/18) in St Magnus Bay was approximately 130 tonnes, which had to be destroyed since the fish were less than 0.5 kg in weight. The cull took place over three days during the first week of September 1998. The fish on the suspect site (ISA 98/19) in St Magnus Bay were culled voluntarily following the removal of the fish from the neighbouring confirmed site.

A third fish farm in Loch Nevis (ISA 98/08) was confirmed infected with ISA on 18 September 1998, bringing the total number of sites confirmed to 10 in 1998. The fish held in five cages on this site in Loch Nevis were not of harvest size and were culled between 9 and 23 September 1998. Because of official surveillance, suspicion was declared on another four sites in December 1998. Two of these were in areas previously thought to be unaffected, Loch Dunvegan on Skye (ISA 98/23) and the Burra area of Shetland (ISA 98/22). The other two suspect sites (ISA 98/24 and ISA 98/25) were in the Loch Linnhe area.

In February 1999 ISAV was isolated at a second site in Loch Dunvegan (ISA 99/01) belonging to the same company that owned the adjacent site (ISA 98/02) in Loch Snizort. The farm was harvested out before the results of testing were known, but since the farm did not contain fish, at the time the infection was discovered, the farm could not be declared officially suspect. Despite this, and with the full cooperation of the company concerned, the site was treated as a suspect farm. It was therefore disinfected and fallowed for six months.

In the Burra area of Shetland two more sites (ISA 99/02, ISA 99/03) belonging to different companies were declared ISA suspect on 23 April 1999. The area is densely populated with fish farm sites and two salmon processing plants had been discharging blood water with no disinfection in the area prior to the ISA outbreak in St Magnus Bay. One of the Burra sites (ISA 99/02) was subsequently confirmed on 25 May 1999. On the same date, suspicion was also announced at a site (ISA 99/04) on the islands off the east coast of Shetland known as Out Skerries. The compulsory slaughter of fish from the ISA confirmed site in Burra had to be undertaken by the Scottish Executive because the company concerned was in receivership. A contract was issued to a private company which cleared over 800,000 salmon averaging about 2 kg from the 19 steel cages on site over a period of two months. The owner of a site (ISA 99/05) in Loch Broom expressed concern regarding abnormal mortalities and as a consequence of sampling and testing by the Official Service this farm was declared ISA suspect on 25 June 1999.

Table 3.2

Chronology of the confirmed and suspect cases of ISA in farmed salmon and their locations in Scotland 1998-1999

Outbreak no	Location	Date suspect	Date confirmed	Link	Type	Date cleared
ISA 98/01	Loch Nevis	06.05.98	15.05.98	Primary	Smolts	02.06.98
ISA 98/02	Loch Snizort	06.05.98	16.05.98	ISA 98/01	Smolts	01.06.98
ISA 98/03	Loch Nevis	06.05.98	09.06.98	ISA 98/01	Broodstock	05.06.98
ISA 98/04	Loch Creran	06.05.98	09.06.98	ISA 98/01	Grower	22.06.98
ISA 98/05	Loch Creran	06.05.98	09.06.98	ISA 98/01	Harvest St	14.09.98
ISA 98/06	Loch Creran	06.05.98	17.06.98	ISA 98/01	Smolts	27.06.98
ISA 98/07	Loch Creran	06.05.98		ISA 98/01	Grower	08.07.98
ISA 98/08	Loch Nevis	06.05.98	18.09.98	ISA 98/01	Smolts	25.10.98
ISA 98/09	Lismore Island	29.06.98	24.07.98	ISA 98/05	Grower	05.09.98
ISA 98/10	Shuna Island	29.06.98	24.07.98	ISA 98/05	Grower	05.09.98
ISA 98/11	Lismore Island	29.06.98		ISA 98/4, 5, 9 and 10	Grower	27.04.99
ISA 98/12	Kerrera Island	24.07.98		ISA 98/4, 5, 9 and 10	Grower	20.10.99
ISA 98/13	Kerrera Island	24.07.98		ISA 98/4, 5, 9 and 10	Grower	20.10.99
ISA 98/14	Kerrera Island	24.07.98		ISA 98/4, 5, 9 and 10	Grower	20.10.99
ISA 98/15	Sound of Mull	24.07.98		ISA 98/4, 5, 9 and 10	Grower	05.09.98
ISA 98/16	Loch Kishorn	24.07.98		ISA 98/05	Grower	28.03.00
ISA 98/17	Sound of Shuna	14.08.98		ISA 98/4 ,5, 9 and 10	Grower	19.11.98
ISA 98/18	St Magnus Bay	21.08.98	27.08.98	ISA 98/02	Smolts	08.09.98
ISA 98/19	St Magnus Bay	27.08.98		ISA 98/17	Grower	09.09.98
ISA 98/20	Loch Spelve	27.08.98		ISA 98/4, 5, 9 and 10	Grower	27.07.99
ISA 98/21	Loch Spelve	27.08.98		ISA 98/4, 5, 9 and 10	Grower	27.07.99
ISA 98/22	Burra	14.12.98		ISA 98/17	Grower	29.11.99
ISA 98/23	Loch Dunvegan	18.12.98		ISA 98/2	Grower	09.12.99
ISA 98/24	Loch Linnhe	18.12.98		ISA 98/4, 5, 9 and 10	Grower	14.09.99
ISA 98/25	Loch Linnhe	18.12.98		ISA 98/4, 5, 9 and 10	Grower	14.09.99
ISA 99/01	Loch Dunvegan	14.02.99*		?	Grower	14.02.99
ISA 99/02	Burra	23.04.99	25.5.99	ISA 98/17	Grower	14.07.99
ISA 99/03	Burra	23.04.99		ISA 98/17 and 30	Grower	01.10.99
ISA 99/04	Out Skerries	25.05.99		ISA 98/17 and 30	Grower	
ISA 99/05	Loch Broom	25.06.99		?	Grower	28.07.00
ISA 99/06	St Magnus Bay	04.11.99		ISA 98/17 and 30	Grower	05.10.00
ISA 99/07	Burra	04.11.99		ISA 98/17 and 30	Grower	27.10.00
ISA 99/08	Wadbister Voe	04.11.99		?	Grower	
ISA 99/09	Orkney	04.11.99		?	Grower	
ISA 99/10	Loch Roag	04.11.99		?	Grower	25.02.00
ISA 99/11	Loch Roag	04.11.99		?	Grower	

*Not officially suspect because no fish present on site at time of diagnosis (virus isolation)

Table 3.4

Possible sources of ISA infection at suspect sites in Scotland 1998-1999

ISA confirmed or suspect sites		Possible source of ISAV
Loch Creran	ISA 98/07	Fish movements to harvest station, well boats and blood water effluent without disinfection
Shuna Island and Lismore Island	ISA 98/11	Movements of staff or equipment, including well boats between sites or proximity to infected sites
St Magnus Bay	ISA 98/19 ISA 99/11	Proximity to infected site
Burra	ISA 98/22 ISA 99/03 ISA 99/07	Proximity to infected site and processing plants
Kerrera Island	ISA 98/12 ISA 98/13 ISA 98/14	Movements of staff or equipment between sites or proximity to infected sites
Loch Spelve	ISA 98/20 ISA 98/21	Movements of staff or equipment, including well boats between sites or proximity to infected sites
Sound of Mull	ISA 98/15	Movement of barge with grading equipment, well boats
Loch Kishorn	ISA 98/16	Divers or well boat movements
Loch Linnhe	ISA 98/24 ISA 98/25	Proximity to infected sites
Loch Dunvegan	ISA 98/23 ISA 99/01*	Movements of staff or equipment between sites or proximity to infected sites
Out Skerries	ISA 99/04	Divers or net washing station
Loch Broom	ISA 99/05	Unknown
Orkney	ISA 98/09	Unknown
East coast of Shetland	ISA 99/08	Proximity to processing plant
West Loch Roag	ISA 99/10 ISA 99/11	Unknown

*Genetic differences were found between the virus isolate from this farm and other Scottish farm isolates

In November 1999, suspicion of ISA was announced on another six sites. Four of these sites were in areas previously thought to be unaffected, namely, the East Coast of Shetland (ISA 99/08), Orkney (ISA 99/09) and West Loch Roag (ISA 99/10, ISA 99/11) on the Isle of Lewis. Detailed investigations into the possible source of infection revealed no specific connections between these sites and other infected areas. Furthermore, these cases were determined on samples taken as part of a surveillance and testing program and were not triggered by the presence of fish showing clinical signs. A summary of the site locations and possible reason for the spread of the ISAV is given in Table 3.4.

In August 2000, the Scottish Government and salmon farming industry published: A Code of Practice To Avoid and Minimise the Impact of ISA) – including:

FOREWORD

This Code of Practice (CoP) represents the culmination of months of effort by a dedicated number of individuals from within the industry and Government. The sole aim of the CoP is to help to provide a more secure and positive future for our salmon farming industry by creating a framework to minimise the threat from infectious salmon anaemia (ISA) and other diseases. I, and my colleagues in the working group, firmly believe that the contents of the CoP will deliver that security, provided ALL salmon farmers do their utmost to follow its recommendations.

I fully appreciate that for a number of companies, large and small, there will be challenges in adopting every recommendation in the CoP. However, it is vital that all companies continuously strive to do so in order to ensure that the risk of ISA is curtailed. We must demonstrate our commitment to this objective at every opportunity. However, the Government, too, must play a role to develop further an aquaculture infrastructure and regulatory environment that helps to promote good husbandry practices.

Today, fish health stands at a very high level within Scottish salmon farms, however there are recommendations within the CoP, which if followed, could lead to further benefits. Furthermore, this CoP should not be seen as the final word on ISA or fish health management. It is, and should remain, a living document that will be updated by the Aquaculture Health Joint Working Group to reflect changing technical achievements and advancements in fish health management.

Finally, I would like to thank all the contributors and editors who have put in so much effort to see this project come to fruition. To all salmon farmers I would say that their efforts can be your rewards.

Dr Graeme Dear
Vice Chairman, Joint Government/Industry Working Group on ISA
August 2000

INTRODUCTION

The Final Report of the Joint Government/Industry Working Group (JWG) on Infectious Salmon Anaemia[1] (ISA) was published in January 2000. It recommends practical measures for salmon farms to minimise the risk of their becoming infected and developing ISA. While some of the recommendations may require new legislation, many can be implemented immediately. These measures, representing good practice, are valid for the control of any fish disease and this Code of Practice, which covers each phase of salmon farming from hatchery to processing plant, should be adopted by all salmon farmers.

1. VERTICAL TRANSMISSION AND OVA DISINFECTION

What are the risk factors?

It is recognised that there are three ways in which infected broodstock may transmit ISA virus (ISAV) to their progeny by:

- True vertical transmission i.e. within the contents of the eggs and sperm;
- External transmission on the surface of the eggs and sperm and in natural secretions and excretions from the parents, for example, ovarian and seminal fluids, mucus;
- Transmission *via* contamination from infected water, personnel, clothing and equipment associated with stripping broodfish and fertilising ova.

How can the risks of contamination be minimised?

Assessment of the current scientific and technical information indicates that the risk from vertical transmission is unlikely, but as a precaution and as part of good husbandry practice, the following steps are recommended:

- Gametes should not be used from ISAV infected broodstock populations.

A Scottish Executive report - "Final Report of the Joint Government/Industry Working Group on Infectious Salmon Anaemia (ISA) in Scotland" - published in January 2000 included:

Final Report of the Joint Government/Industry Working Group on Infectious Salmon Anaemia (ISA) in Scotland

Chapter 2: Vertical Transmission and Ova Disinfection

Whilst neither intra- nor extra-ovum vertical transmission has been shown to occur, a precautionary approach is advocated. The following management recommendations are intended for inclusion in a Code of Practice. To avoid any possibility of intra-ovum vertical transmission, it is recommended that gametes should not be taken from ISA infected broodstock (2.3.1). As a precaution against extra-ovum transmission, recommendations are made for avoidance of contamination of gametes through hygiene protocols and disinfection of ova (2.3.2).

CHAPTER 2: VERTICAL TRANSMISSION AND OVA DISINFECTION

2.1 Risk Factors

There are three ways in which infected broodstock may transmit ISAV to their progeny:

a) True (intra-ovum) vertical transmission within the contents of the gametes.

b) Extra-ovum transmission on the surface of the gametes and in natural secretions and excretions from the parents, eg ovarian and seminal fluids; mucus.

c) Transmission *via* contamination from infected water, personnel, clothing and equipment associated with stripping broodfish and fertilising ova.

2.2 Risk Assessment

2.2.1 Quantitative evidence

There have been three unsuccessful analytical attempts to demonstrate vertical transmission in Norway and Canada.[10,11] In one of these three cases the infectious state of the broodfish was unknown, although it originated from an ISA infected site. In the remaining two cases the broodfish were confirmed ISA positive fish. In one case the eggs were reared to the juvenile stage, although they were disinfected beforehand; in the other two cases an homogenate made from the eggs was inoculated into healthy parr. ISAV was not detected, nor were signs of ISA seen in any of these cases. Some Norwegian researchers[5] suggest that ISAV is not capable of intra-ovum transmission. For intra-ovum or extra-ovum vertical transmission to be established as a genuine risk factor, ISA virus particles must be present in the ovarian fluid, on the egg surface and/or within the eggs themselves.

At present it is not possible to make a quantitative assessment of the risk of vertical transmission. Further research is required to establish the transmission route *via* gametes and repeat transmission experiments from eggs to juveniles.

2.3 Recommendations

2.3.1 ***True vertical transmission has not been proven, and there is circumstantial evidence to suggest that it does not occur. As a precautionary approach it is recommended that gametes should not be used from ISAV infected broodstock populations. This is in line with the Norwegian industry, where at present, fish reared in ISAV affected regions of Norway cannot be used for breeding purposes. This is consistent with current EU control legislation (93/53/EEC).***

2.3.3 ***It is recommended that research is undertaken on:***

a) ***The efficacy of a wide range of disinfectants against ISAV.***

b) ***Further experimental investigation on the quantification of the risk of true vertical transmission of ISAV.***

4.1.1 Characteristics of the disease

Clinically, ISA appears as a systemic and lethal condition that is characterised by anaemia, ascites, congestion and enlargement of the liver and spleen, as well as peritoneal petechiae.[52] Haemorrhages in the eyes may also be seen and hepatocellular degeneration and necrosis are consistent histopathological findings in typical outbreaks.

4.1.2 Characteristics of the virus

ISAV is an enveloped single-stranded RNA virus belonging to the *Orthomyxoviridae* family.[48,50, 51,53] The virus is pleomorphic, with a typical diameter of 100 to 140 nm and mushroom-shaped surface projections of about 10 nm. The genome consists of eight segments ranging from 1.0 to 2.3 kb with a total molecular size of approximately 14.5 kb. ISAV contains four major structural polypeptides with estimated molecular sizes of 71, 53, 43 and 24 kDa. One of the characteristics of orthomyxoviruses is their ability to bind to red blood cells. ISAV has been reported to agglutinate erythrocytes from Atlantic salmon and rainbow trout, but not the closely related brown trout.[53] The maximum rate of virus replication *in vitro* (in salmon head kidney SHK-1 cells) has been observed between 10°C and 15°C. Production of infective virus was reduced by 99% at 20°C and no replication has been detected at 25°C or above.[48]

4.2.1 Clinical signs and macroscopic findings

The clinical signs of ISA can be variable. In classic cases the fish are lethargic, congregate in the upper water levels, gasp at the surface, go off feed and may hang motionless at the sides of the cage. Affected fish may exhibit exophthalmos, ocular haemorrhage, distended abdomen and/or skin haemorrhage.

Internal pathology may include:

- dark, pale or yellow liver;
- ascites;
- pale gills and heart;
- enlarged spleen;
- petechial haemorrhage in visceral fat;
- dark foregut.

None of the above are pathognomonic for ISA. However, the probability of ISA being present increases with increased observation of these signs and lesions.[52]

4.2.2 Evidence of anaemia

Observation of pale gills and heart provides evidence of anaemia, and this may be demonstrated quantitatively by measurement of haematocrit. In advanced cases, haematocrit values are frequently below 10. Although anaemia is a classic feature of ISA, it is not specific to ISA and fish may exhibit anaemia for other reasons, for example, other infections or ulcerative conditions.

Figure 4.1 Salmon head kidney (SHK-1) cells showing cytopathic effect indicative of virus infection.

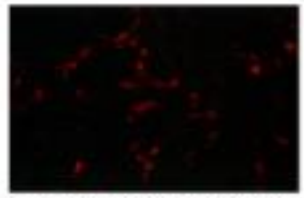


Figure 4.2 Salmon head kidney (SHK-1) cells: identification of ISAV using a monoclonal antibody-rhodamine fluorescent conjugate.

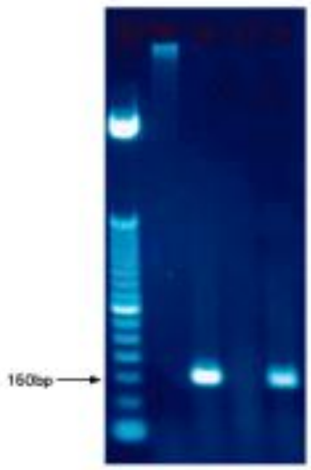


Figure 4.3 Detection of ISAV RNA using reverse transcriptase polymerase chain reaction (RT-PCR) and agarose gel electrophoresis.

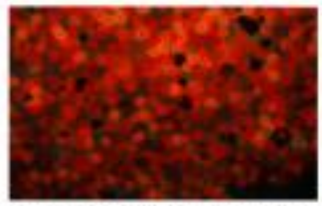


Figure 4.4a Indirect fluorescent antibody test (IFAT) of an Atlantic salmon kidney imprint for ISAV. Positive test showing cells with fluorescence in the cytoplasm surrounding nuclei which have been counterstained red using propidium iodide.

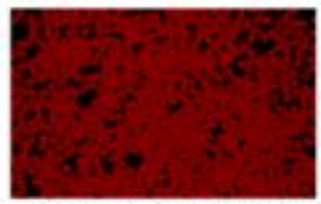


Figure 4.4b Indirect fluroescent antibody test (IFAT) of an Atlantic salmon kidney imprint for ISAV. Negative test. Cell nuclei have been counterstained red using propidium iodide.

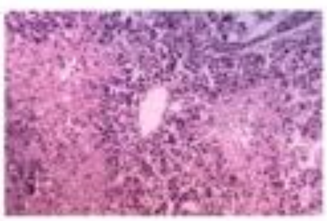
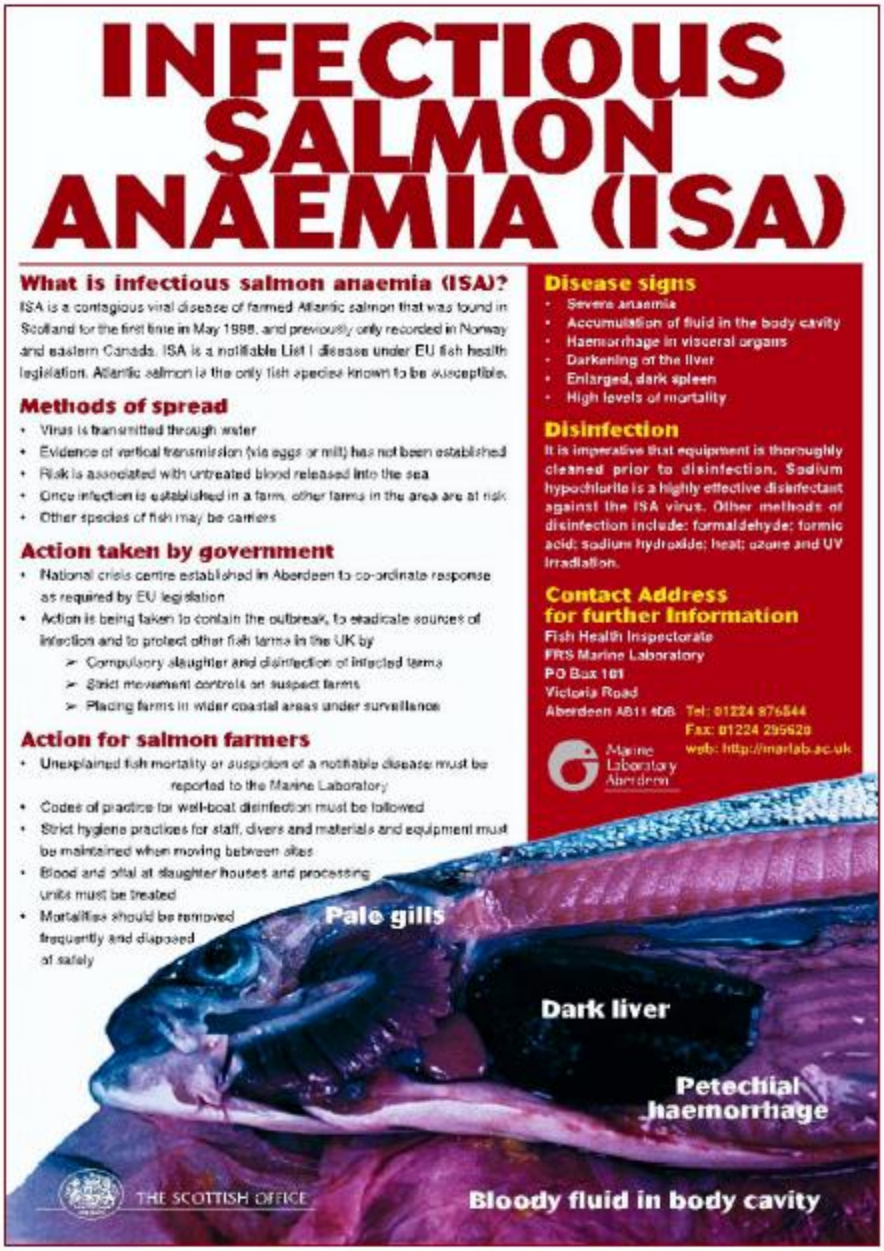


Figure 4.5 Histological section of the liver of an Atlantic salmon showing zonal necrosis associated with advanced stages of ISA. Stained with haematoxylin and eosin.

APPENDIX VI



ISA advisory leaflet issued by FRS MLA to industry following the outbreak of ISA in Scotland.

A scientific paper published in Diseases of Aquatic Organisms in 2000 reported:

Vol. 41: 1-8, 2000 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published May 25

Genetic analysis of infectious salmon anaemia virus (ISAV) from Scotland

C. O. Cunningham*, M. Snow

Fisheries Research Services (FRS) Marine Laboratory, PO Box 101, Victoria Road, Aberdeen AB11 9DB, Scotland, UK

ABSTRACT: The nucleotide sequences of segments 2 and 8 of infectious salmon anaemia virus (ISAV) isolates from Scotland were determined. These were used to predict amino acid sequences of the products of these segments. The sequences were compared with those previously published for ISAV from Norway and North America. Nucleotide and amino acid variations were found in the Scottish isolate, indicating that it is not identical to those Norwegian or North American strains. Although phylogenetic analysis of the sequences was not possible, it was clear that the Scottish isolate is more closely related to the Norwegian strain than the North American. Sequencing further isolates that are geographically or temporally separated will be important in advancing understanding of the epidemiology of this important disease.

KEY WORDS: Infectious salmon anaemia virus (ISAV) · Atlantic salmon *Salmo salar* · Nucleotide sequence

INTRODUCTION

Infectious salmon anaemia (ISA) has led to serious losses in the Atlantic salmon *Salmo salar* farming industries of Norway, Canada and now Scotland. The disease was first recognised in Norway and typical pathological signs include severe anaemia, leucopenia, ascites and haemorrhagic liver necrosis (Thorud & Djupvik 1988, Evensen et al. 1991). Haemorrhagic kidney syndrome (HKS) in Canada has now been recognised as the same disease (Mullins et al. 1998, Bouchard et al. 1999, Lovely et al. 1999). In 1998, the disease occurred in Scotland (Bricknell et al. 1998, Rodger et al. 1998, Turnbull 1999, Stagg et al. 1999).

Characterisation of the etiological agent of the disease first identified an orthomyxovirus-like enveloped virus (Hovland et al. 1994, Nylund et al. 1996). The arrangement of the genome of this virus was found to be typical of *Orthomyxoviridae* (Mjaaland et al. 1997) but no significant similarities were identified when the nucleotide and amino acid sequences of segment 8 were compared with sequences from other orthomyxoviruses. Analysis of the ISA virus (ISAV) polymerase protein (PB1) encoded by segment 2 also showed low sequence similarity with other members of the *Orthomyxoviridae*, but did reveal some conservation of motifs (Krossøy et al. 1999). Phylogenetic analysis carried out using this sequence placed ISAV within the *Orthomyxoviridae* but revealed significant distance between ISAV and the other orthomyxoviruses. The authors proposed that ISAV represents a new genus, *Aquaorthomyxovirus*.

Nucleotide and amino acid sequence comparisons of ISAV segments 2 and 8 from North American and Norwegian isolates demonstrated significant differences between virus from these locations (Blake et al. 1999). Comparisons such as this are of great interest to those investigating the epidemiology and potential sources of disease. Knowledge of the degree of variation between isolates from different regions or countries can provide valuable guidance for the management of the disease, development and application of diagnostic methods and vaccination strategies. Nucleotide sequence analysis produces detailed information on virus similarity. Therefore, sequences of ISAV from Scotland were obtained and compared with those already identified from other countries.

*E-mail: cunninghamc@marlab.ac.uk

BBC News reported in November 1999:

BBC NEWS

News in Audio | News in Video | Newyddion | Новости | Noticias

Front Page
World
UK
UK Politics
Business
Sci/Tech
Health
Education
Sport
Entertainment
Talking Point
In Depth
On Air
Archive

Feedback
Low Graphics
Help

SCOTLAND

Thursday, November 4, 1999 Published at 15:29 GMT

UK: Scotland

Lethal fish infection spreads

Millions of salmon have been destroyed because of the disease

A deadly fish disease is believed to have spread for the first time to wild salmon in Scotland and has been detected on another six salmon farms.

The new scare involving Infectious Salmon Anaemia in the Shetland Isles, the Western Isles and Orkney has prompted the Scottish Executive to embark on an urgent review of the present controls.

A spokesman confirmed these were among the first cases in the world of the ISA virus being detected in wild salmon.

These latest figures bring to 24 the total number of farms in Scotland which have been suspected of having the disease over the last 18 months.

A scientific paper published in 2008 reported:

Bull. Eur. Ass. Fish Pathol., 18(4),115, 1998.

INFECTIOUS SALMON ANAEMIA (ISA) IN THE UNITED KINGDOM

H. D. RODGER, T. TURNBULL*, F. MUIR, S. MILLAR & R. H. RICHARDS
Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, UK
*Hydro Seafood GSP, South Shian, Connell, Argyll, PA37 1SB, UK

Abstract
The first indication of infectious salmon anaemia (ISA) in the UK is described. Clinically the fish presented with severe anaemia, dark brown almost black livers, pyloric caecal petechiae and histologically with multifocal haemorrhagic necrosis in the livers.

Introduction
Infectious salmon anaemia (ISA) is an infectious disease of Atlantic salmon (*Salmo salar*) caused by an orthomyxo-like virus (Falk *et al*. 1997). It is of considerable economic importance to the salmon farming industry in Norway and has recently been isolated from salmon in Canada suffering a significant disease known as haemorrhagic kidney syndrome (Mullins, personal communication). This communication reports the preliminary findings in the first outbreak of ISA in a salmon farm in the UK.

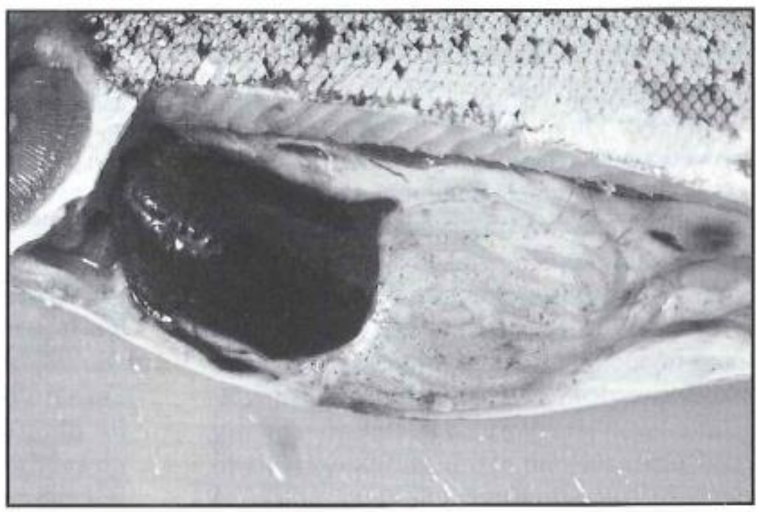


Figure 1 Atlantic salmon from a marine farm in Scotland exhibiting pale gills, dark slightly swollen liver and numerous petechiae on the pyloric caecae.

Clinical history

Approximately 1.3 million S1/2 Atlantic salmon were being reared in one cage site in a remote sea loch in the west of Scotland in May 1998. These fish had been transferred to sea in November 1997 and had experienced no significant health problems other than a low level *Vibrio* sp. infection, which was being treated with antibiotics. Towards the end of April there was an increase in mortalities, with 3% of the stock lost within a one week period. Many of these mortalities had no skin lesions, unlike the fish oreviously affected by *Vibrio* sp., and were generally in good condition externally; the average size was 500g. Water temperature at the time of the increase in mortalities was 8 to 8.5°C.

Clinical findings

Moribund, lethargic fish were obvious near the water surface and on examination had pale gills and some had dark brown, almost black and slightly swollen livers with numerous petechiae on the pyloric caecae (Fig. 1). These fish had no skin lesions and had not fed recently.

Heparinised blood samples revealed mean haematocrits of 11.3% ($\pm$ 10.9) with a range in values from 2 to 26 (n = 9).

Histopathology

Multifocal haemorrhagic necrotic areas were present in the livers with, in some fish, relatively normal areas around the central hepatic vessels giving a distinctive zonal appearance to the hepatic pathology. In the kidneys there were multiple foci of interstitial haemorrhages which in some fish appeared confluent throughout the kidney. Focal haemorrhages were also apparent in the lamina propria of the pyloric caecae and intestine.

Virology

Four pools of tissue extract (kidney, spleen and liver) were inoculated onto CHSE - 214 and salmon head kidney (SHK - 1) cell lines (primary cultures provided by Dr. B. Dannevig, National Veterinary Institute, Oslo) and were incubated at 15°C and 18°C respectively. Three out of the four samples showed a passagable cytopathic effect (CPE) on day four after inoculation of SHK - 1 cells. No CPE was observed on the CHSE - 214 cell lines.

Kidney impression smears were also made on poly - l - lysin coated slides, air dried and fixed in acetone for 10 minutes. These were then forwarded to Dr. K. Falk, National Veterinary Institute, Oslo for screening by a direct immunofluorescent test (DIF) using an anti-ISA virus monoclonal antibody. Seven out of eight samples screened gave a positive reaction.

Interpretation of findings

The combination of the gross pathological findings, histopathology and haematology would by themselves be indicative of ISA (OIE, 1997), however, the further findings assist in confirming the first diagnosis of ISA in Europe.

Acknowledgements

Drs. Birgit Dannevig and Knut Falk of the National Veterinary Institute, Oslo are thanked for their advice and assistance in this case.

References

Falk, K., Namork, E., Rimstad, E., Mjaaland, S. & Dannevig, B. H. (1997) Characterization of infectious salmon anaemia virus, an orthomyxo-like virus isolated from Atlantic salmon (*Salmo salar* L.). *Journal of Virology*, **71**, 9016 - 9023

OIE (1997) Infectious salmon anaemia. In: *Diagnostic Manual for Aquatic Animal Diseases*. 2nd Edition, Office International des Epizooties, Paris, France

BBC News reported in August 1998:

BBC NEWS

News in Audio | News in Video | Newyddion | Новости | Noticias

Front Page
World
UK
UK Politics
Business
Sci/Tech
Health
Education
Sport
Entertainment
Talking Point
In Depth
On Air
Archive

Feedback
Low Graphics
Help

SCOTLAND

Thursday, August 19, 1999 Published at 13:25 GMT 14:25 UK

UK: Scotland

Salmon farming restrictions lifted

A third of salmon farmers have been given the all-clear

A third of Scotland's salmon producers who faced tight restrictions because of a deadly fish virus have been given the all-clear to begin farming again.

Infectious salmon anaemia (ISA) has crippled the fish farming industry - causing job losses and leaving it millions of pounds out of pocket.

BBC Scotland's Andrew McFadyen reports on the lifting of restrictions

But Deputy Minister for Rural Affairs John Home Robertson has announced the end of restrictions on 83 salmon farms affected by the disease.

Seven of these farms had to close their operations as a result of the virus.

BBC News reported in October 1998:

BBC NEWS

News in Audio | News in Video | Newyddion | Новости | Noticias

Front Page
World
UK
UK Politics

Sunday, October 4, 1998 Published at 19:27 GMT 20:27 UK

UK

Virus threat to fish farms

The government is investigating an outbreak of a highly infectious disease that has led to the culling of thousands of tonnes of salmon in Scotland.

BBC Scotland Correspondent Andrew Cassell: Up to a third of fish farms are being monitored

It is thought that the infectious salmon anaemia may have been caused by the illegal importation of live fish from Norway.

Until five months ago Scottish salmon farms had been free of the virus which in the past has devastated the industry in Canada and Norway.

Government officials are still investigating how the disease was transferred to the UK.

Compensation claim

It has now led to the closure of 10 Scottish farms and 150 redundancies.

Up to a third of Scotland's 350 farms are being monitored for signs of the virus, raising fresh concerns of an epidemic in an industry worth nearly £300m and employing around 6,000 people in fragile rural economies.

The virus is highly infectious, causing internal bleeding, but government officials say it cannot be contracted by humans.

Asda and Sainsbury's said their stock comes from areas not affected by the virus.

Intrafish reported in October 1998:

IntraFish Aquaculture Fisheries Processing Markets Feed Opinion Species

Alert me about News

ISA prompts dirty war

Some of UK's leading supermaket chains have been sent letters early this week from anglers' groups warning them that some salmon producers may have recently used Ivermectin - without notifying purchasers - in a bid to fight against ISA.

12 October 1998 8:00 GMT *UPDATED 10 July 2012 8:52 GMT*

The Scottish Anglers' National Association and the Salmon and Trout Association Sea Trout Group has written to supermarkets warning that salmon farmers, under pressure from the ISA crisis, may be harvesting fish treated with the anti-sea lice chemical Ivermectin within the recommended 100-day withdrawal period.

The Scottish Salmon Growers' Association rejected the claim as "irresponsible and unsubstantiated"; adding that this would "be courting commercial suicide" from a farmer's perspective.

Major retailers like Mark & Spencer have adopted a policy to refuse any salmon treated by the anti-sealice chemical. Asda and Sainsbury's say they are reviewing their salmon purchasing policy, and that their stock is from areas which are not affected by the disease.

A spokesman for Tesco told SBR that they had not "changed supplier", but rather "diverted" their sourcing to other non-ISA-stricken farms. Despite the fact that the Scottish Office emphasises that there is no risk to human health from infected salmon, the Tesco spokesperson said that the company had 'to be careful', in particular after the BSE scare. He also dismissed rumours that ISA-infected salmon had been used during last week's "£0.99 per lb" salmon promotion since there is no way such salmon would be allowed to reach the consumers.

Read more via:
Scottish Salmon's 'Ticking Time-Bomb': ISA reported at Scottish Sea Farms on Mull
Media Backgrounder: Scottish Salmon's Recurring ISA Nightmare
Massive Attack on "Rotten Edifice" of 'Scottish' Salmon
Norwegian Salmon Eggs Slip Back Into Scotland (as Infectious Salmon Anaemia plagues salmon farms in Norway)
Norway's Infectious Salmon "Horror Show" Secretly Playing Now In Scotland?
Infectious Salmon Anaemia - Diary of Disease Disaster
Scottish Sea Farms confirms it owns ISA-infected site
Minister criticises industry over ISA
Shetland Islands to cut salmon farms as ISA spreads
ISA will put 27 Scottish fish farms out of action
Shetlanders get £3m ISA loan
New ISA Case Found in Shetland Control Zone
Fish virus detected at third farm
Second ISA case confirmed in Scotland
ISA back to haunt fish farmers
Deadly salmon infection detected

ISA disease suspected at Marine Harvest farm
The scientific issues surrounding the control of Infectious Salmon Anaemia in Scotland
ISA cost Scottish industry £100 million
ISA report published by Scottish Executive
Lethal fish infection spreads
£25.68 million loss in Scotland
ISA can wipe out farms
More ISA found
Fish Farmageddon - The Infectious Salmon Aquacalypse