PD OR NOT PD? – THE QUESTION REMAINS

It was in 2005 that the first meeting of the PD TriNation working group was convened to further our understanding of pancreas disease (PD) and similar pathologies such as Heart and Skeletal Muscle inflammation (HSMI) and Cardiomyopathy Syndrome (CMS). That was 11 meetings ago and since then our knowledge of these conditions has advanced significantly in the areas of viral identification and characterisation, diagnostics, epidemiology, host responses and best management practice for disease mitigation.

These diverse fields formed the basis for the gathering of over 100 delegates at The Scottish Government buildings in Leith on the 18th and 19th September of this year. Given the 18 months which had elapsed since the previous meeting in Belfast there was a general air of anticipation that the meeting would reveal valuable news and updates regarding these diseases and, for the most part, it did.

To kick things off, we were introduced to the new Chairman of the TriNation Group, Charles McGurk of Skretting ARC who has taken up the mantle from Gordon Ritchie of Marine Harvest AS whose role as founder and tireless contributor to the success of the TriNation initiative was applauded by all. Also stepping down was Neil Ruane from the Marine Institute in Ireland whose contribution over the years was also acknowledged and whose role is now filled by Britt Bang Jensen from the Norwegian Veterinary Institute (NVI).

It was Hamish Rodger who, in what must now be regarded as a tradition, opened the first session aimed at updating delegates on the current SAV situation across the TriNation territories. Hamish reported on Ireland and indicated that there has been a marked improvement in the situation there with an average PD associated mortality in 2012 of just 2%, compared to the last figure reported for this Industry in 2010 of 7%, both figures being the lowest recorded in Ireland for over 20 years.

In Norway, the PD situation seems, by and large, to have stabilised since about 2007, oscillating between 75 and 100 confirmed diagnoses per year, the vast majority of which continue to occur below the Hustadvika firebreak in South Trondalag. May to September are the peak months although unusually this year, early spring saw an elevation in the onset of outbreaks.

The big 'PD news' from Norway, however, has been the isolation of the SAV 2 subtype north of the firebreak and away from areas where SAV 3 (formerly the only subtype found in Norway) is present. This news was presented to conference by Britt Bang Jensen who is monitoring the situation, which was further illuminated by her colleague, Monika Hjortaas. Monika reminded us that SAV types 1, 2,4 and 5 have been detected in salmon in Scotland (1,4 and 6 in Ireland) but that SAV 2 had previously been confined to Atlantic salmon and rainbow trout both in the UK and in continental Europe. It has thus come as some surprise that it should appear in Atlantic salmon in mid Norway in 2010 and again in 2011 *and* has caused clinical PD at infected sites.

No real surprise though to Marian McLoughlin and her colleagues at AFBI who are keeping pace with the phylogentic complexities of SAVs! Marian explained that actually there are two distinct strains of SAV 2, the traditional rainbow trout strain which causes sleeping disease and a marine SAV2 strain which is found in Atlantic salmon in Scotland and is very similar to that recently detected in Norway. Marian's up to date summary of SAV strain and type distribution is shown below:

| SAV Subtype | Species | Country |
|---|---|---|
| SAV 1 (PD) | AS (SW)<br>RT[#] (FW) | Ireland, Scotland[# Lester et al 2011] |
| SAV 2 (FW)<br>SAV 2 (SW) | RT<br>AS*ϕ | France, England, Scotland, Italy, Spain, Germany,<br>Scotland* NORWAYϕ |
| SAV 3(PD) | AS & RT in sea | NORWAY only |
| SAV 4 (PD) | AS | Ireland, Scotland |
| SAV 5 (PD) | AS | Scotland |
| SAV 6 (PD) | AS | Ireland only |

*M. McLoughlin 2012*

Strict import controls and detailed analysis meant that the Sernapesca (Chile's National Fisheries and Aquaculture Service) representative, Elena Orellana's long journey to Leith from South America enabled her to deliver the good news that SAVs remains absent from the industry there. Surveillance continues!

The targeting of disease surveillance by the authorities in aquaculture nations is becoming increasingly popular as a way of focussing resources and reducing cost. For this reason, epidemiologists are busy trying to establish models which can predict both the occurrence and severity of PD in affected areas. Saraya Tavornparnich from the NVI and Anne Stene from Ålesund University addressed these areas respectively. Saraya indicated, perhaps not surprisingly, that PD history is a good predictor of future PD, whilst Anne suggested that increasing seawater temperature *per se* is an important predictor for PD outbreaks. The take home message being, avoid farming in areas with a history of PD and avoid stressing fish until temperature is declining or at least stable.

Although not implicated as a vector of infection, the common dab (*Limanda limanda*) has been the subject of some fairly detailed investigations at the Scottish Government's Marine Scotland science laboratory in Aberdeen. David Bruno and Iveta Matejusova have confirmed that this flatfish, which was first identified as a SAV carrier when a positive specimen was caught near Stonehaven in 2009, not only plays host to SAV type 5 but appears to do so in a variety of locations throughout Scottish waters.

Of course, the significance of this finding is largely unknown both in terms of the threat level posed by wild reservoirs as well as the pathogenicity of the virus when resident in wild fish.

An understanding of relative pathogenicity between subtypes is, however, beginning to emerge. Petter Frost of MSD Animal Health explained that it is probably too simplistic to pin clinical expectations on the basis of which SAV subtype is isolated during the course of an infection. Indeed, his experimental challenge study found no correlation between the virulence of different isolates and the subtypes from which they are derived. Thus, it appears quite feasible that some isolates of SAV 2 could be more virulent than those genotyped as SAV 3 and *vice versa*, meaning that it is more helpful to perhaps view virulence from an isolate perspective, independent of genotyping. The reason for this is that subtype or phylogenetic distinctions (i.e. the differences between SAV 1, 2 and 3 isolates etc) are drawn from the accumulated quantity of genetic differences between isolates whereas viral properties such as virulence are more typically determined by where in a gene the difference is as well as what the difference is.

Intra-type variations in pathogenicity were confirmed by Marian McLoughlin who reported on investigations of pancreas disease in Shetland where SAV 2 isolates dominate the scene with SAV 5 isolates being confined to the east coast. In this part of the world, clinical determinants, of which pathogenicity is one, do not appear to be influenced primarily by subtype.

Pathogenic effects from the viral infections covered by the TriNation umbrella are of course usually manifested in a limited range of pathological changes to the tissue of affected animals which are characteristic of that disease. In fish, particularly in the case of PD, HSMI & CMS, clinicians often face quite a challenge when it comes to making a distinction between one disease and another. This can be particularly difficult when concurrent or even sequential infections by these similar but different diseases are at play.

Making these distinctions is not just of academic interest either, hence the title of this article! When insurers are faced with covering a claim for mortality on a fish farm, they will require evidence supporting the claim in order to determine what the fish died *of* rather than what they died *with*. Concurrent or sequential infections can and do occur. Work at the NVI has shown that PD lesions can occur in the same stock from five months to a year after the first diagnosis. Hamish Rodger confirmed that such chronic outbreaks have also occurred in Ireland. Sometimes PD can be followed by infection with other conditions such as CMS. HSMI on the other hand is more likely to occur prior to, or even concurrent with, PD.

It is important therefore that the range of tools available to make distinctions between diseases which can present in quite similar ways is as broad as possible. To this end, the diagnosis of SAV-like infections should involve a multi-disciplinary approach including PCR, histopathology, and antibody monitoring. In addition to this, the field of proteomics is, according to John Tinsley of Biomar, yielding promising results as an additional, if perhaps not front-line, aid in the diagnostic toolbox. The presence and abundance of tissue damage proteins such as creatine kinase as well as proteins associated with an immune response can now be established by monitoring the serum levels of these and other proteins.

Following on from John and in a presentation which fascinated the audience, Patricia Noguera of Marine Scotland Science demonstrated how she has been able to culture self contracting cardiomyocytes (SCCs) *in vitro*. These cells, which visibly contract under the microscope, comprise a novel cell line which may allow investigators to look at and monitor SAV and other virus-host cell interactions *in vitro* without the need for live fish.

Further light into new developments and proposed research projects was shed by Sven Martin Jørgensen from Nofima whose group will be focussing on piscine myocarditis virus (PMCV) which is believed to be the causative agent of CMS. Efforts will be targeted towards host-virus interactions, genetic mechanisms of disease resistance as well as the development of co-habitation challenge models. This work will be conducted over the next four years and will doubtless feature strongly in future TriNation meetings.

As will the impact of the SAV 2 isolates newly identified in Norway in spring 2011. Over the next year this SAV strain will be scrutinised by Hilde Sindre and her colleagues at the NVI, to more fully understand its pathogenicity, viral characteristics as well as its economic impact. In each respect it will be compared to the much more widely distributed SAV 3.

For most of the industry-based attendees of TriNation meetings, it is probably the news about the development, efficacy and availability of vaccines to SAV diseases which is of most interest and in this respect the Leith meeting certainly did not fail to disappoint. For the companies involved at the sharp end of vaccine development, this is understandably a sensitive area and one in which the interests of openness must necessarily be tempered by those of commercial discretion. The session dealing with vaccination and control got off to an informative start with Stephen Mutoloki from the Norwegian School of Veterinary Science giving an academic appraisal of the different types of vaccine and the way in which they promote immunity in the host. Stephen reported that the work of his group, headed up by fish virus guru Øystein Evensen, had indicated that, under experimental conditions, inactivated whole virus vaccines appeared to confer superior protection to sub-unit and DNA derived vaccines at least in terms of challenge by SAVs in Atlantic salmon as published in the scientific journal, Vaccine.

Following this introduction, presentations were made by three commercial companies who are engaged in the supply of fish vaccine to salmonid farmers across the world. MSD, who currently produce the only SAV vaccine, which is licensed for sale, updated conference with data from their experience of Norvax Compact PD in both Norway and the UK. Both speakers confirmed that the desire from farmers to protect their stocks from pancreas disease remains strong. In addition to this, PHARMAQ presented to conference some promising results from their own work with an experimental inactivated whole virus vaccine following a series of both field and lab-based trials in Norway. Finally, it was Novartis's turn with a dazzling animated presentation of how they see the future of vaccination against SAVs, namely with nucleic acid vaccines.

It is no secret that fish farmers are perplexed by the circumstances currently surrounding the world of SAV vaccines. However as one member of the audience commented, they are encouraged to see that a number of different companies remain interested in the development of this important field.

The PD TriNation group continues to be a successful model both for informing industry as well as an international platform for the sharing of results and information about salmon alpha viruses and their impact upon salmon farming in the northern hemisphere. Of course, other diseases, two of which are 'topical' (in both senses of the word!) present equally challenging and seemingly irremediable situations for farmers. However, it has been shown that by working together under the umbrella offered by the PD TriNation's format, movement forward can be both significant and swift. A new website has been set up to capture and centralise information covered by the PD Trinations initiative and can be accessed at http://trination.org

It is anticipated that the next PD TriNations gathering will be in Norway in 2013.