

PD TRI-NATION MEETING 14TH -15TH APRIL 2010

On 14th April 2010 the term 'Volcano plot' was understood by at least some of the 60 delegates attending the 10th PD(+) Trination seminar in Bergen, to refer to specialised statistical representations of gene expression. Twenty four hours later the term had taken on a rather more tangible and immediate meaning as the Eyjafjallajökull volcano both erupted and disrupted in north Western Europe. And whilst even the most hardened of conspiracy theorists could not seriously suggest that this too was a volcano plot (from a fiscally damaged country which had been requested by it's neighbours to send the Cash) the irony was enjoyed by the gathering of scientists – for about 10 minutes. More on volcanoes later.....

Indications from all three member states of the Tri-nation Working Group (TNWG) forum suggested that 2009 saw some improvement in the Pancreas Disease (PD) situation across Northern Europe. Malcolm Hall of FRS reported that the years 2006 to 2008 were the most significant with regard to PD although the disease still remained the prime contributor to disease based losses in the Scottish marine sector. This pattern is largely in agreement with that reported by Hamish Rodger whose Irish statistics indicated a drop in PD positivity across sites in 2009 compared with 2008, but that at 17% mortality these losses still cost the Industry €5 million. Whilst no economic data was presented for Norway it too appears to have seen a peak of outbreaks in 2008 (>100 cases) falling back in 2009 (>90). In the so-called PD zone, the number 'diagnosed' and 'suspect' sites has fallen by 35% since 2008.

Clearly the salmonid alpha virus (SAV) responsible for PD has by no means disappeared, but it seems that a better understanding of the disease, combined with better health management, much of which has come through the coordination afforded by the Trination platform, appears to be yielding results.

One commendable example of sound management through coordination was presented by Cato Lyngøy of Marine Harvest (Norway) who reported to the TNWG on the progress being made by the Norwegian 'PDFRI' Industry committee established in 2007 to stop the spread of PD, and reduce outbreaks as well as losses per outbreak.

Control strategies which have combined to produce a slightly more optimistic outlook in the world of PD are exercised at a macro-level (control of fish movements, disease reporting etc) as well as at the site level through vaccination, diet, and perhaps most importantly, a clear and present understanding of the stock status with regard to PD. In this respect the presentation by David Cox of Fish Vet Group and Alan Dykes reminded the delegates of what can be achieved in the face of PD risk, by regular stock monitoring, sound diagnosis and robust management. They recommend histological and serological examinations of stock in a 4 to 6 week routine, or at least well in advance of any planned stressing operation such as grading. Their preferred method of diagnosis remains histology as this offers the health manager a rich source of information about the progress of the disease as well as, crucially, what SAV condition might be present. Once a disease has been confirmed in samples from moribund fish, David informed the group that it actually makes more sense to begin sampling healthy fish in order to monitor the progress of any outbreak.

At the macro level it is probably the focus of the PDFRI which has trail-blazed a path towards pan-industry cooperation for the greater good. This has not always been easy with very different and often radical intervention required depending on whether a PD positive site is located within or outwith the Hustadvika firebreak.

At this level an understanding of how the virus is transmitted is vital for planning mitigation strategies. Mike Snow from FRS has been paying some attention to the question of natural SAV reservoirs in wild fish stocks. So far his efforts have largely been focussed on demersal species such as the common Dab in areas devoid of salmon farms. Using PCR he did find evidence that some of these fish had previously been exposed to SAV at some point in time, although these were not host to an active infection from which virus could be cultured. In discussion, members of the TNWG suggested that discreet populations of both mackerel and herring found in areas distal to fish farms might also make interesting target species in the hunt for the elusive 'reservoir host'.

An important point made by Mike was that the lack of a particular subtype of SAV (subtype 3, found in Norway) within the British Isles suggests that vertical transmission is unlikely to be a dominant mechanism for the distribution of this virus. This is a point which has been made at previous TNWG meetings and remains the accepted wisdom.

It is a precise and detailed understanding of the variation in SAV subtypes which underpins our knowledge about transmission and distribution of this virus. Increasingly this knowledge is now being applied not just to epidemiology but also in attempts to learn about variations in virulence between subtypes. In both these areas it is David Graham's group at the Agri-Food and Biosciences Institute in Belfast which has led the way. David presented work in which his group compared the 6 SAV subtypes which are distributed across Northern Europe, from the point of view of their differential impact on fish in a co-habitation challenge. The establishment of a reliable co-habitation model for each SAV strain has been an achievement in itself. In addition to this, by using a range of diagnostic methods, including pathology, serology and real-time RT-PCR, David's team has found variations in virulence between the different subtypes. Broadly speaking, subtypes 1 (Ireland) and 3 (Norway) are probably the most virulent with types 2 (UK) and 6 (Ireland) being the least. Subtypes 4 (UK and Ireland) and 5 (Scotland) were found to be somewhere in between. It should be emphasised however that given that only one strain from each subtype was examined, these results are indicative rather than definitive and further work on virulence will need to be carried out.

Another important and novel finding from this work was that the virus can persist in fish mucus and faeces for a number of weeks following infection, suggesting that both of these may be important routes by which virus is shed from infected fish and transmitted to others.

In addition to revealing possible differences in subtype virulence, it has been recognised that a precise knowledge of the way in which these viruses interact with their host will greatly assist in early diagnosis of the disease. Kim Thompson and colleagues at the Institute of Aquaculture in Stirling have successfully shown a number of significant variations in gene regulation in fish which have been challenged

with SAV. By knowing which genes are ‘up’ and which are ‘down’ regulated following infection, it is possible to gain a handle on how the host is responding immunologically to infection and possibly how the virus is exploiting the host’s genome in order to favour its own propagation. Perhaps by understanding which genes assist in the fishes defence, it will be possible to promote these genes in farmed fish. This point was mooted by the author in his short presentation which sought to raise the profile of fish strain, or breed, as a mitigating factor in the battle against SAVs.

Bertrand Collet and his co-workers at FRS appear to have drilled down into the world of host-pathogen interactions and are specifically interested in the interferon response in fish following infection with SAV. Interferon is the main anti-viral cytokine in fish and its presence tends to suppress viral replication. Bertrand has shown that two different subtypes of SAV strain 1 show different abilities to block the effect of interferon. It is therefore probable that the viruses’ competence in, what is effectively a counter-measure to the fishes innate immunity, may be directly related to virulence and perhaps explain some of the differences observed by David Graham and his colleagues in Northern Ireland. Early detection of these virulence factors should also assist in early diagnosis of infection, which is of great assistance to stock health managers in anticipating an outbreak of PD.

Histopathology combined with viral serology and real time RT-PCR was confirmed by David Cockerill of Marine Harvest (Scotland) and Ralph Bickerdike of Biomar as prime methods leading to early detection of PD. Their co-habitation challenge work showed a “progression of pathology” which characterises pancreas disease. Thus immediately following an explosion of virus (actually in the heart) the pancreas marks the onset of pathological changes followed by the heart, the red muscle and finally the white muscle.

Concurrent with a progression of histopathological changes, were spikes in levels of so-called Acute Phase Proteins (APPs) which are associated with tissue damage in animals (and humans) which have sustained some degree of tissue damage. Although not necessarily useful in early diagnosis (these spikes typically occur at six weeks following challenge) the levels of these biochemical markers, which can be measured non-lethally, may well be useful in assessing tissue damage and so help farmers make stock management decisions such as movements and grading.

APP levels may also give a useful insight into how well a stock is recovering following a PD episode. This has important implications for the scheduling of harvests. Turid Mørkøre from Nofima confirmed the undesirable impacts of PD infection at harvest. These include reduced yield, uneven and pale fillet colour as well as an increase in melanin deposition. However no impact on texture or gaping was observed. Some of these effects can be reversed during recovery.

No meeting of the Tri-nation working group would be complete without an update on current as well as potential developments in the vaccine world. In the former category, Marian McLoughlin presented the latest results from the Intervet stable whose product NORVAX® Compact PD remains the only vaccine available for use against pancreas disease. Using their own PD challenge model, Marian was able to show the effect of vaccination in reducing heart lesions (an important and often lethal

outcome of PD) in challenged stock which had been vaccinated. This was corroborated by mortality data from pens where vaccinated fish co-habiting with unvaccinated peer stock appeared to suffer a significantly reduced mortality following challenge. However as Marian pointed out, vaccination alone will never work in isolation of other mitigation strategies which include stock management/husbandry, stock strain, macro or area management as well as diet and feeding.

The future prospects for further development of viral vaccines in aquaculture appear to have reached something of a hiatus as use of the latest technology may not yet be acceptable in the farming of food animals. By artificially constructing components of the SAV genome using recombination technology, Marius Karlsen from the University of Bergen has, with colleagues, produced a 'replicon' construct of SAV. They have gone on to 'infect' fish with the replicon and found that it induces changes in the heart which are similar to that which is characteristic of live SAV. They were also able to transmit the replicon between 'infected' fish and naïve fish in co-habitation trials. Fish which have been exposed to the recombinant virus exhibit a high level of protection when subsequently challenged with wild SAV. However the obvious attraction of only having to vaccinate 25% of the population (which can then transmit the replicon to their co-habiting peers) is somewhat tempered just now by the fact that the current replicon itself is capable of producing some pathological impact! Clearly more work needs to be done to widen the distinction between pathogen and replicon, if this exciting technology is to deliver. Further fine-tune engineering of the replicon should achieve this. At a more fundamental level, though, the application of this technology will only come to pass if the consumer is completely comfortable with its use in food production and this may be the biggest challenge!

Whilst pancreas disease tends to dominate the attention of the Tri-nation group, consideration is also given to other conditions for which SAVs are responsible. Gerrit Timerhaus of Nofima updated the meeting on her work to investigate the immune response of salmon which have been suffering from Cardio Myopathy Syndrome (CMS). This condition, which is characterised by specific pathologies in the atrium and spongy layers of the heart ventricle, causes significant mortalities in farmed salmon usually when they are at harvestable size. As with PD it seems that infected fish mount a detectable innate immune response at least four weeks prior to the onset of clinical pathology. This opens the possibility for early non-invasive detection once suitable immunoassays have been developed to reveal these changes.

Allied to, but distinct from CMS, is Heart and Skeletal Muscle Inflammation (HSMI). So far this distinction has been based upon epidemiological and pathological indicators. Workers at the National Veterinary Institute in Norway have begun to study a novel virus associated with this condition and it was Marie Løvoll who presented their results. They have identified a *reovirus* which is not only found in very high levels in fish suffering from the condition, but can also be recovered from hitherto uninfected fish once they have been exposed to infected material either by injection or co-habitation with HSMI stock. Further work will now follow the well trodden investigative path for new pathogens including virus characterisation, its interplay with the host immune response and modes of transmission to name but a few.

So that concluded two intensive days of presentations and discussion on PD and SAVs. What delegates were soon to learn, however was that Iceland's latest geological disturbances would mean that the journey home for many would become effectively a management training weekend! At this point I should personally like to apologise to the good folk of Orkney for at least having some part in influencing Northlink to divert the ferry away from Stromness over to Bergen to collect the stranded.

Ironically nine colleagues and I arrived in Kirkwall while the ferry was heading east! We had been fortunate enough to hitch a ride on the *Statsraad Lehmkuhle*, Norway's prestigious sail training ship which, we learned at an hour's notice, was making passage from Bergen to Orkney. A rough but interesting and unusual way to return home from a scientific meeting.

Other delegates used a variety of trains, boats and hire cars to get them from Bergen to the channel ports as airline uncertainty continued. The 'Golden Volcano' award for the fastest journey home, should probably go to Hamish Rodger who escaped from Bergen on the morning of Friday 16th April and arrived safely in Galway in time for work on Monday morning! Hats off too to Atlantic Airways who delivered Norwegians back to Bergen from the Faroe Islands and airlifted TNWG newcomer Debes Christiansen back to the Faroes in what was alarmingly described as a "window in the ash cloud"!

Next years meeting is scheduled to be held in Belfast where stability (at least in a geological sense) is pretty much guaranteed!

For those readers who are unfamiliar with the structure and role of the TNWG, the forum which was established in 2005 allows scientists and industry professionals from the salmon farming nations of northern Europe to meet exchange and share information regarding practical research advances and industry experiences pertaining to pancreas disease and other similar conditions. The PD Trination initiative contributes significantly to developing our understanding of PD and related pathologies, closing the knowledge gaps and further improving strategies leading to mitigation. The key success factor in this initiative has been an ethos of openness within the group. Crucially the group is able to condense the sound and practical science by researchers into practical recommendations for the industry, all of which is appreciated by the participants and well recognised outside the group.

Thanks must be extended to Marine Harvest and the Norwegian Veterinærinstituttet for their kind support of the event as well the much appreciated assistance they afforded to the stranded!