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:
 - 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

Journal of Fish Diseases 2015

doi: 10.1111/jfd.12382

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

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Abstract

Introduction

Viral diseases represent serious challenge in 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 salmonids 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 primarily 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 beakers with sea water in the laboratory and stored at different temperature and time conditions. SAV was identified by real-time RT-PCR in the lipid fractions accumulating at the water surface in the beakers. 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, fat with infective pathogenic virus at the surface may contribute to long-distance transmission of SAV.

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

Correspondence A Stene, Ålesund University College, Ålesund N-6025, Norway (e-mail: anne stene@hials.no) Pancreas disease (PD) has negative impact on Atlantic salmon farming in Ireland, Scotland and the western parts of Norway (McLoughlin & Graham 2007). The disease leads to increased mortaling, decreased growth and quality downgrading at slaughter. PD is therefore of importance to both fish welfare and farm economy (Aunsmo *et al.* 2010). The causative agent is a salmonid alphavitus (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 registered for the first time at a marine farming sites in mid-Norway (Hjortaas *et al.* 2013).

SAV is a virus with long half-life in cold, saline water (Graham et al. 2007; Graham, Rowley & McConville 2010) and may therefore be capable of long-distance transport along the Norwegian coast. When introduced into an area with high salmonid 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; Kristoffersen et al. 2009; Aldrin et al. 2010; Tavornpanich et al. 2012). Water contact by ocean currents is the best explanatory variable when it comes to transmission of PD among marine farming sites for salmon in Norway (Viljugrein et al. 2009; Stene 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, Hodneland & «Due to its hydrophobic nature, fat with infective pathogenic virus at the surface may contribute to long-distance transmission of SAV.»

«In this study, we have for the first time identified viable SAV in the lipid fraction leaking from adipose tissue in dead, decaying, infected farmed salmon. This leakage is likely to accumulate on the seawater surface where the water transport is most prominent.»

«...the results indicate that such leakage can be a route for transmission of pathogenic agents among salmon farms.» 🔁 MSD

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Fish

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Journal

PD is transmitted efficiently in the water column, both within and between locations - several publications has confirmed the infectivity of the disease

Year	Journal	Authors	Title
2015	J.Fish.Dis. Doi:10.1111/jfd.12382, early view	A. Stene et al.	Liquid fat, a potential abiotic vector for horizontal transmission of salmonid alphavirus?
2015	J.Fish.Dis. Doi:10.1111/jfd.12353, early view	M. J. Hjortaas et al.	Genetic characterization of salmonid alphavirus in Norway
2014	J.Fish.Dis. Doi:10.1111/jfd.12312, early view	T. Taksdal et al.	Mortality and weight loss of Atlantic salmon, Salmo salar L., experimentally infected with salmonid alphavirus subtype 2 and subtype 3 isolates from Norway
2014	J.Fish.Dis. Doi:10.1111/jfd.12238, early view	M. D. Jansen et al.	Clinical manifestations of pancreas disease outbreaks in Norwegian marine salmon farming – variations due to salmonid alphavirus subtype
2014	J.Fish.Dis. 37, 123-134 Doi:10.1111/jfd.12090	A. Stene et al.	Transmission dynamics of pancreas disease (PD) in a Norwegian fjord: aspects of water transport, contact networks and infection pressure among salmon farms
2014	J.Fish.Dis. 2014, 37, 739-751 Doi:10.1111/jfd.12165	A. Stene et al.	Seasonal increase in sea temperature triggers pancreas disease outbreaks in Norwegian salmon farms
2012	J.Fish.Dis. 2012,35, 945-951 Doi:10.1111/j.1365-2761.2012.01427.x	D. A. Graham et al.	Detection of salmon pancreas disease virus in the faeces and mucus of Atlantic salmon, Salmo salar L., by real –time RT-PCR and cell culture following experimental challenge
2012	J.Fish.Dis. 2012,35, 755-765 Doi:10.1111/j.1365-2761.2012.01401.x	D. A. Graham et al.	Geographical distribution of salmonid alphavirus subtypes in marine farmed Atlantic salmon, <i>Salmo salar</i> L., in Scotland and Ireland
2012	BMC Veterinary Research 2012, 8: 172, 1-10	S. Tavornpanich et al.	Risk map and spatial determinants of pancreas disease in the marine phase of Norwegian Atlantic salmon farming sites
2012	Dis. Aquat. Org. Vol. 102:23-31, 2012 Doi: 10.3354/dao02529	B. B. Jensen et al.	Cohort study of effect of vaccination on pancreas disease in Norwegian salmon aquaculture
2011	J.Fish.Dis. 34, 273-286 Doi:10.1111/j.1365-2761.2010.01234.x	D. A. Graham et al.	A comparative study of marine salmonid alphavirus subtypes 1-6 using an experimental cohabitation challenge model
2010	J.Fish.Dis. 2010, 33, 723-736 Doi:10.1111/j.1365-2761.2010.01176.x	M. D. Jansen et al.	Pancreas disease (PD) in sea-reared Atlantic salmon, <i>Salmo salar</i> L., in Norway; a prospective, longitudinal study of disease development and agreement between diagnostic test results.
2010	J.Fish.Dis. 2010, 33, 391-402 Doi:10.1111/j.1365-2761.2009.01131.x	M. D. Jansen et al.	Salmonid alphavirus (SAV) and pancreas disease (PD) in Atlantic salmon, Salmo salar L., in freshwater and seawater sites in Norway from 2006 to 2008
2010	Preventive Veterinary medicine 93 (2010) 51-61	M. Aldrin et al.	A stochastic model for the assessment of the transmission pathways of heart and skeleton muscle inflammation, pancreas disease and infectious salmon anaemia in marine fish farms in Norway
2009	Preventive Veterinary medicine 90 (2009) 127-136	A. B. Kristoffersen et al.	Risk factors for pancreas disease (PD) outbreaks in farmed Atlantic salmon and rainbow trout in Norway during 2003-2007
2000	Dia Aquat Ora V/al 89:25 44 2000	H Viliugrain at al	Integration of hydrodynamics into a statistical model on the spread of

PD detections & suspected cases 1995-2014 -distribution per county



- 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



From: M. J. Hjortaas et al. *Journal of Fish Diseases* 2015, doi:10.1111/jfd.12353 «Genetic characterization of alphaviruses in Norway»

Legend

AV3 Atlantic samon

W3 rainbow trout

300 Klometer



Regions:

- 1. Rogal., Hordal., S&F
- 2. Sunnmøre
- 3. Romsdal
- 4. Nordmøre
- 5. Sør Tr. Lag
- 6. Nord Tr. Lag
- 7. Nordl., Troms, Finnm.

- 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 fallowing periode 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





Numbers of PD detections all counties 2006-2014 -accumulated





-Most PD detections in the summer; May-August -Rest of the year the detections are more evenly distributed per month





Official PD statistics 2015

Source:Norwegian Veterinary Institute

Distribution of PD cases (suspicions & detections):

County	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Total No (April)	Total %
South of Hustadvika									27	93				
Rog	1	1	3	2										
Hord	2	9	1	2										
SF	1	4	0	0										
MR	0	0	0	0										
North of Hustadvika								2	7					
MR	0	0	0	0										
S.Tr.	1	1	0	0										
N.Tr.	0	0	0	0										
Nord	0	0	0	0										



PD-detections 2006-2014

Source: Norwegian Veterinary Institute



Most PD detections in 2014:

- 1. Hordaland
- 2. Sør Trøndelag
- 3. Rogaland



Regions with highest density of farmed fish in Norway

From: P.A. Jansen et.al. Proc.R. Soc. B, 2012



Sea transferred salmon over 20 years (1994-2014) -per county

Source: Directorate of Fisheries

Numbers x1000





Numbers of salmon in sea 2014 -average per month





County



Sea transferred rainbow trout over 20 years (1994-2014) -per county

Numbers x1000

Source: Directorate of Fisheries



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



Disease	Transmission route	R ₀	Level of the population needed to be vaccinated			
Diphtheria	Saliva	6-7	85%			
Measles	Air	12-18	83 - 94%			
Mumps	Air/secretion	4-7	75 - 200			
Whooping cough	Air/secretion	12-17	individual			
Polio	Intestinal-mouth		hat can be infected from one 83 - 85%			
Rubella	Air/oc	te in average t				
Smallpox	ther of individ	luals in ave	83 - 85%			
EL Ro- Max	imum number of	1-2	Test vaccine			
PD	Waterborne/contact	??	??			

Animal Health



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 <u>SPDV virus/virus genetic material in heart</u> 4 weeks after addition of vectors

Positive Negative





How much constitutes PD in rbt of the total detected / suspected cases -period 2009-2014





County

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 were₂PD vaccinated



Fallow areas in Hordaland

Source: Lovdata







Sea transfer of S1 per 19th May 2015, Hordaland



Tegnforklaring

Sea transfer of S1 per 8th May 2015, Rogaland



Source: AkvaGIS

Eic





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



PD-SAV2 & SAV3; Møre & Romsdal south of Hustadvika Status september 2014



Any contact between these areas?



Wellboat routesM&RPeriod: 60 days august-october 2014

Source: MarineTraffic





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»

Vestfold

-Ander

Østfold





SAV2 now also registered south of SAV3 areas PD status October 2014, Nordfjord



The first registered co-infected site with SAV2 + SAV3 from the field. Ref. PD report December 2014 Veterinary Institute 32

Proposed new SAV2 zone regulations for Nordfjord

Sources: Norwegian Food Safety Authority, AkvaGIS

New SAV2 regulation decided for Nordfjord, May 5th 2015

Sources: Norwegian Food Safety Authority, AkvaGIS

PD-regulations: Will there be changes?

Source: Norwegian Veterinary Institute

Temperatures in sea January-March 2015

Source: Akvafakta

Temperatur

Gjennomsnittstemperaturen i mars var 5,65 °C, mot et snitt i perioden 2008 - 2013 på 4,59°C

Effect of temperature on growth of SAV in different

cell lines

Journal of Fish Diseases 2008, 31, 859-868

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doi:10.1111/j.1365-2761.2008.00946.x

Cultural characteristics of salmonid alphaviruses influence of cell line and temperature

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Abstract

Laboratory studies were carried out to investigate the cultural characteristics of salmonid alphaviruses (SAV) from Atlantic salmon (AS, Salmo salar) and rainbow trout (RT, Oncorhynchus mykiss), particularly in relation to cell line and temperature. In an initial study, SAV was isolated from 12 viraemic sera and passaged in Chinook salmon embryo (CHSE-214) cells at 15 °C. Geometric mean titres (GMT) after initial isolation were found to be significantly higher ($P \le 0.05$) relative to those after two or four passages. Primary isolation of SAV was conducted from 12 viraemic sera (six AS and six RT) in seven different cell lines at 15 °C: CHSE-214, rainbow trout gonad (RTG-2), TO (derived from Atlantic salmon head kidney leucocytes), salmon head kidney (SHK-1), blue fin-2 (BF-2), fat head minnow (FHM) and Epithelioma papulosum cyprini (EPC). Overall, significant differences were found between cell lines in both the numbers of strains where growth was detected and in the GMT obtained. For both AS and RT strains, GMT values were significantly (P < 0.01) higher in both TO and BF-2 cells relative to the others, including CHSE-214 and RTG-2, the cell lines conventionally used for SAV. The effects of temperature of (1997) reported the first isolation of sleeping disease 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.

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Keywords: cell line, culture, growth curve, salmonid alphavirus, temperature.

Introduction

The first isolation of alphaviruses from fish was described by Nelson, McLoughlin, Rowley, Platten & McConnick (1995). The isolate was obtained by serial passage of tissues from affected fish in Chinook salmon embryo (CHSE-214) cells at 15 °C, with viral growth identified by the development of a cytopathic effect (CPE) after cocultivation and serial passage. This isolate, desig nated F93-125, was obtained from Atlantic salmon, Salmo salar L., (AS) during an outbreak of pancreas disease (PD). Such outbreaks are characterized by pancreatic and cardiomyocytic necrosis and red and white skeletal muscle degeneration (McLoughlin, Nelson, McCormick, Rowley & Bryson 2002). This virus was named salmon PD virus (SPDV; Weaver, Delgarno, Frey, Huang, Kinney, Rice, Roehrig, Shope & Strauss 2000), with F93-125 as the reference strain. Subsequently, SPDV was shown to be an alphavirus (Weston, Welsh, McLoughlin & Todd 1999). Castric, Baudin Laurencin, Bremont, Jeffroy, Le Ven & Bearaotti virus (SDV) from farmed rainbow trout (RT), Oncorbynchus mykis (Walbaum), in fresh water suffering from a condition called sleeping disease (SD). This was characterized by histopathological changes in the pancreas, heart and skeletal muscle similar to those seen in PD. Isolation of this virus, designated \$49p, was based on the development of CPE following serial passage in CHSE-214 and rainbow trout gonad (RTG-2) cells at 14 °C. Despite this, Villoing, Bearaotti, Chilmonczyk, 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

Sesonal changes in water temperature and impact on PD virus

Journal of Fish Diseases 2014, 37, 739-751

Journal of Fish Diseases 2014, 37, 739-751

doi:10.1111/Jd.12165

Seasonal increase in sea temperature triggers pancreas disease outbreaks in Norwegian salmon farms

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Abstract

Pancreas disease (PD) is a viral disease causing negative impacts on economy of salmon farms and fish welfare. Its transmission route is horizontal, and water transport by ocean currents is an important factor for transmission. In this study, the effect of temperature changes on PD dynamics in the field has been analysed for the first time. To identify the potential time of exposure to the virus causing PD, a hydrodynamic current model was used. A cohort of salmon was assumed to be infected the month it was exposed to virus from other infective cohorts by estimated water contact. The number of months from exposure to outbreak defined the incubation period, which was used in this investigation to explore the relationship between temperature changes and PD dynamics. The time of outbreak was identified by peak in mortality based on / monthly records from active sites. 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. Hydrodynamic models can provide information on the risk of being exposed to pathogens from neighbouring farms. With the knowledge of temperature-dependent outbreak probability, the farmers can emphasize prophylactic management, avoid stressful operations until the sea temperature is decreasing and consider removal of cohorts at risk, if possible.

Correspondence A Stane, Ålesand University College, Ålesund, N+8023 Norway (e-mail: anne-stene®hlate.no) *Present address: Det Norske Veritas AS, Technical Advisory Trondheim, Norway Keywords: Atlantic salmon, disease dynamics, pancreas disease, sea temperature changes.

Introduction

Pancreas disease (PD) is a severe viral disease in salmonid farming. The causal agent is salmonid pancreas disease virus (SPDV), and at least six subtypes exist Fringuelli *at al.* 2008). Subtype 3 (SPDV3) if the dominating subtype responsible for PD in Nerway and affects both farmed Atlantic salmon, Sulmo salar L., and rainbow trout, Oncorlym chose motios (Walbaum) (Hodneland *et al.* 2005).

The transmission pathway of SPDV is horizontal during the growing period in the sea. The major virus reservoir in the marine invironment is infected farmed salmon. Wild organisms have not been identified as important transmitters of SPDV (Snow et al. 2010; Stepe et al. 2010). Neither has transmission from a previous salmon cohort to new smolts on the same site after fallowing (Aldrin a al. 2010; Werlman et al. 2011; Kilburn et al. 2012). Water contact, close proximity, sharing ownership with infected farms and movement of fish have been found to increase the risk of PD (Rodger & Mitchell 2007: Kristoffersen et al. 2009: Viliusrein et al. 2009; Aldrin et al. 2010; Stene et al. 2013). For example, Aldrin et al. (2010) estimated that 80% of the PD cases in Norway could be explained by horizontal transmission among farming sites within close proximity.

For horizontally transmitted pathogens, survival outside the host is crucial. SPDV has a long survival in cold, clean and full strength sea water (Graham «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 »

Fish Diseases

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Journal

Use of wellboats

> over long distances between countries between counties \succ locally inside the fjords between different sites between harvesting plants smolt & fish for harvesting \succ through infected areas > treatments; sea lice, AGD capasity challenges?

Hordaland county: PD-detections & suspected cases per 07.09.14 -transportation between sites in heavely infected areas

Sources: Directorate of Fisheries, MarineTraffic

Sør & Nord Trøndelag: PD detections & suspected cases per 08.09.14 -transportations between infected areas

Sources: Directorate of Fisheries, MarineTraffic

Wellboat routes Scotland-Orkney-Shetland August- October 2014

Source: MarineTraffic

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

Nordfjord

Ålfotbreen

Vartdalsfjorde

Sognefjorde

Shetland

Mainland

275

Stølsheime

E39

Naustdal-Gjengeda

Bergen

E3

E39

Geographical distribution of SAV in Scotland and Ireland

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.

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