March in Bergen marked the time and venue for the PD TriNations meeting which this year included a workshop on CMS. Camilla Wilson of MSD, the new Chair of the TriNations group, opened proceedings with a welcome message before Marian McLoughlin, of the Fish Vet Group gave a brief update on the cardiomyopathy situation in Scotland, suggesting that in 2017 confirmations of HSMI were on the increase as was CMS, the latter appearing earlier in post transfer smolts. PRV had also been detected in Rainbow trout.

Fish Vet Group Ireland's, Susie Mitchell revealed that of the 13 operational sea sites in Ireland, 11 tested positive for PD in 2017, the isolates being SAV 1 and SAV 4. Mortalities ranged from 6% to 15%. She suggested that although mortality, which peaks in both the spring and autumn, doesn't appear to correlate well with the use of vaccine on a site-by-site basis, levels of mortality have fallen from 38% in 2014 to 10% in 2017. Susie mentioned that CPK levels could be used as an indicator of disease severity with values of 9000 being 'normal', 40,000 mild, and 79,900 'severe'. In the meantime, she anticipated that vaccination would increase in 2018 but suggested that further development of PD vaccines should remain a priority. Her parting advice to fish health personnel was "keep on top of AGD" and "avoid intervention during any clinical PD event."

The Norwegian situation was updated by Brit Hjeltnes of the Norwegian Veterinary Institute, a time served contributor to the TriNations group! As in Ireland, there appears to be some seasonality in Norway with peaks in summer and autumn. In 2017, 176 outbreaks were confirmed compared with 137 in 2015. This, combined with a gradual move north of SAV 2, has resulted in new regulations and control measures being introduced by the Norwegian Food Safety Authority in 2017 which were outlined to delegates by Martin Binde of that organisation. At an estimated cost to Industry of €5 million each site in Norway which farms salmonids must submit 20 fish each month for molecular screening of SAV, unless the virus has already been detected at the farm. The border between the PD-zone and the surveillance zone has been moved north, from Hustadvika to Skjemta, in the middle of the Trøndelag county. However, SAV3-outbreaks north of Hustadvika can still invoke compulsory slaughter.

Britt Bang Jensen, also of the NVI, emphasised that screening is only useful if it is conducted for a specific purpose such as tracking of SAV progress, feasability of eradication or the limitation of spread. An understanding of the trade-offs between sensitivity and specificity are crucial for making informed decisions within the context of the sampling objective; the implications of false positives and negatives are clearly different depending upon whether they occur during screening or diagnosis.

Not for the first time the conference was reminded that the economic impact of fish disease is notoriously hard to document particularly when the condition under consideration is not always fatal. An economic assessment of losses from PD using combined site data by Jostein Mulder Petterson, from Patogen, together with Industry and NVI partners, showed that the cost of PD in mid production (2Kg fish) was actually greater than at the end (4Kg fish) which is counterintuitive and contrary to accepted wisdom. Such costs can range between 12 and 17 million NOK per site.

Hilde Sindre, of NVI gave an overview of best practice in the testing and screening for SAV. Hilde confirmed that heart ventricle remains the tissue of choice for screening purposes and that the vaccination status of the fish is an important consideration in the interpretation of results from both RT-PCR (currently the preferred choice for screening) as well as tests that

involve serum neutralisation. In the latter case an additional risk to that arising from vaccine derived antigen can come from cross reactivity from antibodies raised against other antigens such as PRV.

In work jointly funded by the Fish Vet Group and MSD, the former's Marianne Kraugerud shared some experiences from using a variety of blood biochemical markers as PD indicators. Of particular note is Creatine Kinase (CK) the levels of which correlate with muscle damage and may thus be useful as a prognostic tool to predict improvements in muscle quality of PD afflicted stocks. Likewise, Aspartate Transaminase (ASAT) was also shown to correlate well with red muscle damage. The potential benefit is that these, and possibly other markers, could help in the management of PD through non-lethal sampling.

Liam Doherty from MSD went on to explain how serology is used as a tool both for on-site monitoring of SAV as well as for mapping the bigger SAV picture. Mapping has revealed that since 2015, SAV1 has extended its geographical reach in Scotland (now including both the Western Isles as well as the Shetland Islands). SAV5 is now also present in this northern archipelago. On at least 2 sites there has been evidence of change in the SAV type identity (SAV4 to SAV 1 in one case and SAV 5 to SAV 1 in the other). One site showed a concurrent infection of SAV 4 and SAV 5.

Mitigation of PD in the form of dietary intervention has been presented at TriNations before and Biomar's Trygve Sigholt, working in conjunction with Nofima and the Norwegian University of Life Sciences, gave an interesting update from the world of Krill as a feed component. A study was established to compare two types of Krill based diet, one richer in EPA and DHA lipids than the other. In both compositions heart pathology in fish challenged with SAV was lower than control fish fed a triglyceride diet low in both fatty acids. The observed difference was possibly due to the anti-inflammatory impact of the DHA as well as elevated cell membrane integrity. The Krill enriched feed was also shown to dampen the growth arrest normally associated with SAV3 infection.

Rodrigo Belmonte from MSD reminded delegates that both salmon and trout can be affected by SAV in seawater although the outcome is not so severe in the latter. However, trout can shed virus if infected and this may be a significant infection reservoir where the disease is endemic in those areas holding unvaccinated fish. Tank trials have shown that trout vaccinated with a monovalent PD vaccine subsequently shed significantly less SAV than unvaccinated peer fish following SAV 3 challenge. Also evident in the vaccinated group, was a significant reduction in clinical heart and pancreas damage compared to the non-vaccinates. Additional studies showed that the vaccination of trout with this PD vaccine was safe and free of any check to growth.

DNA constructs (in an SAV vector) derived from two non-structural proteins of PRV (μNS and σNS) formed the basis of a presentation by Espen Rimstad of NMBU. Espen described how these proteins produced a moderate protective effect (based upon histopathology scores) in fish vaccinated with them. This was enhanced with the addition of a cell attachment protein also from PRV (σ1).

In a benchmarking trial conducted by SalMar and supported by MSD, two vaccines, one containing a SAV antigen (Aquavac PD7) and the other without (ALPHA JECT micro 6) were compared for safety and efficacy under commercial conditions over a 15-month period. Whilst a significantly lower level of SAV RNA (an indicator of viral load) was found in fish

vaccinated with the PD antigen, subsequent differences in mortality, Spielberg score and growth were found to be not significant between the two groups.

Industry practitioners are often perplexed as to why mortality outcomes following infection with SAV can vary so much from site to site. Ingunn Sommerset of MSD working with partners at Marine Harvest, The Norwegian Veterinary Institute and NMBU set out to explain whether these differences could be explained by strain differences in SAV 3, based upon an examination of 11 isolates. The study revealed no obvious relationship between heterogeneity in the E2 gene and mortality in stocks infected by isolates of SAV3 which were heterogenous in this region of the genome.

A look at SAV2 genomes has revealed a common ancestor that likely existed around 2008 and could be the incipient isolate for this strain in Norway. Whilst the Norwegian SAV 2s are related to Scottish ones, an epidemiological link between the two has not yet been established. We do, however, have an improved understanding of the relative virulence of this strain and how it has changed over the last 6 six years. Marius Karlsen from PHARMAQ explained how a comparison of virulence between Norwegian SAV2s shows that whilst the 2011 isolate was quite a mild strain, ones from subsequent years (2012, 2013 and 2017) were found to be quite virulent confirming that it is often better to regard virulence from an *intra*-strain perspective than an *inter*-strain one. At the molecular level, these differences can be attributed to a few amino acids.

Whole genome sequencing is a costly and highly specialised process. Michael Gallagher of the University of Aberdeen presented a new mobile sequencing platform, the Minion whole-genome sequencer. This small device can separate out SAV genomes with a high degree of accuracy although, it was conceded, not quite as accurately as the established, if less portable machines. Low cost and high speed, it seems, come at the expense of accuracy and output levels.

Continuing the R&D theme, Anne Aas-Eng from PHARMAQ presented results from a large field trial conducted in Norway for testing PHARMAQ's new PD vaccine, ALPHA JECT micro 1 PD. Despite of the challenges commonly associated with such trials in obtaining valid data, outbreaks of PD occurred at several locations, and the vaccine was found to reduce mortality from PD by almost 50% compared to the Compact PD vaccine which was used as a control in this study.

By way of an introduction to the session on transmission and infection, Sonal Patel from Norway's Institute of Marine Research, described a diverse project tasked with improving our understanding of the macro and micro environmental factors, as well as the immunological ones, that determine the progress of infection by SAV into clinical disease. Sonal showed how well-adapted but SAV infected, salmon smolts that have been in the sea for 9 weeks or more, shed less virus than newly transferred ones. One reason, demonstrated in this programme, is that the immune framework, including the antigen presentation apparatus in the pancreas, heart and pyloric caecae, has been activated by this stage. The study also showed SAV induced changes to the skin microbiome (so called dysbiosis) where harmless taxa are replaced by more pathogenic ones including *Flavobacteriaceae* and *Tenacibaculum.*

A ploidy comparison of salmon in the same project, showed that in triploid fish the accumulation of SAV positivity in the triploid population was slower than in the diploid one. Noelia Nuñez-Ortiz, Institute of Marine Research indicated that this observation was supported by a higher level of up-regulated antiviral immune genes and pattern recognition

receptors in the triploid group. Weight gain, as a measure of response to vaccination, was also found to be improved in lines of fish bred for enhanced resistance to SAV.

Using established artificial transmission / challenge techniques, Bjørn Olav Kvamme of the Institute of Marine Research has been able to demonstrate that Sea trout (*Salmo truttae*) post smolts are actually less susceptible to SAV3 and PRV than their Atlantic salmon cousins, and in the case of the latter virus *"much less so"* and were *"not expected to develop HSMI"* at the time of publication during this ongoing study.

Neil Ruane from the Marine Institute in Ireland continued the 'wild 'theme, but with cleaner fish. Irish salmon farmers deploy wild caught wrasse in salmon cages as cleaner fish. The first 60 individuals from each capture operation are health screened. In 2017 a pooled sample with material from 5 Ballan wrasse was confirmed positive for SAV 6. The last such finding was in 1996 and the significance of this recent one, according to the authors, has more to do with the taxonomy of Alpha viruses than the epidemiology of salmon pancreas disease in the Irish Industry, to which it was not believed to be linked.

The PD component of the Bergen TriNations meeting ended with an applied look at PRV infection in Atlantic salmon; specifically, its influence on the host's tolerance to hypoxia through two mechanisms, one by virtue of the infection itself and the other from the clinical outcome of infection – HSMI. Erythrocyte loading with PRV results in a reduction in haemoglobin Oxygen affinity which negatively impacts the carrying capacity of the blood for this molecule. Cardiac inflammation, characteristic of clinical HSMI, leads to reduced cardiac performance which in turn diminishes the fishes' ability to cope with sub optimal dissolved oxygen episodes, occasionally found in the pen environment.