It was fitting perhaps that the **3rd** meeting of the **Trinations** conferences to be hosted in Ireland convened at **Trinity** College Dublin on June **3rd** in a year that *nearly* saw the host nation win the Triple Crown! Must **tri** harder?

To set the scene for the 14th meeting of the PD Trination gathering, Brit Hjeltnes from the Norwegian Veterinary Institute updated everybody about the Salmonid Viral Myopathy (SVM) situation in Norway. In 2014 there were 142 confirmed cases of Salmon Alpha Virus (SAV), up from just under 100 in the previous year. Slightly more alarming was her observation that whilst there were 53 cases of Cardio Myopathy Syndrome (CMS) in Norway in 2010, last year 107 cases were recorded!

PRV (Piscine Orthoreovirus) on the other hand was confirmed on 181 sites in 2014 an apparent elevation in the number of cases of a condition which has also spread to northern Norway. Whilst the relationship between this virus and clinical Heart and Muscle Skeletal Inflammation (HSMI) remains poorly understood it has been established that whilst the virus is largely ubiquitous it is significantly elevated in fish which are suffering from HSMI and is associated with their erythrocytes. It has also been diagnosed in hatcheries.

Brit's observations and comments about HSMI were echoed by Hamish Rodger who reported an absence of HSMI in Ireland but nevertheless ubiquitous, albeit low levels, of PRV. By contrast Ireland saw a single case of CMS in 2014 (last recorded in 2012) which was serious enough to warrant accelerated harvest to keep mortality at <0.5%.

Pancreas disease has historically been an Industry blight in Ireland second only, in terms of impact, to that in some parts of Western Norway. In 2014 SAV 1 accounted for up to 10% mortality on some affected sites (average 5%). Of the 10 smolt inputs 8 became positive for SAV, some as early as three months following transfer. Vaccinated fish accounted for 38% of this input and where the disease was diagnosed in vaccinates it presented as chronic emaciation rather than outright mortality. To this extent at least, the impact of PD in Ireland in 2014 was widely regarded as having diminished compared to previous years.

Hamish's colleague, Angela Ashby, informed the conference that viral myopathies remained challenging conditions in Scotland. Although the distribution and incidence of the disease had been reduced when compared to previous years it had, as in Ireland, presented as a condition characterised by wasting and chronic debilitation. For those of us old enough to remember, these characteristics were typical of PD in the early '90s when mortality was not necessarily the main concern.

Both CMS and HSMI remain on the Scottish salmonid disease agenda, whilst the former may be diminishing (in contrast to Norway) the latter appears to be slightly elevated.

A similar picture was painted by David Sutherland who reported that in Shetland the three myopathies follow a fairly similar pattern to mainland Scotland with SAV 2 dominating. CMS has not disappeared in these islands and can continue to cause significant economic impact in harvest sized fish in mid-winter.

The Scottish Industry remains scared of SAV 3 appearing on this side of the North Sea according to Iain Berrill of the Scottish Salmon Producers Organisation whose access to, and marshalling of, PD Industry data means that they would be quick to learn if this Norwegian strain emerged.

So too it seems would MSD whose AQUAVAC Monitor programme in conjunction with the University of Stirling amongst others, has and continues to monitor the occurrence of PD up and down the coasts of Scotland and Ireland. Through assessing viraemia, antibody levels as well as SAV subtypes it has been possible to map the presence of different subtypes of SAV as well as their clinical impact. In terms of the former it would appear that little has changed since 2008 with SAV 1 dominating Ireland and south west Scotland. SAVs 4, 5 and 2 follow latitudinally in a northerly progression.

Much of the basis for the findings of the speakers reporting on the SAV situation in the Western Atlantic was, and still is, based upon viral sequencing and serology conducted by Filipe Nunes and Paul Savage at the Agri-Foods and Biosciences Institute (AFBI) in Belfast. . With RTQPCR primers for all six of the SAV subtypes as well as the ability to subtype through sequencing, AFBI has been at the vanguard of SAV phylogenetics. By combining this area of expertise with serological techniques they have produced a tabulated guide to diagnostic scenarios shown here:

SAV Diagnostic Scenarios

	SAV Neutralizing Antibodies (serum)	SAV Virus Isolation (serum)	SAV RT-qPCR (serum)	SAV RT-qPCR (tissue)	Comment
Scenario 1	-	-	-	-	Most likely not SAV Infection
Scenario 2	+	-	-	-	Previous SAV infection
Scenario 3	+	+	+	+	Current SAV infection
Scenario 4	+	-	-/+	+	Late stages of SAV infection
Scenario 5	-	+	+	-/+	Early Stages of SAV infection

afbi Agri-Food and Biosciences Institute

This approach was largely endorsed during the first of 2 conference workshops, (chaired by Dr Marian McLoughlin, Fish Vet Group) which convened to address the work of diagnosticians. A consensus was reached that whilst PCR remains a very powerful diagnostic tool it is vital that the process and laboratory in which it is conducted is accredited and that it is not used in isolation of other techniques including histopathology and serology.

If the 2015 Trination meeting felt deficient in any respect it was perhaps from the lack of input from Industry practitioners. This though was confined to quantity not quality. Bård Skjelstad of Salmar reported that for him smolt quality (and by extension, freshwater origin) is the key determinant of clinical outcome from infections with myopathic viruses. In fact his best performing crop in the 2013 input was positive to SAV from soon after transfer! But the smolts were good. It is this type of

observation which can never be discovered through PCR, antibody assessments or even epidemiology, which makes Industry participation at these meetings so vital.

Additional Industry experience came during the 2nd conference workshop (chaired by Chris Mitchell, PHARMAQ) which had been set up specifically to explore mitigation of myopathic viral infections through farm management. Ole Waagsbø-Kjellsen, a former Marine Harvest employee who now works with FoMAS – Fiskehelse og Miljø AS (a veterinary services and training consultancy based in Haugesund), suggested that farmers should *"…..assume all fish are infected from the outset and manage stocks on this basis".* This mantra which wouldn't sound out of place in a hospital (fish reference excepted!) has involved a cultural shift in the way site operators regard their stocks. Improved inter-site and inter-company communication combined with practical initiatives such as only stocking well boats during the hours of daylight as well as stress testing smolts prior to loading led to real improvements such as reducing time to market.

Support tools available to Industry faced with myopathies include, vaccination, breeding as well as the emergence of nutritional support including that presented by Ralph Bickerdike of BIOMAR who are continuing to explore the benefits of including krill derived phospholipids in their Qardio functional feed product. Ralph presented data which showed a reduction in PRV loads in fish challenged with this virus and fed Qardio which also improves heart size and reduces pathological changes in this organ following challenge with PMCV.

Comparative histopathology and viral loading are also used by breeders to indicate advances in breeding for resistance to the diseases being discussed here. In the case of CMS, Sissel Kjøglum from Aquagen suggested that inter-family variation in mortality to this disease correlates well with histopathological changes as well as viral loading and that if you are a fish that is homozygous for protective alleles your heart lesion score, following infection, will be lower than your heterozygous (and homozygous "–ve") peers. She also presented the economic benefit of using QTL stock for this disease.

Salmobreed on the other hand appear to have focussed their efforts on promoting resistance to PD and have identified a QTL of "major significance" in this respect which affords carriers a common resistance mechanism regardless of lifecycle stage.

By deploying genomic selection using a newly developed SNIP chip, Ashie Norris at Marine Harvest has been able to improve the heritability score associated with PD resistance in her stocks from 0.35 to 0.47. This technology will greatly accelerate the genetic gains in each generation to up to 15% and will double the accuracy of broodstock selection.

Whilst breeding appears to be offering some exciting prospect in the prevention of myopathies, traditional vaccination remains a key component of preventative management. Tore Hovland from MSD gave a detailed and wide ranging presentation on risk factors associated with SAV transmission and the propagation of infection. These included the transport of viable virus through lipids leaking from dead fish as well as insufficient levels of vaccination resulting in an absence of herd immunity. Whilst this has been well documented in airborne viruses little is known about these dynamics in water borne infections. It was therefore no surprise to Tore that in 2013 25% of SAV detections occurred in non-vaccinated Rainbow trout which constituted 20% of the fish stocked in that county. The "herd" should not just be defined by species!

Whilst the impact of non-vaccinated fish in the herd is largely unknown, Tore's colleague Ingunn Sommerset has been comparing viral shedding rates between these fish and vaccinates. Interestingly, but perhaps not surprisingly, serum and faecal virus loads are far greater in non vaccinates than vaccinates. Ingunn went on to show that this has a real and measurable impact on viral dissemination through the water column so demonstrating the role of vaccination in not just protecting the individual but also reducing infection pressure in the surrounding environment.

Further information from MSD was presented by Kristian Ulven who re-emphasised last year's presentation by Petter Frost by claiming that vaccines based upon one strain of SAV would confer protection against other strains. To support this Kristen presented data which demonstrated a degree of protection (as measured by heart and pancreatic lesion scores) in separate challenge studies using SAV 3 and SAV 2 against fish vaccinated with the currently available commercial vaccine based upon a SAV 1 isolate.

The vaccination theme continued with PHARMAQ's Karine Lindmo who presented novel data on the composition and structure of the recently discovered piscine totivirus, the causative agent of CMS. Because cultivation of this virus is challenging, Karine is interested in developing constructs or VLPs (virus-like particles) which might be vaccine candidates.

A detailed understanding of host pathogen interactions has led Sonal Patel and colleagues at the Norwegian Institute of Marine Research to conclude that post smolts are most susceptible to SAV infection during the initial phase following their transfer to sea. In fact susceptibility to challenge seems to diminish as the interval between transfer and challenge is extended.

An additional influence on the outcome of SAV challenge is co-infection with PRV according to Morten Lund. Morten, a PhD student at the Norwegian Veterinary Institute presented evidence suggesting that whilst PRV may actually suppress SAV 3's ability to replicate, the same cannot be said for SAV 2, at least in heart tissue. This type of differential influence at the genomic level may have important implications at a geographic one. Given the more ubiquitous presence of PRV than the different strains of SAV, it may be one of the controlling influences on clinical outcomes following SAV infection.

Either way these nuances will not affect the regulatory efforts now being put in place by the Norwegian government in what Martin Bind from the Norwegian Food Safety Authority described as a three pronged strategy for combatting pancreas disease in Norway. New regulations will be constructed with consideration to the type, scope and occurrence of the two SAV strains now established in Mid and Southwest Norway. The accepted wisdom has hitherto been mitigation, mitigation and monitoring and ultimately eradication with each becoming more intense as the prevalence (and hopefully incidence) of these viruses diminishes with increasing latitude.

In summary the take-home message from Dublin conference was that while major advances in molecular and genetic technologies will undoubtedly transform the development of vaccines and fish stocks, traditional good husbandry as well as robust diagnostics remains as foundations in combating these diseases.