ISA: Challenges related to epidemiology, detection, control and documentation of the ISA situation including questions related to identifying the source of new disease outbreaks

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Background and rationale

- Norwegian aquaculture is facing a new legal framework through the implementation of the new Animal Disease Legislation (ADL) from April 2021.
- ADL now only allows «classical» eradication programmes with a defined time-frame
- Thus, Norway is loosing the legal framework underlying our (successful?) ISA control programme which have been in function since the early 1990-ties.
- Q: How shall Norway prevent the potential devastating effect of ISA in the future, and what challenges are we facing when attempting to control this disease?

ISA and Norwegian marine salmon production

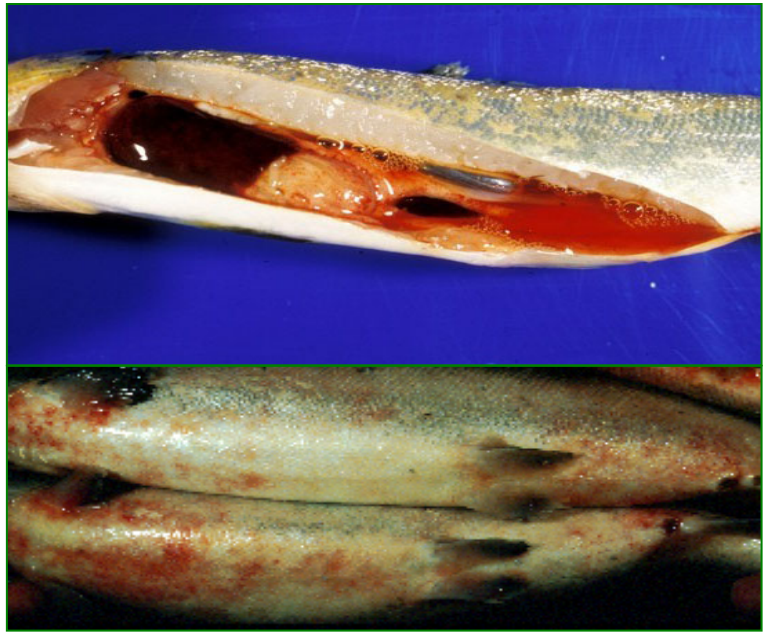
- 450-500 active ongrowing sites
- Average number of fish per site approx. 1 million fish
- Slaughter at 5 kg, i.e. each fish has a value of EUR 30 ++
- During the last 25 years: 1-20 annual ISA outbreaks
  for the last 5 years: 10-15 annual outbreaks
  <u>Overall annual incidence rate: 2-3%</u>

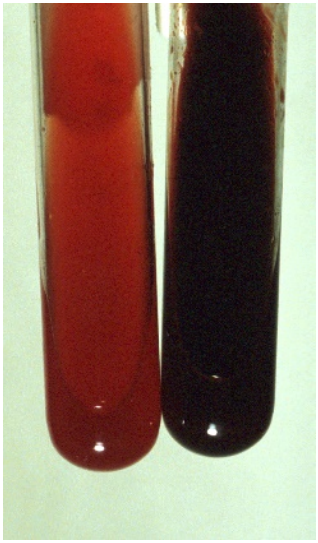
ISA occurrence, characteristics and epidemiology

- Initially, ISA usually appear as an insidious infection affecting one or a few netpens. Such situation may persist for weeks or months, and may be difficult an require experience to detect.
- Typical daily mortality in an affected netpen is around 0.05 – 0.1 %
- If noting is done to limit infection, a more acute appearance may develop with more netpens affected and higher mortality.
- Outbreaks may only affect a single farm in an area, or appear as small epidemics affecting several farms
- Outbreaks occur in salmon farms along the whole Norwegian coast

ISA and ISAV infections

- ISA is caused by virulent HPR-deleted ISA-virus
- The infection is initiated on mucosal surfaces, followed by infection of the endothelium of the circulatory system.
- Clinical and pathological findings are characterised by circulatory disturbances, bleedings and anaemia





ISAV HPR0

- It is now generally accepted that the HPR0-type, which does not cause disease, is the origin of virulent HPR-deleted virus through deletions in the HE-gene and mutations/inserts in the F-gene
- ISAV HPR0 causes a common, sub-clinical, and transient infection of the mucosal epithelium. HPR0 virus is prevalent in all production stages and is also a common finding in wild salmon.
- Formal reports and anectdotal suggest that all salmon populations experience one or several HPR0 infection episodes during their life cycle.
- However there are several unknown's:
  - We don't know the frequency of the HPR0 to HPR-del transition
  - We don't know what drives the transition
  - We don't know the risk of transition to virulent HPR-del virus if HPR0 is found
  - Q: Will detection of HPR0 increase the risk of getting ISA?
- Unsolved questions:
  - Which role does HPR0 infection play associated with general population immunity?
  - Are there any unknown reservoirs for ISAV? (HPR0 and HPR-del)

Current Norwegian ISA control strategy

- The current strategy was established in the early 1990-ties, and was initially claimed to be an eradication programme.
- However, in practical terms, the programme is a procedure to lower infection pressure and prevent further spread of the infection.
- The programme include detection of new cases, establishing a control zone surrounding infected farms (combat + surveillance zone) followed by fallowing and various control measures.
- The programme also include a number of rules and actions aimed at improving biosecurity.
- The programme has been successful in limiting spread of the disease, but has not eradicated ISA

There are now three major challenges associated with ISA control:

1. Detection of outbreaks and infected populations/sites
2. Identification of the source of new infections
3. Documentation of freedom of infection

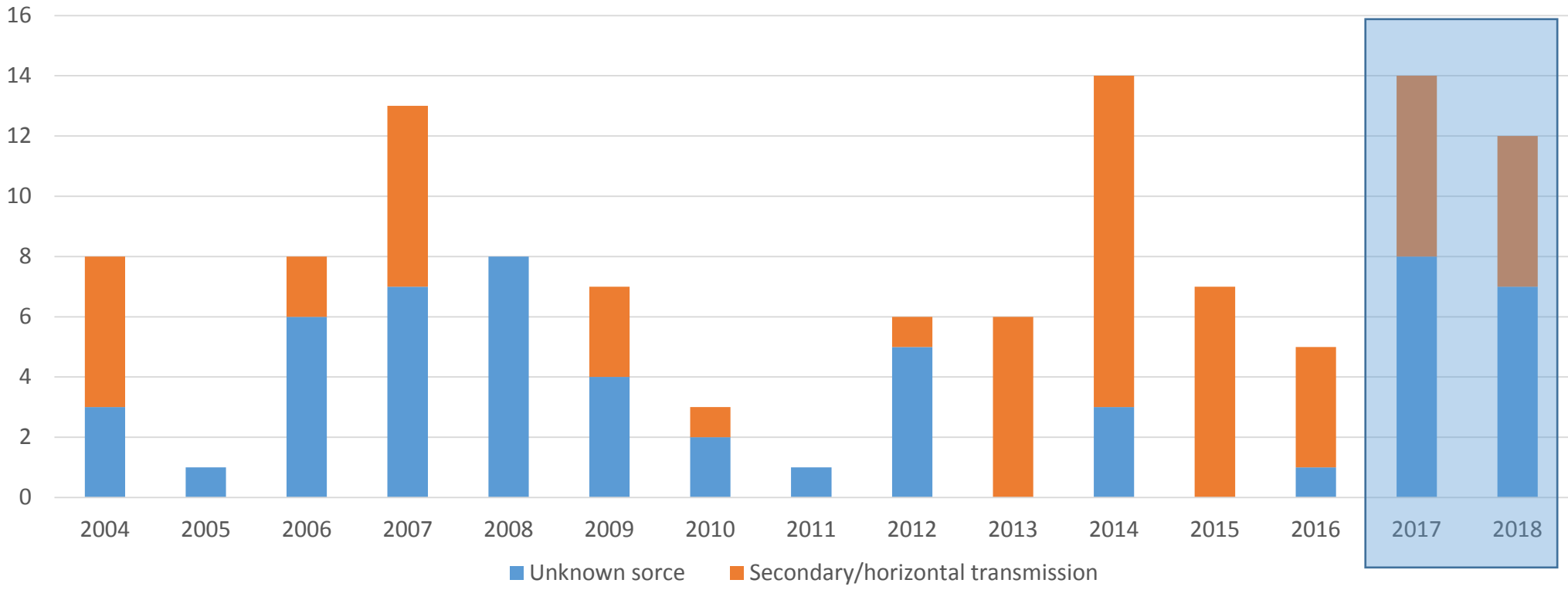
1. Detection of outbreaks and infected populations/sites

- Clinical observations and classic disease diagnostics are key factors for detecting disease cases at early stages. The skills and experience of the farmer and fish health services are thus essential.
- Diagnostic methods include
  - clinical and gross pathological examinations
  - IHC
  - qPCR
  - Virus cultivation, sequencing and phylogenetic analyses
- qPCR alone is a powerful tool to detect ISAV infection in diseased fish, but has significant limitations associated with screening for infected fish. It can be challenging to find ISAV by qPCR even in known infected populations.

2. Identification of the source of new infections:

Approx 40% of the ISA outbreaks have an unknown source

(The figure is modified from Lyngstad et al. 2018: Risk Factors Associated With Outbreaks of Infectious Salmon Anemia (ISA) With Unknown Source of Infection in Norway.



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2. Identification of the source of new infections

- Possible sources:
  - Horisontal transmission from known or unknown sources
    - Transmission from neighbour farms
    - Transmission from smolt facilities
    - Transmission through transport, equipment, personell etc
  - Vertical transmission from broodfish
  - New transitions from HPR0 to virulent HPR-del virus
  - Transmission through virus persisting in the environment or from an unknown reservoir.

3. Documentation of freedom of infection

- A “classical” eradication programme require:
  - Efficient detection of infected animals
  - Ability to document freedom of infection
  - As apparent incidence is reduced, it becomes more challenging to detect new cases
- Field observations suggest:
  - The diagnostic/epidemiological predictive value of a negative qPCR may be very low.
  - qPCR testing is an excellent tool to detect ISAV in diseased fish, however it can be challenging to detect virus in fish that don’t show typical sign of disease – even in populations known to be ISAV infected.
  - qPCR testing to document both freedom of infection, or to test for presence of infection must be evaluated critically and with great care.
- A couple of examples illustrates this uncertainty:

Example 1:
qPCR-testing of salmon from 4 confirmed ISA outbreaks

		N	Kidney	Brystfinne	Gjelle	Ryggfinne
1	A	28	**0**	0	0	0
	B	30	6	17	4	24
	C	30	8	17	14	23
2	A	30	**0**	0	4	1
	B	30	6	27	26	25
	C	30	29	29	29	27
3	A	30	**0**	0	0	0
	B	30	0	0	0	0
	C	30	11	14	22	23
4	A	30	**0**	0		
	B	30	0	0		0
	C	9	0	0		0
	Totalt	337	80	104	99	123
	%		31	45	59	67

A: Healthy fish, healthy cage
B: Healthy fish, cage with diseased fish
C: Fish with clinical ISA

Example 2:

Last ISA-outbreak on the Faroe Islands in 2016 – summary of testing (from Debes Christiansen)

Date	Number of fish	ISAV RT-qPCR	HPR0	HPRdel	Undet.
14. Mars	5	0			
06. April	40	1	1	0	0
08. July	30	11	0	7	4
15. July	188	0			
20. July	38	0			
11. Aug	40	2	2	0	0
16. Sept	77	5	3	0	2
20. Oct	80	3	1	0	2
22. Nov	79	0			
Total	577	22	7	7	8

Further history:

5. JanuarY: 5 qPCR positive out of 90 fisk, clinical sign of ISA observed

17. Januar: Testet approx 60% out of 117 fish tested positive => slaughter

Sequencing suggested that the virus detected in July and January was the same,

I.e. the virus had persisted in the farm, but where and why so many negative tests?

Conclusions/summary

- The Norwgian salmon industry is facing a major challenge related to ISA control within the framework given by ADL.
- The benefit-cost of a classical eradication program will be uncertain because of:
  - The size of the industry
  - The nature of the disease
  - The low apparent incidence of new outbreaks
  - The lack of reliable screening procedures
- However, if nothing is done to limit spread of the infection, small outbreaks may easily develop into devastating epidemic situations.