Aberdeen gets SAVy - An Update from the PD TriNations 2016 Chris Mitchell.

It seemed appropriate that once again the meeting of the TriNations, which this year saw a focus on Salmon Alpha Virus (SAV), should convene in the laboratory where, in 1984, Munro, McVicar and Ellis (or Alan, Alasdair and Tony) first described "An exocrine pancreas disease of farmed salmon in Scotland" (Helgolander Meeresunters 37: 571-586).

A great deal has changed since those days not least the widespread incidence of the disease. In 1985 when Pancreas Disease (PD) was believed to have a nutritional aetiology, Alasdair McVicar reported that 20% of the Scottish Industry appeared to be affected. Thirty one years later and incidentally 22 years after PD 'went viral' following Marian McLoughlin and her Belfast colleague's discovery of this aetiology, there appears to have been no real decrease in the number of new PD cases per year over the last 5 years. This was reported to delegates by Marian herself and was based on histological diagnoses, with the trend showing an increase this year (2016). The picture for HSMI is similar with pathology characteristic of this condition being observed increasingly year upon year and is now similar to the number of PD cases. This however is not associated with any significant clinical impact. The number of cases of CMS meanwhile has remained fairly stable over the last 5 years. However exact figures detailing both the prevalence and incidence of viral myopathies in Scottish aquaculture remain disappointingly scant when compared to corresponding Norwegian data. Marian went on to suggest that centrally collated data possibly through the SSPO could help change this as well as inform decisions about the real impact and control of these important cardiomyopathies.

Meanwhile in Norway there are suggestions that whilst the alarming levels of SAV 3 outbreaks recorded in 2007 and 2008 have not been surpassed it would appear that levels have plateaued at between 135 and 142 sites affected on an annual basis (SAV 2 included). As far as severity goes June remains the time, and Hordaland the place, for peak outbreaks.

Of the 87.5 million fish vaccinated against PD in Norway in 2016, 11% were vaccinated in the region north of Hustadvika where SAV 2 is endemic. Interestingly in a separate presentation reporting on the findings of 'on farm' interviews with site personnel, Mona Dverdal Jansen (Norwegian Veterinary Institute) suggested that some farmers believed that vaccination against PD in Norway is not permitted in this area! Other findings in this survey of experiences in the field included a belief that good quality (and possibly PD QTL) smolts mitigate the outcome of PD infection as does coordinated management of sea lice treatments. Areas where more information was deemed to be required by interviewees included more documentation on the effects of both vaccine and functional feeds in the field. This extensive epidemiological study was funded by FHF the main funding body of the TriNations initiative.

The distribution of HSMI associated with PRV remains more widespread than SAV infection. In 2015 incidences were confirmed all the way up the coast of Norway with 187 sites recording infection. A variant of PRV has been detected in rainbow trout and whilst **intra**-species transmission has been demonstrated for both rainbow trout and salmon, transmission **between** these two species has not yet been investigated.

Marit Stormoen from Marine Harvest gave delegates an update on her company's experience of pancreas disease in Norway, from which came two key observations. 1). the level of mortality which they have experienced on farms in Norway has shown a declining trend from 2013 and 2). Those farms that do suffer mortality reveal wide variation between cages. This so-called 'cage effect' is both un-explained and not confined to pancreas disease. Some discussion ensued amongst delegates about what R&D could be conducted to try and elucidate the underlying causes of this phenomenon.

An Industry view from the Scottish perspective was given by Abby Irish from the Scottish Salmon Company who emphasised the benefits of close stock monitoring in the run up to what she termed 'high risk periods' (September through October). Once a diagnosis of PD is confirmed Abby's advice is to avoid handling for at least a month, although she did recommend that if AGD was identified it is better to get on top of this immediately. Abby now holds the chair of the TriNations committee.

The wild reservoir of SAV is not something that the PD TriNations group has heard much about since David Bruno updated the 2012 conference in Leith on his studies in the Marine environment. So it was interesting to hear a current perspective on this from Abdullah Madhun from the Institute of Marine Research, Norway. RT-PCR testing of 843 wild seatrout collected from different counties failed to confirm a single positive for SAV with only 1.3% of these fish registering as positive for PRV. This finding was repeated in northern and western Norway in returning wild Atlantic salmon but with a PRV occurrence in 8% (5-35 %) of the fish analysed. Here no evidence of SAV infection was found in the examined salmon. The same fish were negative for ISAV-HPRΔ with 7 % being positive for the HPR-0 type. Investigations into the viral status of wild migrating smolts revealed that low levels of PRV (4.5 %) could be detected in these fish but no SAV or ISAV.

Testing of fish deemed to be 'escaped farmed salmon' revealed, perhaps not surprisingly, a slightly different picture. In Hardanger (River Steinsdalselva) of the 58 fish examined, 98% tested positive for SAV whilst 100% were PRV positive. In the River Etna were 129 fish were examined the figures were 12% and 79% respectively. Abdullah also reported on wild parr in three other rivers, all of which have, to date, revealed no evidence of SAV, ISAV or IPNV in these riverine fish. Interestingly one river (The Os) yielded a 3% level of PRV in salmon parr. Proximal habitation studies whereby smolts were 'incubated' in sea cages close to salmon farming operations in Hordaland in May and June 2015 also failed to induce detectable infection in these fish with either SAV or ISAV.

	Virus		
Collected fish	**SAV**	**PRV**	**ISAV (HPR-del)**
Sea trout **Northern and western Norway**	**ND**	**1.3 (0-23 %)**	**NT**
Returning Atlantic salmon Northern Norway Western Norway	 ND ND	 8 % (5-35 %)	 7 % (0-20%) (HPR-0) 1 % (0-2 %) (HPR-0)
Migrating smolt Western Norway	 ND	 4.5 % (2-6 %)	 ND
Juvenile salmon (parr) Northern Norway Western Norway	 ND ND	 ND 3 % (0-8 %)	 ND ND
Escaped farmed salmon Northern Norway 2010 Western Norway 2012 Western Norway 2014	 ND 98% 12 %	 86 % (50-100 %) 100 % 79 %	 5 % (0-50%) NT 1%

ND: not detected, NT: not tested.

So far these studies have given lie to the often accepted wisdom that farmed salmon, either captive or escaped, pose a serious threat to their wild cousins, at least from the perspective of viral diseases.

Tore Hovland from Patogen presented his conviction that with adequate screening for the detection of the two SAV types (2 & 3) now endemic in Norway their control and containment is achievable. Virus detection, using molecular techniques occurs on average 90 days in advance of more classical diagnosis. Different strategic approaches ranging from management through monitoring to ultimately slaughtering (depending upon which SAV is identified in which area) will, according to Tore, go a long way to controlling the disease at the County level.

The cost of failing to control PD, or indeed any other disease, is often difficult to quantify; not something that put off Arnfinn Aunsmo of AquaGen who has been looking at this from both a macro as well as micro viewpoint. With an estimated cost of 55 million Nok per 1 million smolts in a farm clinically infected with SAV 3 the case for widespread control is a strong one. However if you are the farmer subjected to compulsory slaughter under an eradication programme your cost is to the benefit of your neighbours. Arnfinn suggested that where a common resource is being shared, those sharing the benefits should also share the costs!

In the first of a series of presentations examining next-generation vaccine technologies, Catherine Collins of Marine Scotland Science outlined her work as part of the EU Targetfish programme, investigating DNA vaccine efficacy and safety to protect fish against PD. The principle of DNA vaccination involves constructing a nucleic acid plasmid which, following introduction into the vaccinate host, is transcribed into the antigenic protein required to generate protection in that host. So far Catherine's group have reported complete suppression of viraemia in challenged vaccinates as well as no myocardial degeneration or cardiac inflammation. However whilst some cytokine response to vaccination could be demonstrated, the role of antibodies in observed protection was currently an area of uncertainty. Catherine's analysis relied on histopathological techniques which are currently used to assess outcomes of cardiomyopathy vaccine trials.

This subject area was eloquently addressed by Jeffrey Wolf who described his team's successful use of histopathology as a tool for measuring vaccine efficacy. The non-lethal SAV3 challenge requires that criteria more sensitive than mortality must be used to evaluate efficacy. In cooperation with Elanco, Dr Wolf at Experimental Pathology Laboratories in Sterling, Virginia created a standardized system for assessing the morphologic effects of SAV3 exposure in routine histology sections. With near 100% success, the pathologist was able to distinguish between tissues collected from vaccinated challenged fish or unchallenged controls from fish that were virus challenged but received no vaccination. The study also documented the chronological development and subsequent resolution of SAV 3 induced lesions in the heart and skeletal muscle of challenged fish.

Ingunn Sommerset of MSD Animal Health continued the session on vaccine development by reminding the audience of the challenges faced by pharma companies seeking to develop safe and efficacious vaccine for aquatic organisms. This is primarily due to the environmental variation such as temperature, stock type and quality as well as the pathogen strain and challenge pressure to which cultured fish are exposed in a way that doesn't apply to other intensively farmed terrestrial animals. This necessitates good follow-up in the field in order to continue gathering information AFTER the product has been authorised for the market. Ingunn illustrated how variations in the environment can influence outcomes with data showing mortality due to PD in vaccinated fish ranging from 1.8% to 12.3% on different sea sites but with smolts from the same supplier. She also revealed that findings so far suggest that single vaccination with MSD's new Aquavac PD7 has improved outcomes in the face of PD challenge when compared to double, sequential vaccination (circa 1.2% and 4.4% respectively).

Apart from presenting a new vaccine on an existing platform, Mia Hikke revealed some of MSD's work on exploring new technologies in collaboration with colleagues at Wageningen University and the Norwegian School of Veterinary Medicine. Following principles similar to those described in Catherine Collins work, Mia and her colleagues have established that a competent immune response can be induced against SAV in fish that have been vaccinated with replicons of the virus (partial edited sequences of the viral genome) as long as those replicons contain **both** of the major structural proteins. Mia commented that current 'gentechnology' regulations were outdated and this needs to be addressed in order to keep up with advances in nucleic acid vaccine technology.

A slight cautionary note to the above was presented by Hetroney Mweemba Munangandu on behalf of Elin Petterson at the NMBU whose recent work has demonstrated that, when administered to fish, virus RNA produced from nucleic acid plasmids from SAV 3 can recombine to form viable SAV which can be grown in tissue culture. In addition to this the recombined virus was demonstrated to produce pathological changes in the heart and pancreatic tissue. Hetroney speculated about the possible implications of recombination amongst different SAV subtypes.

The R&D theme continued with Laurent Bigarre from the ANSES agency in France describing the first identification of piscine reovirus (PRV) in France and in *Salmo trutta*, a fish not normally at the front of the R&D queue in salmonid disease research! Laurent showed images of gross pathology indicating "numerous haemorrhages on fat tissue" with underlying vacuolisation of the pericardium. Phylogenetically the isolates were similar to ones identified in rainbow trout from Norway and Coho salmon from Chile.

The presence of SAV in rainbow trout is something that concerns Rodrigo Belmonte of MSD Animal Health. Not only do these trout harbour the virus but can act as vectors for the virus in onward transmission to Atlantic salmon as well as to other trout and should be considered as a risk factor to neighbouring farms. Rodrigo went on to show that viral shedding in trout could be significantly curtailed in fish that were vaccinated against SAV. Of course this vaccination would be primarily for the benefit of neighbouring salmon farmers and echoes Arnfinn Aunsmo's sentiment of those who share the benefit should perhaps share the costs!

The role of PRV in the context of co-infections was the subject of Magnus Vikan Røsaeg's presentation based upon Industry experience at Salmar. Field observations led Salmar to further investigate whether infection with PRV affected outcomes of subsequent infection by SAV. Studies indicated that late infection of PRV infected fish by either SAV 2 or SAV 3 could mitigate tissue damage normally associated with these pathogens when present alone.

At the Dublin meeting in 2015 Sonal Patel of The Institute of Marine Research presented results showing that post smolts are at their most susceptible to SAV in the initial phase following transfer to seawater. Since then Sonal and colleagues have demonstrated these fish are also strong shedders of virus suggesting that viral replication is maximised at this time. The team have also demonstrated that tissue damage and viral shedding rates are dose dependent.

The only presentation addressing the potential role of functional feeds in the mitigation of viral myopathy was given by Julia Mullins of Skretting. Julia described how two commercially available functional feeds were shown to reduce peak inflammation pathology in fish experimentally infected with PRV and subsequently shown to have developed HSMI. The severity of the condition was diminished in these fish.

The final presentation at the 2016 TriNations meeting was given by Julie Svendsen of the Norwegian Veterinary Institute on Cardiomyopathy syndrome (CMS). Julie outlined a new wide ranging study,

once again funded by FHF to fully describe the epidemiology, risk factors, transmission routes etc of PMCV in the farming environment. The project which is divided into 5 work packages and is already underway will include an important assessment of whether this virus can be vertically transmitted currently a ‘known unknown’!