PD along the coast of Norway - can we prevent further spread?

Trination meeting, Dublin
June 3-4th 2015
Tore Hovland, MSD-Animal Health
Risk factors and PD-infection

1. Important factors, but not discussed further here:
   o Smolt quality, handling incl. dead fish, escapees, water current

2. PD areas
   • Development of numbers of PD cases in salmon & rainbow trout
   • Development of fish stock kept in sea
   • Vaccination strategy
   • Fallow areas
   • PD legislation

3. Environment
   • Temperature

4. Transportation
   • Open/ closed transport
   • Transport routes
   • Movement of infected fish

5. Harvesting plants
   • Localization & centralization
   • Open holding pens
Viable SAV from dead fish can be transported by the surface currents

Liquid fat, a potential abiotic vector for horizontal transmission of salmonid alphavirus?

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⁴ PanGen Analyse AS, Åsund, Norway

Abstract

Viral diseases are a serious challenge to marine farming of Atlantic salmon (Salmo salar L). Pancreas disease (PD) caused by a salmonid alphavirus (SAV) is by far the most serious in northern Europe. To control PD, it is necessary to identify virus transmission routes. One aspect to consider is whether the virus is transported as free particles or associated with potential vectors. Farmed salmon have high lipid content in their tissue which may be released into the environment from decomposing dead fish. At the seawater surface, the effects of wind and ocean currents are most prominent. The aim of this study was to identify whether the lipid fraction leaking from dead infected salmon contains SAV. Adipose tissue from dead SAV-infected fish from three farming sites was submerged in buckets with sea water in the laboratory and stored at different temperatures and time conditions. SAV was identified by real-time RT-PCR in the lipid fractions accumulating at the water surface in the buckets. SAV-RNA was also present in the sea water. Lipid fractions were transferred to cell culture, and viable SAV was identified. Due to its hydrophobic nature, far with infective pathogenic virus at the surface may contribute to long-distance transmission of SAV.

Keywords: Emergent disease in aquaculture, Salmon Pancreas Disease Virus.

Introduction

Pancreas disease (PD) has negative impact on Atlantic salmon farming in Ireland, Scotland and the western parts of Norway (McDoughill & Gesham 2007). The disease leads to increased mortality, decreased growth and quality downgrading at slaughter. PD is therefore of importance to both fish welfare and farm economy (Autonuevo et al. 2010). The causative agent is a salmonid alphavirus (SAV) and six subtypes are registered (Fringuelli et al. 2008). In Norway, subtype 3 (SAV3) was the dominating subtype responsible for PD until 2010 when subtype 2 was recorded for the first time at a marine farming site in mid-Norway (Hjorten et al. 2013).

SAV is a virus with long half-life in cold, saline water (Graham et al. 2007; Graham, Rowley & McConnell 2010) and may therefore be capable of long-distance transport along the Norwegian coast. When introduced into an area with high salmoid farming activity, SAV has the capability of spreading to nearby farms. Close proximity, shared ownership with infected farms and movement of fish have been suggested as potential risk factors for SAV transmission (Rodger & Mitchell 2007; Stensfjord et al. 2009; Akhtar et al. 2010; Taneesupaphat et al. 2012). Water current by ocean currents is the most explanatory variable when it comes to transmission of PD among marine farming sites for salmon in Norway (Viljugrein et al. 2009; Sven et al. 2014).

To our knowledge, SAV is not identified in sea water in field, only in seawater laboratory studies (Graham et al. 2007; Andersen, Høidal &...
PD is transmitted efficiently in the water column, both within and between locations — several publications has confirmed the infectivity of the disease.

<table>
<thead>
<tr>
<th>Year</th>
<th>Journal</th>
<th>Authors</th>
<th>Title</th>
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<tbody>
<tr>
<td>2012</td>
<td>BMC Veterinary Research 2012, 8:172, 1-10</td>
<td>S. Tavornpanich et al.</td>
<td>Risk map and spatial determinants of pancreas disease in the marine phase of Norwegian Atlantic salmon farming sites</td>
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</table>
SAV3 appeared in the Norwegian aquaculture in the 1980s with a gradual spread to an endemic zone that covered practically the entire south-west part by the end of 2006.

The first cases of marine SAV2 were reported in 2011 and this subtype spread rapidly between fish farms outside the PD endemic zone and is responsible for disease outbreaks at an increasing numbers of sites.
Sequence analysis of the E2 gene revealed that all marine SAV2 included in this study were nearly identical, suggesting a single introduction into Norwegian aquaculture.

The first samples positive for marine SAV2 originated from Romsdal, in June 2010.

This study also support the generally recognized hypothesis of a single introduction of the SAV3.

Figure 2 Map showing the distribution of salmonid alphavirus (SAV) subtypes in Norway in years 2007–2012. Red dots represent SAV2, the dark blue cross SAV3 from Atlantic salmon and turquoise dots SAV3 detected in rainbow trout. The magnification shows the spread of marine SAV2 in Regions 2, 3 and 4 in 2010–2012. Marine SAV2 detected in 2010 is represented by yellow dots while detections in 2011 and in 2012 are marked orange and red, respectively.
Pancreas Disease (PD) is still the most important viral disease in Norwegian Fishfarming

The statistics constitutes numbers of new positive locations or new locations after the following period i.e. the real number of infected sites every year is much higher, since additional numbers of infected fish is already present in sea from the year before

The most important reservoir for infection is infected farmed fish
Differentiation of mortality after sea transfer

Hordaland: S1-2012

2012

Mortality causes

2013

Without Diagnosis

Transportation

Loser Fish

Wounds

Mortella/Winter ulcer

Gill Infection

Handling

CMS

PD

Mortality due to PD often occurs late in the sea phase
Most PD detections in the summer; May-August
-Rest of the year the detections are more evenly distributed per month
### Official PD statistics 2014

#### Distribution of PD cases (suspicions & detections):

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<th>County</th>
<th>Jan</th>
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**Hordaland:**
- Most detections
- Mortality?
- Atlantic salmon or rainbow trout?
- Vaccinated?

**Sør Trøndelag:**
- 2. most detections
- Mortality?
- Atlantic salmon or rainbow trout?
- Vaccinated?
## Distribution of PD cases (suspicions & detections):

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Most PD detections in 2014:
1. Hordaland
2. Sør Trøndelag
3. Rogaland
Although more salmon in sea in Nordland, the density of farmed fish in sea in Hordaland is higher...and that also applies to Rogaland and Trøndelag...
Sea transferred salmon over 20 years (1994-2014) - per county

Source: Directorate of Fisheries

Numbers x1000

Increasing number of potential hosts for diseases
Numbers of salmon in sea 2014 - average per month

Nordland is the county containing most salmon in sea

Source: Directorate of Fisheries
Sea transferred rainbow trout over 20 years (1994-2014) - per county

Source: Directorate of Fisheries

18.6% of the fish in sea in Hordaland is rainbow trout (2014)

Numbers $\times 1000$

County

Finnmark, Troms, Nordland, Nord-Trøndelag, Sør-Trøndelag, Møre og Romsdal, Sogn og Fjordane, Hordaland, Rogaland, Others, Total

Increase in Hordaland
Herd immunity

• Maximum protection due to vaccination is achieved when a major portion of the population is vaccinated.

• The percentage that must be vaccinated for controlling the disease will depend on the infectivity of the disease.
How herd immunity influences the risk of outbreaks of diseases - i.e. MMR vaccination (vaccine against mumps, measles & rubella) in UK

<table>
<thead>
<tr>
<th>Disease</th>
<th>Transmission route</th>
<th>$R_0$</th>
<th>Level of the population needed to be vaccinated</th>
</tr>
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<tbody>
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<td>Diphtheria</td>
<td>Saliva</td>
<td>6-7</td>
<td>85%</td>
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<tr>
<td>Measles</td>
<td>Air</td>
<td>12-18</td>
<td>83 - 94%</td>
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<tr>
<td>Mumps</td>
<td>Air/secretion</td>
<td>4-7</td>
<td>75 - 86%</td>
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<td>Whooping cough</td>
<td>Air/secretion</td>
<td>12-17</td>
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<td>Polio</td>
<td>Intestinal-mouth</td>
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<td>Rubella</td>
<td>Air/secretion</td>
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<td>Smallpox</td>
<td>Air/secretion</td>
<td>5-7</td>
<td>83 - 85%</td>
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<tr>
<td>Ebola</td>
<td>Body fluids/secretion</td>
<td>1-2</td>
<td>Test vaccine</td>
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<td>PD</td>
<td>Waterborne/contact</td>
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$R_0$ = Maximum number of individuals in average that can be infected from one individual
PD in rainbow trout (rbt):

- Marine SAV2 in rbt was registered for the first time in the county Sør-Trøndelag and this fish was kept in the same site as SAV2 infected salmon.

- PD in rbt has been diagnosed annually since 1996 in the SAV3 area, with a peak in 2011 with 18 registered sites.

- In the county Møre & Romsdal PD SAV3 is limited to the area Storfjorden.
Can SPDV infected rbt transmit virus to rbt and salmon?
- presence of SPDV virus/virus genetic material in heart 4 weeks after addition of vectors

![Bar chart showing frequency of infection in vectors, rbt, and salmon.]

- **Vectors (rbt):**
  - Positive: 20%
  - Negative: 80%

- **Rbt:**
  - Positive: 30%
  - Negative: 70%

- **Salmon:**
  - Positive: 100%

Yes, infected rbt is able to transmit PD to rbt and salmon via the water.
How much constitutes PD in rbt of the total detected / suspected cases? - period 2009-2014

Hordaland 2013:
- 25% of the PD detections were registered on rbt and these constitutes 20% of the fish sea transferred in that county
- <80% of the fish in Hordaland were PD vaccinated
Fallow areas in Hordaland

Source: Lovdata
Sea transferring of S0-2014 in Hordaland (13.10.14)

-What can be expected of PD development in this area?

Source: AkvaGIS

PD-vaccinated and unvaccinated fish are sea launched in areas already containing PD infected salmon & rbt from different generations.

RBT= Rainbow Trout
RBT= Rainbow Trout previous generations
Sea transfer of S1 per 19th May 2015, Hordaland

Date: May 29th 2015
New suspicions

AGD detections:
- sites with PD
- sites without PD
- different fish generations

Sources: AkvaGIS, Lovdata

Tegnforklaring

Anlegg: Utsett alle
- Vår 2015
- Høst 2014
- Vår 2014
- Høst 2013
- Vår 2013

Anlegg: Ikke aktiv

Vår 2015

AGDA - Positiv

AGD - Positiv

PDV - Positiv

PD - Positiv

= Holding pens

Date: May 20th 2015
New suspicions
Sea transfer of S1 per 8th May 2015, Rogaland

Source: AkvaGIS

AGD detections:
- sites with PD
- sites without PD
- different fish generations

Tegnforklaring

Anlegg: Utsett alle

Vår 2015
Høst 2014
Vår 2014
Høst 2013
Vår 2013

Anlegg: Ikke aktiv

AGDA - Positiv

AGD - Positiv

PD - Positiv

Slakteri (Kilde: Fiskeridirektoratet)

= Holding pens

AGD detections:
- sites with PD
- sites without PD
- different fish generations
Overview on localization of the different fish generations present in sea and PD status is not available on the AkvaGIS map north of Hustadvika.

Source: AkvaGIS
PD-status in Sør-Trøndelag per March 2015

Source: Directorate of Fisheries

Tegnforklaring:
- Slaktemer: Slaktemer = Holding pens
- PD: PD Mistanke = PD suspicions
- PD påvist = PD detections
Any contact between these areas?
Wellboat routes
Period: 60 days August-October 2014
Source: MarineTraffic

SAV2 salmon
SAV3 rainbow trout
PD detections in the observation zone: Nord Trøndelag
- movement of PD positive fish from the observation zone into the combat zone

Bondøya 10256, Vikna
PD suspected 17.09.14
PD confirmed 03.10.14
Fish removed within 14.11.14
PD positive site in Meløy, Nordland - October 2014
«Stamping Out»

Source: Fiskeridirektoratet

Loc. 13125 Storvika, Meløy
PD detected Oct. 24th 2014
The loc. emptied in 14 days

Tegnforklaring
Slaktemerd:
= Holding pens
PD:
= PD suspicions
= PD detections
SAV2 now also registered south of SAV3 areas
PD status October 2014, Nordfjord

Sources: AkvaGIS, Mattilsynet

The first registered co-infected site with SAV2 + SAV3 from the field.
Ref. PD report December 2014 Veterinary Institute

Separate SAV2 regulations under preparation for Nordfjord by the Norwegian Food Safety Authority
Proposed new SAV2 zone regulations for Nordfjord

Date picture: 16th April 2015

PD detection period Nordfjord: October 2014-February 2015

Sources: Norwegian Food Safety Authority, AkvaGIS
New SAV2 regulation decided for Nordfjord, May 5th 2015

SAV2 observation zone
SAV2 combating zone

Sources: Norwegian Food Safety Authority, AkvaGIS
PD-regulations: Will there be changes?

Source: Norwegian Veterinary Institute

- New! SAV2 regulation in Nordfjord
- Hustadvika
- SAV2 regulation
- SAV3 regulation
- Observation zone
- Endemic zone

35
Temperatur

Figur 8: Gjennomsnittstemperaturen på lokalitetene

Gjennomsnittstemperaturen i mars var 5,65 °C, mot et snitt i perioden 2008 - 2013 på 4,59 °C
The effects of temperature of incubation (4, 10, 15 and 20 °C) on growth in TO, CHSE-214 and RTG-2 were investigated. In TO and RTG-2 growth was optimal at 15 °C, whereas in CHSE-214 results at 10 and 15 °C were more similar. Little or no growth was detected at 4 or 20 °C.

Cell lines:

TO: Derived from Atlantic salmon head kidney leucocytes
RTG-2: Rainbow trout gonad
CHSE-214: Chinook salmon embryo
Survival analysis demonstrated that cohorts exposed to virus at decreasing sea temperature had a significantly longer incubation period than cohorts infected when the sea temperature was increasing.

The farmers can emphasize prophylactic management, avoid stressful operations until the sea temperature is decreasing and consider removal of cohorts at risk, if possible.
Use of wellboats

- over long distances
- between countries
- between counties
- locally inside the fjords
- between different sites
- between harvesting plants
- smolt & fish for harvesting
- through infected areas
- treatments; sea lice, AGD
- capacity challenges?
Hordaland county: PD-detections & suspected cases per 07.09.14 - transportation between sites in heavily infected areas

Sources: Directorate of Fisheries, MarineTraffic

Example: Wellboat routes
Period: July-September 2014
Period: 60 days

During the period only a few times the routes are in open waters
Sør & Nord Trøndelag: PD detections & suspected cases per 08.09.14 - transportation between infected areas

Sources: Directorate of Fisheries, MarineTraffic

Example: Wellboat routes
Period: July-September 2014
Period: 60 days
Wellboat routes Scotland-Orkney-Shetland
August- October 2014

Source: MarineTraffic
Wellboat routes from Shetland to North-West coast of Norway, October 2014

Source: MarineTraffic
SAV2 is dominated in Shetland & Orkney
The way forward...

Consensus on zone strategy?
- Will additional PD detections in Nord Trøndelag and further north along the coast lead to change of the regulations?
- Will we see expanded use of the coast line and alternative forms of production for fish farming in the future?

Will stricter regulations for transport of fish have positive effects on the spread of infection?
- Closed transport, disinfection of transportation water...

What about the harvesting plants?
Adaptation to a stricter regime as for the wellboats?
- Closed holding pens, harvesting directly from the wellboats, handling of wastewater...

An overall initiative should be taken for managing PD for avoiding the fragmented practice as seen today. This will be beneficial for the fish farming industry in the future.
Thanks for your attention

«Sunset from Algrøy, Sotra»