15 Years of TriNation

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Epidemiology & Risk

Nation	SAV 1 (PD)	SAV 2 (PD) (Sleeping disease)	SAV 3 (PD)	SAV 4 (PD)	SAV 5 (PD)	SAV 6 (PD)	PRV (HSMI)	PMCV (CMS)
Ireland	✓				✓	✓	✓	✓
Scotland	✓	✓		✓	✓	✓	✓	✓
Norway		✓	✓				✓	✓

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Epidemiology & Risk

- **Risk factors for SAV (and maybe *et al.)***
  - Movements of marine stocks.
  - > 1 input per seawater site.
  - Site holds >250,000 fish.
  - Over feeding.
  - Recovered stocks are a risk to naïve ones.
  - Lipid exudate from carcasses.
  - Poor smolt quality.
  - Non vaccinates in proximity to population / herd.
  - Period immediately following smolt transfer (higher susceptibility, greater shedders of virus)

Epidemiology & Risk

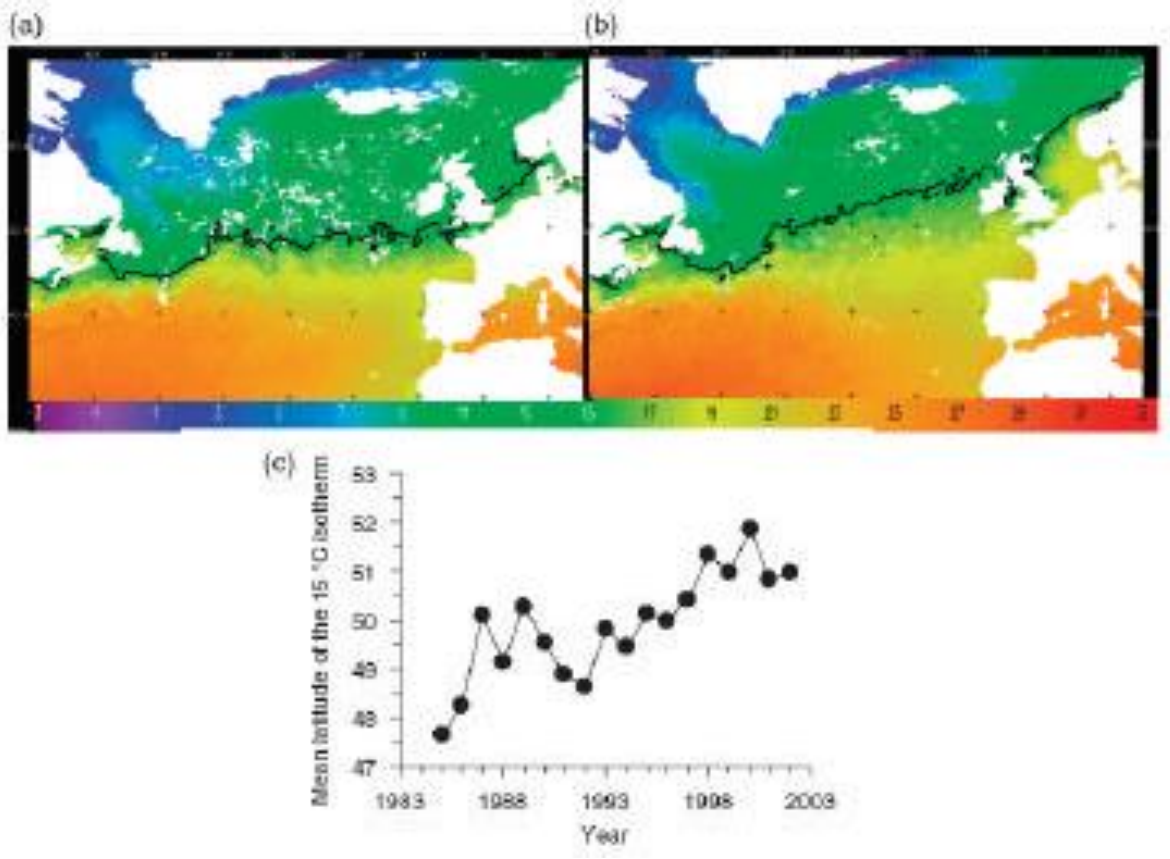
- **Ireland.**
  - 2008 – 2009 saw a fall in PD positivity (losses valued at €5M).
  - Reports from 2010 showed the lowest mortality from PD in 10 years (7.1%) possibly attributed to adoption of vaccination.
  - Improvement continued into 2012 with PD attributed mortality falling to 2%.
  - 2013 saw an elevation in PD related mortality at 13% - suspected to be related to AGD incidence.
  - 2014 to 2017 saw some improvement with maximum site mortality of 10% - attributed to vaccination.
  - Although mortality had improved - still significant levels of emaciation in affected fish.

Epidemiology & Risk

- **Norway.**
  - 2007 saw 99 recorded cases of PD – described as a ‘devastating year’.
  - 2008 – 2009 saw a fall in PD ‘diagnosed’ and ‘suspect’ sites by 35%.
  - Further fall-off in 2010 attributed to monitoring, smolt quality and vaccination.
  - By 2012 the situation had stabilised, but!
  - SAV 2 first reported north of the Hustadvika PD ‘firebreak’.
  - By 2015 135 to 140 sites were reported as positive for (SAV 2 & 3) a ‘plateau’.

Epidemiology & Risk

- **Seasonality & Temperature.**
  - In Ireland mortality from PD peaks in both spring and autumn.
  - In Norway a similar picture.
  - Mortality occurs during a temperature window of between 10° and 15°C.
  - In 1985 the North Atlantic 15°C isotherm was located 47.5° latitude. By 2000 this had moved north to 52° latitude (Brest to South Wales!)



The Viruses & Clinical Outcomes

- **What are the viruses?**
  - **Pancreas Disease**. Caused by the Salmon Alpha Virus (*Togaviridae*) currently recognised in 6 subtypes SAV 1-6.
  - **Sleeping Disease.** Affects Rainbow trout and is caused by a SAV 2 variation.
  - **Heart and Skeletal Muscle Inflammation** is caused by Piscine Orthoreovirus (PRV) (*Reoviridae*).
  - **Cardiomyopathy Syndrome** is caused by Piscine Myocarditis Virus (PMCV) (*Totiviridae*).

The Viruses & Clinical Outcomes

- **SAV similarities and variations – taxing taxonomy!**
  - SAVs contain a highly conserved gene which appears to be vital for replication (a potential target for vaccine development etc(.(J.Guo)
  - Different types of SAV 1 have been shown to vary in their ability to block the effect of the anti-viral cytokine, Interferon. May be an indicator of virulence.(B.Collet)
  - Differential sensitivities to MX protein (an anti-viral protein induced by interferon) has also been recorded between different SAV types. (B.Collet)
  - ***Intra***-strain differences in SAV type (especially SAV 2) may be as or even more important as determinants of virulence than ***Inter***-strain differences Phylogenetic distinctions are constructed chiefly from **accumulated** genetic difference rather than **individual** gene differences can have **big** phenotypic outcomes.(P.Frost).**BUT** a study of 11 isolates of SAV 3 did not show heterogeneity in E2 gene to be a predictor of mortality in infected fish.(I.Sommerset)

The Viruses & Clinical Outcomes

- **Impacts.**
  - PD can affect value of harvest through reduced yield, loss of pigmentation as well as melanin deposition.(T.Mørkøre)
  - There is a *progression of pathology* characteristic of PD, following an 'explosion' of virus in the heat, pathological changes begin in the pancreas followed by the heart, red muscle and finally white muscle.(D. Cockerill)
  - PRV can infect, reside and replicate in up to 50% of the host's erythrocytes and all this prior to entry into the heart ventricle where subsequent HSMI pathology develops. This not only reduces the O_2 carrying capacity of the erythrocytes but also cardiac performance (probably reducing aerobic scope CGM)
  - SAV can induce dysbiosis to the skin microbiome potentially replacing harmless microbial taxa with pathogenic ones. (S.Patel)
  - PRV infected fish subsequently challenged with SAV 2 or 3 (co-infected) have been observed to suffer less tissue damage. (M Røsaeg)
  - Some evidence that SAV 3's ability to replicate may be diminished in fish co-infected with PRV. (M.Lund)

The Viruses & Clinical Outcomes

Species	SAV 1 (PD)	SAV 2 (PD) + (Sleeping disease)	SAV 3 (PD)	SAV 4 (PD)	SAV 5 (PD)	SAV 6 (PD)	PRV (HSMI)	PMCV (CMS)
Atlantic salmon								
Rainbow trout								
Brown trout								
Balleen Wrasse								
Dab								

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The Viruses & Clinical Outcomes

- **Host perspectives.**
  - Salmon can be categorised as ‘high’ or ‘low’ responders following challenge with PMCV on the basis of their ability to initiate an active cellular immune response.(G.Timmerhaus)
  - The gill tissue of fish infected with SAV 1 provides an area where the virus can replicate within apparent impunity. It is once in the heart that interferon mediated anti-viral response occur (causing pathology in this tissue). So called tissue tropism (T.Herath)

Mitigation

- **Vaccination.**
  - Vaccination outcomes presented in 2010 revealed fish vaccinated with NORVAX® Compact PD showed less heart pathology and improved survival when compared to non vaccinates in a tank study. The mortality pattern was corroborated by field data. (M. Mcloughlin)
  - Vaccines containing antigen from one strain of SAV (1) will cross protect against challenge from other strains (SAV 2 and SAV 3). (K. Ulven MSD)
  - A 'proof of concept' trial with a RNA construct of SAV showed that not only could the replicon transmit through a cohabiting population, but that protection was also delivered. However the replicon was also capable of inducing some pathology on its own! (M. Karlsen)
  - Experimental nucleic acid vaccines have been demonstrated to give protection provided that the replicons used contain code for both structural proteins. (M. Hikke)
  - Rainbow trout will shed SAV when infected, but less so if vaccinated. (R. Belmonte)
  - Results from a field trial with a new commercial PD vaccine, ALPHA JECT micro 1PD, produced favourable results in terms of reducing mortality when compared to an older alternative product. (A. Aas-Eng PHARMAQ)
  - Single vaccination with Aquavac PD 7 lowered mortality in the face of PD challenge when compared to double sequential vaccination (1.2% and 4.4% respectively). (I. Sommerset)

Mitigation

- **Breeding and Genetics.**
  - Field observations from Ireland in 2014 suggested that some strains of salmon faired better than other in terms of clinical outcome following PD infection. (S Mitchell)
  - Using heart lesions as an indicator, PD vaccine efficacy has been observed to be improved in stocks of fish which are either homozygous or heterozygous for IPN QTL. (N. Santi +MSD)
  - A major QTL for PD resistance has been mapped and validated in salmon. (B Hillestad, Salmobreed)
  - Stocks of fish that are homozygous for protective alleles to CMS show lower heart lesion scores than heterozygous fish following challenge. (S Kjøglum Aquagen)
  - The use of SNIP chip technology PD resistance heritability scores to from 0.35 to 0.47. The technology has doubled the accuracy with which broodstock are selected, (A Norris MOWI)
  - The accumulation of SAV in infected triploid fish is slower than in diploid ones. This observation was supported by a higher level of up-regulated antiviral immune genes and pattern recognition receptors in the 3n fish. (N. Nuñez-Ortiz).

Mitigation

- **Nutrition.**
  - Functional feeds, Protec™ and React™ demonstrated to reduce PD related mortality, levels of creatine kinase and C-reactive protein.(C. McGurk)
  - Diets containing anti-inflammatory factors and optimal balance between omega 3 and omega 6 fatty acids delivered reductions in pathology scores in fish suffering from all three cardiomyopathies. In cases of HSMI and CMS, genes associated with inflammation were down-regulated and those for involved in fatty acid desaturation (also anti-inflammatory) were up-regulated. (S. Wadsworth)
  - Krill oil composed of phospholipids rather than triglycerides, as a dietary inclusion was shown to reduce mortality and pathology in fish suffering from both CMS and HSMI probably through improved cell membrane integrity.(G.Molland)

Mitigation

- **Disinfection.**
  - SAVs do not tolerate high or low Ph making both ensiling and alkaline hydrolysis safe for carcass disposal (in this context). (D. Graham)
  - SAV can be destroyed by composting, if temperature exceeds 60°C. (D.Graham)
  - Usual farm disinfectants including peroxy compounds, iodophores and chloramine T were all shown to be effective against SAV when used according to the label. (D.Graham)

Mitigation

- **Husbandry.**
  - SAVs

VIRAEMIA/SEROLOGY/RT-PCR for PD & CMS DIAGNOSIS

- Convenient and practical SAV diagnostic method + CMS +

SAV Antibody Serum	Virus Serum	RT-PCR Serum	RT-PCR Heart	Interpretation
-	+	+ +	+/- +/-	**Early SAV infection- No disease**
+	+	+ +	+ +	**Current infection**
+	-	-	+/- +/-	**Late stage disease**
+	-	-	-	**Previous infection**

Number to sample - minimum of 10 to be 95% certain to detect ≥1 positive if 25% positive

Interpretation aided by serial sampling or increasing the number of fish sampled by using non lethal sampling

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Diagnostics & Screening

- **Tools.**
  - The first recommendations for routine screening came in 2010 with serological and histological examination of stock every 4 to 6 weeks being the suggested protocol.(D.Cox and A. Dykes)
  - In the same year other methods of diagnosis including RT-PCR as well as the tracking of Acute Phase Proteins which spike at 6 weeks following challenge, were also presented. (D. Cockerill and R, Bickerdyke)
  - Immunoassays targeted at the innate immune system have been suggested as potential non-invasive super-early detection tool for PMCV. (G.Timmerhaus)

Diagnostics & Screening

- **Tools cont.**
  - Insurers settling claims arising from mortalities on fish farms are primarily concerned with what the stock died ***of*** rather than what they died ***with***.
  - Clinicians must therefore bring a range of analyses to bear on cases particularly where co-infection is suspected.
  - To this end serology, PCR and histology all have role to play. In addition to these proteomic assays for tissue damage proteins (CK etc) as well as those associated with the immune response could further assist in both discriminatory diagnosis (J.Tinsley) as well as the management of affected stocks.
  - The use of CK monitoring in stock management has also been extended to guide harvesting. Myopathy has a negative effect on carcass quality and CK levels corollate quite well with customer complaints! (D.Cockerill)
  - Another proteomic biomarker mooted as possibly useful in informing farm decisions is Enolase 3, a white muscle specific biomarker. (M. Braceland)

Diagnostics & Screening

- **Strategy.**
  - The road to successful control of the spread of SAV can be achieved through screening programmes as part of a wider strategy of containment. (T. Hoveland)
  - The case for widespread control of SAV 3 in Norway was supported by an estimate that the cost of infection from this virus is 55M NOK per 1 million smolts. (A. Aunsmo).
  - The current programme of screening and monitoring In Norway requires 20 fish from farms –ve for SAV to be tested each month at a cost to the Industry of circa €5 million per annum. (M.Binde)
  - The border between the PD zone and the surveillance zone has moved from Hustadvika to Skjemta in Trøndelag county (reported 2018).(M.Bind)
  - The purpose of any screening must be clearly established before being undertaken. There are trade-offs between sensitivity and specificity and the implications of false +ves and false –ves differ depending upon whether they have come from a screening or diagnostic test. (B. Bang Jensen)
  - Screening by RT-PCR must consider the vaccination status of the fish as should those using serum neutralisation. In the case of the latter the risk from vaccine derived antigen is joined by one from potential cross reactivity from antibodies raised against other antigens such as PRV. (H. Sindre)