European Union Reference Laboratory for Fish Diseases
National Institute of Aquatic Resources, Technical University of Denmark

Report of the

22nd Annual Workshop of the National Reference Laboratories for Fish Diseases

Kgs. Lyngby, Denmark

May 30th – 31st 2018

Lumpfish in experimental trial at DTU

Dissection of fish during a 2017 EURL training course

Organized by the European Union Reference Laboratory for Fish Diseases,

National Institute of Aquatic Resources, Technical University of Denmark, Kgs. Lyngby

Contents

Introduction and short summary

The 22nd Annual Workshop of the National Reference Laboratories for Fish Diseases was held 30th – 31st of May, at DTU Aqua, 2800 Kgs. Lyngby, Denmark. This annual workshop was the second to be held at our premises in Kgs. Lyngby.

A total of 67 participants from 35 countries attended over the two days period. All presenters arrived to the workshop, thus, no last minute changes were made in the programme. There were five sessions with a total of 28 presentations, three of which were given by invited speakers; 1) Satu Viljamaa-Dirsk from Finland (IHN outbreak in Finland), 2) Britt Bang Jensen from Norway (Salmonid Alphavirus) and 3) Sven Bergmann from Germany (Koi herpesvirus (KHV). Furthermore, there was a Working Group activity during session 1. The scientific programme of the Annual Workshop was again this year wide and covered many interesting topics.

The workshop was opened with "Welcome and announcements" by Head of the EURL for fish diseases, Niels Jørgen Olesen and EURL coordinator, Nikolaj Reducha Andersen. The scientific part was opened with the traditional Session 1 "Update on important fish diseases and their control", in which participants had the opportunity to present new findings from their respective countries.

Initially, an overview of the disease situation and surveillance in Europe 2017 was provided on the basis of the results obtained from the Survey & Diagnosis questionnaire. A report compiling all information is available at the EURL website www.eurl-fish.eu . Secondly, the fish disease situation in Norway was presented; a detailed report in Norwegian is available at https://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2018/fiskehelserapporten-2017. An English version will be available later. The two final presentations in Session 1 were an update on the disease situation in aquatic organisms in the Mediterranean and a presentation of a recent outbreak of Infectious hematopoietic necrosis (IHN) in Finland 2017.

The second half of the morning was allocated to the Working Group activity, introduced for the first time during the Annual Workshop in 2014. Briefly, each participant was asked to choose one of four groups, divided into fish species, and rate the different fish diseases for that certain fish species in his or hers country. All participants received four tables listing the most renowned pathogens for the most important farmed fish species in Europe. During the Working Group activity, participants discussed and agreed on a common rating for all the diseases. Each Working Group lastly presented their results to the rest of the participants at the workshop. A more detailed outcome of the Working Groups can be found later in this report under the chapter "Working Groups: Perception on the impact and risk of infectious fish diseases in Europe". The afternoon of the first day was allocated to emerging diseases and in the evening, a banquet was held at Restaurant "Viva" in Copenhagen.

The second and last day was opened with a session on results from ongoing research on listed and emerging fish diseases. Traditionally, this fourth session faced several different topics covering molecular characterization of pathogens, development of new diagnostic techniques, including conventional PCR and Real Time PCR, vaccines and characterization and description of new fish pathogens.

The Annual Workshop ended with the traditional fifth session on updates from the EURL. The results of the two proficiency tests sent out in 2017, PT1 and PT2, were presented. The programme and application system for the annual training courses, which will be provided by the EURL in October 2018, was described and participants were given the opportunity to suggest topics for future courses. The planned EURL activities in year 2018 were presented and proposals for the EURL work plan for 2019-2021 were discussed. It was informed that the work plan will include tasks for both fish and crustacean diseases.

Employees from DTU Aqua took minutes from the meeting: Jacob Günther Schmidt, Lone Madsen, Argelia Cuenca Navarro, Niccolò Vendramin, Sofie Hansen and Dagoberto Andres Sepulveda Araneda. Nikolaj Reducha Andersen has assembled a draft of the report, which has been sent to all the presenters and participants, who asked and answered questions during the presentations, for correction in order to avoid misunderstandings.

We would once again like to thank all the presenters for their great contribution, without them the meeting would not have been a success. The workshop and meeting was organized by a team consisting of Teena Vendel Klinge, Nikolaj Reducha Andersen and Niels Jørgen Olesen, with the help from the rest of the fish disease section at the National Institute of Aquatic Resources, DTU. The meeting next year is tentatively planned to be held at mid June 2019, also at DTU Aqua. More details will follow.

We wish to thank all of you for participating and we are looking forward to seeing you next year.

Niels Jørgen Olesen and Nikolaj Reducha Andersen

Programme

Wednesday May 30th

Annual Workshop of the National Reference Laboratories

08:50 – 9:20	**Registration**
09:20 – 09:40	Welcome and announcements *Nikolaj Reducha Andersen and Niels Jørgen Olesen*

SESSION I: Update on important fish diseases and their control

Chair: Olga Haenen and minutes: Jacob Günther Schmidt

09:40 – 10:00	Overview of the disease situation in Europe *Niels Jørgen Olesen*
10:00 – 10:20	Update on the fish disease situation in Norway *Brit Hjeltnes*
10:20 – 10:40	Update on the disease situation in aquatic organisms in the Mediterranean *Niccolò Vendramin*
10:40 – 11:00	Infectious hematopoietic necrosis (IHN) in Finland *Tuija Kantala*
11:00 – 11:20	***Coffee break***
11:20 – 12:50	Perception of the impact and risk of infectious fish diseases in Europe: Group 1: Atlantic salmon, Group 2: Rainbow trout, Group 3: Seabass and Seabream and Group 4: Cyprinids, Sturgeon, Eel and Tilapia *Nikolaj Reducha Andersen*

13:00 – 14:00 ***Lunch***

SESSION II: Emerging diseases

Chair: Eann Munro and minutes: Sofie Hansen

14:00 – 14:20 Piscine orthoreovirus (PRV-3), a new pathogen for farmed rainbow trout?
Niccoló Vendramin

14:20 – 14:40 Occurrence and control of Salmonid Alphavirus in the North Atlantic
Britt Bang Jensen

14:40 – 15:00 Confirmation of SAV 2 and SD in Austria and in Arctic Char (*Salvelinus alpinus*)
Eva Lewisch

15:00 – 15:20 Growing insect culture in Europe as protein in fish feed: risk of pathogen transfer?
Olga Haenen

15:20 – 15:40 ***Coffee break***

SESSION III: Control and surveillance of relevant pathogens in the EU

Chair: Uwe Fischer and minutes: Argelia Cuenca

15:40 – 16:00 Listing of fish diseases in EU legislation
Niels Jørgen Olesen

16:00 – 16:20 Implementation of the new animal health law
Fiona Geoghegan

16:20 – 16:40 Cardiomyopathy syndrome (CMS) on the Faroe Islands
Debes Christiansen

16:40 – 17:00 Economics of diseases in aquaculture - how to quantify losses
Britt Bang Jensen

17:00 – 17:10 Merging of the EURLs for fish and crustacean diseases
Nikolaj Reducha Andersen/Niels Jørgen Olesen

17:30 – ***Bus transport to Hotel Cabinn City***

19:30 – ***BANQUET dinner at restaurant "Viva"***

Thursday May 31st

Annual Workshop of the National Reference Laboratories

SESSION IV: Results from ongoing research on listed and emerging fish diseases

Chair: Brit Hjeltnes and minutes: Niccoló Vendramin

09:00 – 09:20	30 years of disease diagnostics in Switzerland *Thomas Wahli*
09:20 – 09:40	Results of the 2nd VER Inter-laboratory Proficiency Test *Anna Toffan*
09:40 – 10:00	Infectious hematopoietic necrosis virus (IHNV) RT-qPCR: validation and implementation in EU and OIE manuals *Marine Baud*
10:00 – 10:20	Viral hemorrhagic septicemia (VHS) and infectious hematopoietic necrosis (IHN): recent advance in molecular characterization *Argelia Cuenca*
10:20 – 10:40	Development of a vaccine against viral nervous necrosis (VNN)/viral encephalopathy and retinopathy (VER) *Sofie Hansen*
10:40 – 11:00	***Coffee break*** *Chair: Debes H. Christiansen and minutes: Dagoberto Sepúlveda*
11:00 – 11:20	Update on red mark syndrome (RMS) *Jacob Günther Schmidt*

11:20 – 11:40	An update on koi herpesvirus (KHV) and KHV disease research *Sven Bergmann*
11:40 – 12:00	Isolation of salmonid alphavirus (SAV6) from wild caught ballan wrasse in Ireland *Neil Ruane*
12:00 – 12:20	Recent research from Australia and new master programme in tropical aquaculture *Ellen Ariel*
12:20 – 12:40	Epidemiological aspects of the infectious hematopoietic necrosis (IHN) outbreak in Finland *Satu Viljamaa-Dirks*
13:00 – 14:00	***Lunch***

SESSION V: Update from the EURL

Chair: Niels Jørgen Olesen and minutes: Lone Madsen

14:00 – 14:20	Results of the Proficiency Test, PT1 and PT2, 2017 *Niccolò Vendramin and Teena Vendel Klinge*
14:20– 14:30	EURL Training Courses. Topics and organization of courses 2018 *Nikolaj Reducha Andersen and Tine Moesgaard Iburg*
14:30 – 14:45	EURL activities in 2017 *Niels Jørgen Olesen*
14:45 – 15:00	EURL Work Plan for 2018 and ideas and plans for 2019-21 *Niels Jørgen Olesen*
15:00 – 15:10	Next meeting and end of 22nd Annual Workshop *Niels Jørgen Olesen*
15:10 –15:30	***Coffee, cake and goodbyes***

SESSION I: Update on important fish diseases and their control

Chair: Olga Haenen

Overview of the fish diseases situation and surveillance in Europe in 2017

Niels Jørgen Olesen, Niccolò Vendramin and Nikolaj R. Andersen

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Abstract

The Questionnaire on Surveillance and Diagnosis (S&D) which is collated annually is the only comprehensive overview of the disease situation in fish farming in Europe. The information has been made available on the EURL web site (www.eurl-fish.eu), where all raw data can be obtained. The questionnaire comprises 4 parts:

1. General data on aquaculture fish production: Number of fish farms, and the health categorization according to Council Directive 2006/88/EC, and information on national surveillance programmes.
2. Epidemiological data on the disease situation in each Member State with focus on the listed diseases (information on number of out breaks and increase or decrease in number of infected farms and severity of outbreaks) but also including other diseases of interest.
3. Laboratory data from the NRLs and other laboratories, including the numbers of samples examined, and diagnoses of fish diseases made.
4. A National report describing health and surveillance situation in general. These reports are compiled into one and can be found on the website and in the present booklet.

Production data from FEAP

The data on the European aquaculture production was this year again obtained from the "European aquaculture production report 2008-2016" Prepared by the FEAP secretariat October 2017. The report does not include information on the number of fish farms, and therefore these data were obtained directly in the questionnaire. The report only provides data from back to the end of 2016 as data from 2017 will only be available in autumn 2018.

The total fish production in aquaculture in Europe decreased again a little after a steady increase until 2014 and is now at 2.297.571 t. Among the EU Member states the production has been almost horizontal in the past 10 years with a total production of 648.935 t., while the 4 non-EU countries Iceland, Faroe Islands, Turkey and Norway produce 1.648.634 t and also experienced a minor decrease in 2016 compared to previous years.

The Atlantic salmon production, account for 1.49 mill ton in 2016 against 1.55 mill ton in 2015, and is by far the largest contingency in Europe. The production of large rainbow trout in sea water has increased quite significantly in the recent years and accounts now for 153.954 t while the production of portion rainbow trout has decreased and was 233.654 t in 2016 production (maximum in 2013 with 259.970 t). After several years of increased production Turkey have experienced a 20% reduction from 2013 to 2016 but is still the largest contributor of table size rainbow trout with > 100 000 t production. The carp production is still mainly in the Eastern part of Continental Europe and is very stable with 58.995 t produced in all. Both the production of sea bream and especially sea bass also increased in the Mediterranean countries with a

production of 160.563 t and 157.698 t, respectively. Among other fish species of interest are eel (with 6.098t in 2016 no significant change since 2010 despite difficult access to elvers), sturgeon which is a promising species especially in view of its caviar production has been very stable in the past 10 years (2.635 t) while the caviar production increased with 20% from 2015 to 124 t in 2016, turbot (decrease from 12.748t in 2012 to 7.823t in 2013 and increased again to 9.907 t in 2016), the cod production have collapsed from 22.729t in 2009 to <1 t in 2016! The production of cleaner fish as lumpfish for lice control is increasing significantly (several cod farms took over the production of cleaner fish) but the total production has not been possible to retrieve.

Number of fish farms in Europe:

The total number of authorised/licensed fish farms in Europe was reported to be around 27.806 farms, with the largest contingency in Germany with 12.961 farms having a high number of very smal production. Norway having by far the largest production in Europe license almost 1.400 farms/sites. An overview of the number in each country can be found in Annex 1.

Health categorization of fish farms:

Almost all Member States did reply to the questionnaire and provided very clear and correct answers. This year in all 12.743 farms with species susceptible to VHS were reported in categorized zones, 11.473 to IHN, 6.519 to ISA and 11.225 farms with cyprinids susceptible to KHV; 1.523 farms were reported as non-categorized

79% of the authorised trout farms in Europe are situated in category III zones for VHS and 76% for IHN, with 20% and 23% respectively in Category 1. For both diseases the remaining 1% of the farms are situated in category II, IV or V. In all countries except Norway almost all salmonid farms are in Category I for ISA with 67% in Category I and 21% in category III. Only very few carp farms are approved KHV free in Category I (1%) and almost all are placed in Category III (97%) or in Category II 2%.

In Europe there are still several different views on how categorisation shall be performed, e.g. should VHS free marine rainbow trout farms be placed in Category III or I? Considering the risk of infection with VHSV from the marine environment.

Commission Decision 2015-1554 provide the guidelines for obtaining disease-free health statuses with regard to ISA and to contain infection with HPR deleted ISAV, saying that detection of Isavirus HPR0 will not compromise the health status of a fish farm and is not notifiable to the EU (in contrast to OIE where detection of ISAV HPR0 is still notifiable). Some Member states do not include small registered APBs in the categorisation (e.g. hobby farms) but according to 2006/88/EC Annex III health categorisation comprise all APBs in the Member states, zones and compartments for each category. Only fish species listed as susceptible for the given listed disease shall be included in the categorization. Therefore important aquaculture species as sea bass, sea bream, meagre, eel and pike-perch are not included in the European health surveillance for specific diseases.

The new Animal Health Law is now adopted and includes all aquatic animals; in this connection the categorisation system will be simplified and be made more transparent on the other hand more lists will be adopted compared to the present lists of exotic and non-exotic diseases (from present 2 to 5 lists). Annex 2 provide the full list of farms in categorized zones.

Outbreaks and severity of listed diseases in Europe

Only few participants reported that they observed major changes in the epidemiological situations in their respective countries. Overall only few new outbreaks of **VHS** were observed (Thuringia and Saxony-Anhalt (Germany), Switzerland) while a few reported decrease in severity (Czech Republic, Bavaria). For **IHN** increase was reported from Bavaria (from 2 to 4 cases) and especially the new and first epidemic in Finland have attracted attention (3 farms and 2 put-and take lakes - will be reported during the workshop). No new outbreaks of IHN were reported from Croatia or the Netherlands and decrease in Poland..

For **ISA** Norway reported 12 new sites with ISAV HPRΔ in 2016 and reported 14 in 2017. Unfortunately no report was received from the Faroe Islands. ISA was only reported from Norway.

Concerning **KHV** Czech republic reported one more outbreak compared to 2016, and UK reported 35 infected sites. Germany experienced increases in number of cases in Lower-Saxony, Saxony, Baden-Württemberg and in Bavaria. Lithuania observed a decrease in number of KHV positive samples as did Poland. Croatia has not encountered KHV before 2016 and did not find it in 2017. Annex 3 provides the full list of reports.

Other fish diseases problems in Europe

A whole range of other disease problems in 2017 were reported:

In **rainbow trout** the major concerns are flavobacteriosis (RTFS), red mark syndrome, puffy skin, enteric redmouth, and infectious pancreatic necrosis but also, lactococcosis, proliferative kidney disease, ichthyophtariasis, saprolegniosis, columnaris and furunculosis (especially in brown trout). More and more report BKD (bacterial kidney disease) as an increasing problem- possibly due to increased number of RAS in Europe. First outbreaks of HSMI-like disease in rainbow trout caused by PRV3 reported end of 2017.

In **salmon** farming it is pancreas disease, heart and skeletal muscle inflammation, cardiomyopathy syndrome, yersiniosis! (Norway) amoebic gill disease, and moritella and in addition flavobacteriosis, furunculosis, and saprolegniosis (Baltic salmon).

In **pike-perch** farming 1 new outbreak of perch rhabdovirus.

In **cleaner fish** it seem like Flavivirus is one of the major problems. Lack of knowledge and poor management is likely the most important problem

In **Carp** it is primarily CEV, *Aeromonas hydrophila*, SVC (in Romania) and parasites in general.

In **seabass** and **seabream** it is primarily VNN/VER, tenacibaculosis, *Vibrio harvey*, *Sparicotyle chrysophrii*. *Aeromonas veronii* and *Lernathropus kroyeri* infection.

In northern European countries the most common problems in the salmon production are thus sea lice, PD, and AGD, in addition several countries reported finding of Winter Ulcer Disease in salmon caused by *Moritella viscosa*. Cardiomyopathy sundrome caused by PMCV is of increasing concern in Norway (reported later during the workshop) as is piscine reovirus infection in both Atlantic salmon (PRV1) and rainbow trout (PRV3). In continental Europe it is primarily bacterial diseases like RTFS, ERM and Aeromonas infections, and AGD – but also red mark syndrome is causing severe problems. Parasite infestations as Ich is still a very serious problem especially in view of the foreseen prohibition of use of formalin, while problems in the Mediterranean countries are the same as in continental except for Lactoccocosis wich is more common in Southern Europe and Nodavirus infection in mariculture which definitely plays an important role and as a bottleneck for especially the seabass production.

Laboratory examinations

There are very large differences between countries on how many samples are tested on cell cultures, ranging from < 100 to several thousands. Annex 5 provide the total number of laboratory examinations conducted in Europe in 2017 on VHSV, IHNV, ISAV, KHV, SVCV, CEV, IPNV, SAV, and Nodavirus, respectively (Annex 5 will be provided when data from the remaining laboratories has been received).

Questions and comments:

Sven Bergman: *"Has SAV (sleeping disease) been found in brook trout in Europe?"*

Niels Jørgen Olesen: *"No."*

Eva Lewisch: *"But in arctic char."*

Charlotte Axén: *"When you talk about numbers tested for VHS it is important to consider method (PCR, cell culture) and pooling of samples."*

Niels Jørgen Olesen: *"Yes, it is true that when you are testing on cell culture you are often testing pools of 10 fish, whereas it is often single fish by PCR."*

Update on the fish disease situation in Norway

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Abstract

In 2017, Norway produced 1.207800 tons of Atlantic salmon (*Salmo salar*), 60000 tons of rainbow trout (*Oncorhynchus mykiss*) 5500-6000 tons captive wild caught Atlantic cod (*Gadus morhua*), 1600 tons of Atlantic halibut (*Hippoglossus hippoglossus*), 500 tons Arctic char (Salvelinus alpinus) and 2-300 tons turbot (*Scophthalmus maxima*).

Salmon lice (*Lepeophtheirus salmonis*) infestation represents one of the most significant challenges to Norwegian aquaculture, and increased resistance to anti sea lice chemicals is a problem of great concern. The fish farming industry, the Norwegian government and research institutions are investing in the development of non-chemical methods to control sea lice. Cleaner fish eating sea lice are used by a large number of fish farmers. In 2016, fish health personnel reported that mechanical de-liceing resulted in an increased level of mechanical injury and mortality in treated fish. This is still the situation in 2017.

Infection with salmonid alphavirus (SAV) remains the most serious virus disease in sea-farmed salmonids. In total, 176 new sea-farms were registered affected in 2017. This is a significant increase compared to 2016. Change in regulations with more mandatory screening for SAV is probably contributing to the increase.

Infectious salmon anaemia (ISA) was diagnosed in 14 farms in 2017 compared to 12 farms in 2016.

Infectious pancreatic necrosis (IPN) was diagnosed in 23 - 27 salmonid farms in 2017. This is much lower than the peak year of 2009 when IPN was diagnosed in 223 farms. Use of QTL strains of salmon combined with increased focus on eradication of ‘house strains’ of virus is probably the most important reasons behind the reduction in number of cases in recent years.

Heart and skeletal muscle inflammation (HSMI) was in 2014 removed from the Norwegian national list of notifiable diseases. Reported cases from the Norwegian Veterinary Institute, NVI (93) and private laboratories (90) indicate a similar situation in 2017 as in 2016.

Cardiomyopathy syndrome (CMS), also known as ’heart rupture,’ wasdiagnosed by NVI on 96 sites in 2017. Considering reported cases from private laboratories (100 cases), this indicates an increase over recent years.

While AGD (*Paramoeba perurans*) remains an important parasitic infection, the disease was not as severe in 2017 as it was in 2014. Gill disease occurs during all phases of salmonid culture. Chronic gill inflammation is a particularly significant and recurring problem. Bacterial ulcers continue to be a problem in farmed fish particularly in Northern Norway. Yersiniosis (*Yersinia ruckeri*) continues to affect an increasing number of farms and in recent years, there appears to be an increasing trend towards clinical outbreaks in large sea-farmed salmon especially in Mid-Norway.

Production losses remain a significant problem in Norwegian aquaculture.

Questions and comments:

Niels Lorenzen: *"Is PD increasing? How many farms vaccinate?"*

Brit Hjeltnes: *"In the endemic area it used to be a huge number that vaccinated in the south-western and western part of Norway using the traditional vaccine. However, Brit Bang-Jensen did a survey some years ago which showed that the vaccine was not very efficient, and was not solving the situation."*

Niels Lorenzen: *"Maybe make a new DNA vaccine that will solve the problem?"*

Brit Hjeltnes: *"I hope so. I hope we can be PD free again, but it needs a lot of commitment from the fish farmers. Vaccines can be useful by reducing the shedding of the virus, making other measures more effective."*

Update on the disease situation in aquatic organisms in the Mediterranean

Niccoló Vendramin

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Abstract

The Mediterranean basin represents an interesting area for aquaculture. The production in the area includes historically established freshwater aquaculture farming of salmonid (rainbow trout, brook trout and charr) and cyprinids.

Marine Aquaculture comprising land based hatcheries and sea cages has developed in the last 20 years and contributes yearly with a production of approximately 315.000 Tonnes of European Sea bass and Gilthead sea bream.

The aim of this initiative, which started in 2012, is to set up a platform that can link authorities and stakeholders aiming to target the main sanitary issues in the basin and focus future research activities on these topics. Currently two large initiatives named MEDAID and Performfish, to support Marine Mediterranean Aquaculture, have been funded by EU and are aiming to improve Key Perfomance Indicators (KPI) of the industry.

Health management represents a key aspect for development and sustainability of the industry; in order to map health issues and infectious diseases in the area, a simple questionnaire asking to rank the three most important diseases for marine and fresh water sector was delivered to a panel of experts.

Contributions from 13 experts were obtained about disease situation in the Mediterranean basin for 2017. Data will be presented and discussed showing comparison with previous years focusing both on important known diseases and emerging pathogens.

Data and presentation will be uploaded on the website of the EURL for fish diseases at the following link: http://www.eurl-fish.eu/Activities/annual-meetings

Questions and comments:

Brit Hjeltnes: *"I have a comment about the way of scoring the diseases. We previously used the questionnaire in the same way, but found out that it is better to give each disease a score instead of prioritizing them. The latter can lead to overestimation of impact of some diseases. Are you thinking about changing the system?"*

Niccoló Vendramin: *"Yes, that is definitely a possibility. I think the point here is that when I plot the results, there may be a bias in that one respondent may be answering based on 10 farms, and another based on one farm. However, I am not sure how to deal with this, as there are also things like confidentiality to consider, and presenting the data like this means that everyone is acknowledged for their work, but is anonymous."*

Brit Hjeltnes: *"You mentioned that parasitic problems are building up, and that it could be linked to build-up of organic material under the cages. For many years in Norway it has been mandatory to have rotation of*

sites, but that is impossible in the Mediterranean. However, it is so important to change site, and that will improve the situation."

Niccoló Vendramin: *"It is not all-in-all-out in the Mediterranean as you do wisely in Norway. There are some situations where there are several farms existing in the same bay, and each often have different generations, so for example in a farm there are 12 cages, and four are 0+, four are 1+ and four are 2+. That is an interesting epidemiological challenge."*

Brit Hjeltnes: *"It is the same as what you do in agriculture. You do not grow potatoes in the same site year after year - then you are asking for trouble."*

Infectious hematopoietic necrosis virus (IHNV) in Finland

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Abstract

Infectious hematopoietic necrosis (IHN) is a fish disease notifiable to the European Union and the World Organisation for Animal Health. The causative agent, infectious hematopoietic necrosis virus (IHNV), is a single-stranded RNA virus belonging to the genus *Novirhabdovirus*, family *Rhabdoviridae*. IHNV causes clinical disease and mortalities in several salmonid species, including Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), and the virus is considered to be an economically important pathogen worldwide. Until recently, Finland, like other Nordic countries, has maintained an official IHNV-free status.

In November 2017, IHNV was detected for the first time in Finland in a fish farm located in Northern Ostrobothnia in the Gulf of Bothnia. The farm was a winter storage net cage farm producing rainbow trout for food. Cytopathic effect typical for IHNV was detected on EPC cell line from two cultured samples in routine screening. The virus was identified as IHNV in ELISA, real time RT-PCR, RT-PCR, and sequencing. Immediately after the first detection of IHNV, testing of contact farms started to determine the prevalence of the disease, together with epidemiological investigation. Shortly after the first case, the second case was detected in the neighbouring winter storage net cage farm that was run by another operator.

In December 2017, IHVN was detected in a government-owned broodstock and nursery facility in continental Finland in Northern Savonia (municipality of Tervo) in the River Kymi basin. The facility was one of the largest farms producing juveniles in Finland. Testing of all contacts to which live fish or eggs had been delivered from this facility, as well as other farms in the Riven Kymi basin started immediately. The marine winter storage net cage farm where the second case was detected in November 2017, had received fish from this facility during the previous summer. In the end of December 2017, the fourth case was detected in a catch and release fishing pond close to the positive broodstock and nursery facility.

In January 2018, the fifth case of IHNV was detected in samples from a small privately owned backyard put and take fishing pond in River Vuoksi basin, and the sixth case in a fishing pond in northern parts of River Vuoksi basin. Both ponds had received rainbow trout from the positive broodstock and nursery facility in Tervo during summer 2017.

Partial G (glycoprotein) and N (nucleocapsid) genes of the IHNV genome were sequenced from PCR positive samples from the positive farms. The Finnish isolates were highly similar with each other, but they did not clearly cluster with any of the known IHNV genogroups. The closest relative was an IHNV isolate RU-FR-1 (GenBank accession FJ265715) isolated in Russia.

No clear clinical symptoms of IHN were reported in the positive fish, and no mass mortality was detected at the infected farms. Although the origin of the infections in the marine farms and the fishing ponds was tracked to the continental broodstock and nursery facility, the initial origin of the virus has so far not been found out in the epidemiological investigation.

Questions and comments:

Vlasta Jenčič: *"How did you sample wild fish? How many were checked, and were they caught just for sampling?"*

Tuija Kantala: *"Yes, we asked sport fishermen to catch them for us for sampling."*

Vlasta Jenčič: *"How many were positive?"*

Tuija Kantala: *"All wild fish were negative."*

Niels Lorenzen: *"You say that you sent culled fish into the food chain. Did you secure that disease was not spread to/from slaughterhouses?"*

Tuija Kantala: *"We took care of it".*

Sven Bergman: *"When you sequenced, did you not even see a single nucleotide difference between the marine and the freshwater IHN?"*

Tuija Kantala: *"Maybe one or two, but the problem was that we did not get very long sequences. However, they were maybe 98 or 99% similar."*

Sven Bergman: *"Do you consider to also include serology? Because in these subclinical cases it is more important."*

Tuija Kantala: *"We have discussed it, but not yet decided."*

Working Groups: Perception on the impact and risk of infectious fish diseases in Europe

Group 1: Atlantic salmon, Group 2: Rainbow trout, Group 3: Seabass and Seabream and Group 4: Cyprinids, Sturgeon, Eel and Tilapia

Nikolaj Reducha Andersen

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Abstract

The overall aim of this part of the 22nd annual workshop is to evaluate the impact and risk of relevant fish diseases in Europe. Not only impact in production, economy and legislative consequences will be evaluated, but also the risk of increasing significance in the future. Again, this year groups are divided into fish species.

The participants will be divided into 4 groups; Group 1: **Atlantic salmon**, Group 2: **Rainbow trout**, Group 3: **Seabass and Seabream** and Group 4: **Cyprinids, Sturgeon, Eel and Tilapia**. A facilitator has been assigned to each group (Group 1; Brit Hjeltnes, Group 2; Richard Paley, Group 3; Niccoló Vendramin and Group 4; Niels Jørgen Olesen).

The participants will receive a table beforehand (found in the workshop folder) which lists the most relevant diseases for the respective fish species, and this table must be used in the rating process. Furthermore, each group has to assign a presenter for presenting the agreed results for the rest of the participants (cannot be the facilitator). Thus, the tasks are to discuss the important fish diseases, provide an agreed score and select the five most important fish diseases, present these most important diseases and describe why they have been selected as the most important.

Time schedule:
11:20 – 11:25 Nikolaj will explain how to use the tables
11:25 – 11:30 Dividing into groups by "show of hands"
11:30 – 11:35 Each participant uses 10 minutes to fill in the table
11:35 – 11:40 Groups will join and move to location
11:40 – 12:25 Evaluation and rating of fish diseases (each facilitator will be given the table in A3 size to fill out an overall table representing the groups agreed decision
12:25 – 12:45 Plenary presentation from the groups (5 minutes each)

Working group's summary

In order to integrate data provided through the questionnaire on Survey and Diagnosis in Europe with direct inputs from the NRL (National Reference Laboratories) representatives, we arranged this Working Group activity. It is the fifth time, starting in 2014, that we conduct this activity during the Annual Workshop. Again, this year the groups were divided by fish species, and not by geographical region. The participants were divided, after own choice, into one of these four groups:

Group no.	Fish species	Facilitator

1	**Atlantic salmon and cleaner fish**	Brit Hjeltnes
2	**Rainbow trout**	Richard Paley
3	**Seabass and Seabream**	Niccoló Vendramin
4	**Cyprinids, Sturgeon, Eel and Tilapia**	Niels Jørgen Olesen

At first, participants were asked to fill out a table with a list of relevant diseases for each fish species with a view to the sanitary status in their home countries. For each disease, the participants were asked to give a score on four different parameters characterizing the impact of the disease:

1) The perception of the impact on production, meaning the severity of losses in terms of mortalities, reduction of growth, etc.

2) The impact on the economy, indicating the cost of prevention (i.e. vaccination and biosecurity measures), treatment and reduced value of the product.

3) Consequences due to trade restrictions, national plans for control/eradication, suspension time after antibiotic treatment etc.

4) The risk of increasing significance in the future.

Based on discussions, each group was asked to agree on a score (from 1-10) for each of the four parameters for each disease, select the five most important diseases for each fish species and to select a representative to describe the outcome of the work for the other participants of the Annual Workshop. It should be noted that since the working groups were organized equal to last year, the outcome of the discussions and results are very similar to last year.

The output of the Working Groups was:

Group 1; Atlantic salmon and cleaner fish

The group agreed that the most important fish disease for Atlantic salmon was the crustacean parasitic **sea lice**. This major threat was appointed a high score (>5) in all four categories; 1) impact on production (score of 10), 2) impact on economy (score of 10), 3) legislative consequences (score on 5) and 4) risk of increasing significance in the future (score of 7-8). As noticed in last year's identical session, the sea lice are still considered the bottleneck for future development of the salmon production in Norway. The economic impact of this disease is mainly related to increased resistance to treatment.

The disease scoring second highest (5-10) was **pancreas disease (PD)** caused by a viral infection of the salmon alphavirus (SAV). Due to national legislation in some areas of Norway, there is a special focus on this disease and it scored 7-10 in the legislative impact category (0 for countries where the disease is not relevant).

Cardiomyopathy syndrome (CMS) scored third highest (1-7) with the highest score (5-7) under impact of production and economy. The viral disease, **heart and skeletal muscle inflammation (HSMI)** disease were appointed the fourth highest score. The viral disease, **infectious salmon anemia (ISA)** where appointed the fifth place. The situation with ISA is relatively under control, but may increase in recirculation systems with smolt production in the future. The problems noted in cleaner fish are bacterial issues, however, also flavivirus.

Group 2; Rainbow trout

As noted last year, a prioritized list was difficult to make, since some important viral diseases are only found in certain countries and absent in others. However, the most important disease was agreed to be the **viral hemorrhagic septicemia (VHS) virus** with a high score (5-10) in all categories. The second most important disease was decided to be **infectious hematopoietic necrosis (IHN)**. The score for VHS and IHN were almost identical and both diseases must be considered important in rainbow trout production in Europe.

After the viral diseases VHS and IHN the most important diseases are bacterial, mainly **rainbow trout fry syndrome (RTFS)** caused by *Flavobacterium psychrophilum*. This disease is followed by **enteric redmouth disease (ERM)** caused by *Yersinia ruckeri* and **furunculosis** caused by *Aeromonas salmonicida*. As opposed to the viral diseases, the bacterial diseases are found in almost all countries producing rainbow trout. Parasites are generally less important. ICH and PKD were also noted as serious diseases.

Group 3; Seabass and Seabream

For European seabass, the diseases **tenacibaculosis,** caused by *Tenacibaculum maritimum*, was considered the most important disease. Following tenacibaculosis were **vibriosis**. Last year**, viral encephalopathy and retinopathy (VER),** also known as **viral nervous necrosis (VNN)** were considered the most important disease, however, this year it was agreed to be the third most important disease.

For gilthead seabream the most important diseases were considered to be the parasite *Sparicotyle chrysophrii*, followed by **red rash** which is of unknown aetiology. Third was the VER-VNN.

Group 4; Cyprinids, Sturgeon, Eel and Tilapia

For cyprinids, the ranging of important diseases was as follows; 1) **koi herpesvirus disease (KHVD), carp edema virus (CEV)** and **cyprinid herpes virus (CyHV-2)**. For sturgeon, **White sturgeon iridovirus (WSIV)** was agreed to be the most important disease overall followed by herpesvirus. For eel, viral **anguilla herpesvirus 1 (AngHV1)** was the most important. For Tilapia, three diseases where ranged, most important were **tilapia lake virus (TiLV)** followed by **Tilapia parvo-like virus (TPLV)** and problems *Francisella* sp. infections.

SESSION II: Emerging diseases

Chair: Eann Munro

Piscine orthoreovirus-3 (PRV-3), a new pathogen for farmed rainbow trout

Niccolò Vendramin [1], Dhamotaran Kannimuthu [2], Argelia Cuenca [1], Øystein Wessel [2], Maria Dahle[3], Lena Teige [3], Anne Berit Olsen [3], Tine Iburg [1], Espen Rimstad [2], Niels Jørgen Olesen [1]

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Abstract

Piscine orthoreovirus – PRV have emerged as relevant pathogens for salmonid aquaculture worldwide.

Currently three different subtypes with specific host are described for this viral species.

PRV-1 is the causative agent of heart and skeletal muscle inflammation (HSMI) in Atlantic salmon and and is associated with jaundice syndrome in farmed Chinook salmon

PRV-2 causes erythrocytic inclusion body syndrome (EIBS) in Coho salmon.

PRV-3 causes heart pathology resembling HSMI in rainbow trout.

PRV-3 was firstly discovered in 2013 in Norway during disease outbreaks affecting farmed rainbow trout. A first series of experimental trials conducted in a joint project involving DTU, NVI and NMBU were performed to assess its pathogenicity and pathogenesis in *O. mykiss* and *S. salar*.

The Norwegian PRV-3 isolate has been further characterized analyzing its genome and antigenic features.

An experimental infection study with purified virus demonstrated that PRV-3 infects rainbow trout and induces pathological heart lesions similar to HSMI, and thus fulfill Koch's postulates. Furthermore, the infection upregulates IFN production, and induces specific antibody response in later phases.

In late 2017 the presence of PRV-3 was also reported in different countries in Europe including Scotland, Germany, France, Italy and Denmark. Interestingly, these viral isolates appear to be genetically distinct from the Norwegian isolate leading to proposition of two separate clades within PRV-3 viral type (PRV-3a and PRV-3b).

In Denmark the virus has been associated with severe disease outbreaks in recirculating aquaculture systems. Clinical signs are represented by reduced appetite followed by uncoordinated swimming behavior and increased mortality; necropsy findings include severe anemia and ascites. Such outbreaks are complex disease cases where different bacterial (including *Flavobacterium psychrophilum* and *Renibacterium salmoninarum*) and viral pathogens (IPNV) are present at the farm.

Notably PRV-3 load increases in the target organs (heart, spleen) before the clinical disease appear, whereas the other pathogens are not detected in a systematic pattern.

In 2018 in cooperation with the Danish Aquaculture industry a project mapping the prevalence of PRV-3 in the country, investigating its virulence and the risk for vertical transmission, was funded and initiated. An overview of the results will be presented.

Questions and comments:

Marine Baud: "W*hich PCR do you use?*"

Niccoló Vendramin: *"qPCR"*

Hege Hellberg: *"Phylogenetic three - which fish species were tested?"*

Niccoló: *"PRV1: Atlantic salmon, PRV 2: coho salmon from japan, PRV 3: mixture of rainbow trout, brown trout and coho salmon from Chile".*

Eann Munro: *"From the sequence data, have you noticed putative virulence markers in PRV3".*

Niccoló Vendramin: *"Not enough data available yet, hope to conduct more whole genome sequencing and comparison with PRV1 strains."*

Occurrence and control of Salmonid Alphavirus in the North Atlantic

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Abstract

Salmonid alphavirus causes Pancreas disease in salmonids in the North Atlantic. The virus is widespread in Ireland, Scotland and Norwegian salmonid aquaculture, and is the cause of large economic losses and decreased welfare for the fish. In Ireland, most of the farms are affected, and in both Scotland and Norway more than half the farms are affected. In recent years, Cardiomyopathy syndrome (CMS) has become more widespread, and is an even greater concern than PD in some areas.

PD is a notifiable disease to the OIE, but not the EU. It is on the national list of notifiable diseases in Norway, and since September 2017 it has been mandatory to test salmonids in seafarms for SAV by PCR every month. Norway is further divided into an endemic and a non-endemic zone, and different restrictions apply if SAV is detected in each zone.

Control of PD has been mainly by stamping-out. Several vaccines are now available, and all have been shown to reduce the consequences of SAV-infection, but not to prevent infection.

Questions and comments:

Debes H. Christiansen: *"You were talking about outbreaks, what is the definition of an outbreak?"*

Britt Bang Jensen: *"Good question. In the project we have now agreed on when cumulated mortality is above 1% of the total fish population, you would say it is a severe case of PMCV. Out of 500 diagnosed farms, 150 meets this criterion. 0.1-0.5% cumulative mortality: mild outbreak (half of the 500 diagnosed farms qualifies as mild to severe outbreaks). When we report 96 cases of CMS, its individual farms – it can be everything from one to all of the fish on each farm."*

Niels Jørgen Olesen: *"Surveillance for PMCV in the wild, is it prevalent?"*

Britt Bang Jensen: *"It has been found, but in low prevalence and with low virus load (High Ct values). We do not consider wild fish as a reservoir for PMCV in Norway. The wild fish program screen for it and it is rarely found"*.

Uwe Fischer: *"SAV infections – should they be notifiable, what does the industry think?"*

Britt: *"The infection is notifiable in Norway, not just the disease must be present (specified in legislation last year). Authorities propose mandatory ring vaccination. Not yet implemented, but they can be if they want. The institute's advice to the authorities is that as long as the vaccine is not better, it should not be mandatory"*.

Niels Jørgen Olesen comment: *"New animal health law, five categories, it is possible that SAV will be included, but possibly in group B, where only surveillance is needed and not vaccination"*.

Fiona Geoghegan, EU: *"If it becomes listed, it will probably be as a category C"*.

Salmonid Alphavirus in Arctic char (*Salvelinus alpinus*)
First confirmation in the species and first evidence of SAV in Austria

1. E Lewisch[1,*], T Frank[2], H Soliman[1], O Schachner[1], Adina Friedl[1], M El-Matbouli[1]
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Abstract

(This abstract has been submitted for publication and can therefore not be published here)

Notes for presentation

Questions and comments:

Uwe Fischer: *"Technical question: when you use NSP3 PCR, and get a positive band, do you sometimes also get a sequence from the salmonid host? Last time we got sequences from marena fish and rainbow trout"*.

Eva Lewisch: *"No, I don't think so. My technician would have told me"*.

Uwe Fischer: *"We frequently get host sequences with the NSP3 PCR, annoying problem."*

Growing insect culture in Europe as protein in fish feed: risk of pathogen transfer?

Olga Haenen[12], A. Borghuis[1], G. van Duijvendijk[1], E. Weerman[1], B. Schoelitsz[1], L. Bonte[1], L. Dingboom[1], H.J. Roest[2], P. de Cocq[1]

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Abstract

In the last few years, there is a fast growth of culture of edible insects in Europe. In particular, mealworms (larvae of beetles, like the buffalo worm), crickets and grasshoppers, and fly larvae, like black soldier fly (BSF) larvae are cultured in intensive, industrialized farms.

These insects are cultured as a valuable and new protein source, for use in animal feed, like for fish, and also in food. Various pilot studies have been done for application of BSF in f.i. salmon feed, to partly replace fish meal, and thereby make fish feed more sustainable. Results are promising. Based on the first results, some farms are expanding ten-fold.

Insects are produced on substrates. These may vary from leftover streams to commercial insect feed. Which viruses, bacteria, fungi, parasites, and even TSE's s could be present in these substrates, as risks to animals and humans? This was an unexplored field. Rules on the use of use of substrates, as basis for risks of pathogen transfer from insects to animals and humans were needed.

European legislation has been formulated and is still in development: 999/2001/EC, 1069/2009/EC (art.35), 142/2011/EC (substrate for insect culture may only be vegetal, egg, or dairy based), and 893/2017/EC (allowing insect meal in aquaculture feed). Legislation to allow use of insect meal in chicken feed is in draft, and further legislation related to other animal branches probably will follow.

To support this new field of protein production, since 1st of January 2018, a new lectureship started at HAS University of Applied Sciences, in close cooperation with the NRL for Fish and Crustacean diseases and epidemiologists of Wageningen Bioveterinary Research at Lelystad (WBVR). Its title is: *Novel proteins: Insects and Fish, Healthy, sustainable and safe*. The project runs from 2018-2022.

Aims of this lectureship are: Extend and integrate knowledge, experience, and education on healthy and safe insect and fish culture: Investigate risk factors and support the use of healthy and safe insects in aquaculture feed in cooperation with feed processors. Students of HAS will do field research at insect and fish farms, and feed mills, in close cooperation with WBVR. Example 1: Safe and healthy insect culture on industrial and restaurant leftover streams, Example 2: Relation of bacterial and fungal flora at insect and fish farms with culture conditions.

In this presentation, an overview will be given of this new branch of insect culture, the interaction with the fish culture branch and humans, and risks of pathogen transfer, contact-zoonotic, and veterinary (insects and fish), apart from risks for food safety. There is still much to explore.

Questions and comments:

Uwe Fischer: *"Bright future! How is the omega fatty acid content in fish after feeding with insects?"*

Olga Haenen: *"There has been looked at it, I cannot remember, but there are published results to be found".*

Debes H. Christiansen: *"Are insects cost efficient compared to the existing feed in salmon production."*

Olga Haenen: *"Not yet, but it's getting more and more industrialized."*

Hege Hellberg: *"Do you collaborate with the ref lab for bee diseases, as far as I know they are the only one working with insect diseases? They have methods that could be implemented."*

Olga Haenen: *"No, not yet, where are they located?"*

Hege Hellberg: *"At Friedrich Loeffler Institute".*

Nikolaj Reducha Andersen: *"If we change the fatty acid composition, fish get more or less resistant to diseases. Do you only talk about the insects as protein source?"*

Olga: *"No, fat is also important. Especially black soldier fly larvae are pretty fat. International groups look into this, I know from a recent insect conference at Ede, the Netherlands. I do have some contact persons, if you like".*

SESSION III: Control and surveillance of relevant pathogens in the EU

Chair: Uwe Fischer

Listing of fish diseases in EU legislation

Niels Jørgen Olesen

EURL Fish Diseases, National Institute of Aquatic Resources, Technical University of Denmark, Denmark

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Abstract

An important task for implementing the new Animal Health Law is to determine the listing of diseases in a "delegated act" comprising both aquatic and terrestrial animals.
The listing is changed from 2 (exotic and non-exotic diseases) to the following 5 categories: Category a): Diseases not normally occurring in the Union. Category b): Diseases which must be controlled in al MS Category. c): Diseases subject to voluntary control in the MS, Category. d): Diseases for which movement restriction measures may apply. Category e): Diseases which shall be subject to surveillance.

Common for all these categories is the need to determine exactly which species are susceptible for the diseases in question, which species can act as vectors for transfer and which species are refractory. How the various fish diseases fit into these categories is still a matter of discussions. In order to provide the necessary background for assessment of susceptible species the EURL Fish was asked to provide updated information based on scientific studies.
Simultaneously an ad hoc group was settled in 2017 to assess the listing of species susceptible to the 10 OIE listed diseases according to the rules given in Chapter 1.5. 'Criteria for listing species as susceptible to infection with a specific pathogen' in the *Aquatic Code*. A work I am involved in together with colleagues from Australia, Japan, US and Canada. In order not to duplicate the work it is decided to make a provisionally list for the fish diseases listed according to the Animal health law, and to await the OIE for providing a final list. There are however, some discrepancies between the two bodies as the OIE operates with the following 3 lists: 1: Susceptible species for the Aquatic Code, 2: not enough evidence for susceptibility for the Aquatic Manual, and 3: proved to be non-susceptible for the Manual. The provisional list have been proposed for the listed diseases: VHS, IHN, ISA, EHN and KHV infection for the annex to the Animal Health Law and will be presented.

Some issue might cause some controversy as the listing of Atlantic salmon as susceptible for VHS, delisting of rainbow trout as susceptible for infection with ISAV HPR deleted, listing of other species than cyprinids as susceptible for KHV infection. Also the possibility for grouping susceptible species into families or genera is discussed in order to simplify the lists.

Questions and comments:

Olga Haenen: *"Do you also use the old EFSA report?"*

Niels Jørgen Olesen: *"Yes".*

Britt Bang Jensen: *"In the dissemination meeting it was discussed why CMS was not being listed, as it seems that this disease fulfill all the requirements".*

Niels Jørgen Olesen: *"It is not included because nobody has proposed it. ISAV was included just after Norway proposed it."*

Fiona Geoghegan: *“You need to be aware that the disease needs to fulfill certain requirements to be included.”*

Implementation of the new animal health law

Fiona Geoghegan

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Abstract

An update of the implementation of the new animal health law. Components of the work, timeline etc.

Notes

Questions and comments:

No comments

The Faroese CMS Mapping Project

(Cardiomyopathy syndrome (CMS) on the Faroe Islands)

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Abstract

Atlantic salmon farming is one of the most important industries in the Faroe Islands. Since the beginning in the 1970's the production has increased from a few tons to an annual production of around 80.000 tons for the last four years. The salmon export value was a record 3500 million DKK in 2017 and accounted for almost 50 % of the total Faroese export value.

Cardiomyopathy syndrome (CMS) is a serious viral disease affecting farmed Atlantic salmon and was a serious disease in the 1990's in Faroe Island. Following a complete reorganization of the industry and the implementation of new legislation on fish farming in the beginning of this millennium, CMS apparently disappeared for almost a decade despite a fast increase in production. In 2014, CMS re-emerged at a marine grow-out site in a fish group originating from eggs imported from Norway. Subsequently the annual cases of CMS has increased and has been associated with increased morbidity and mortality, raising concern in the Faroese farming industry and for the authority. However, knowledge on the risk factors for CMS is non-existent in the Faroes. Thus, there is an urgent need for increased scientific knowledge on CMS in order for the Faroese authorities and the farming industry to develop efficient management strategies against CMS.

The main objective of the present project is to elucidate if CMS in general is an emerging threat for Faroese aquaculture and specifically if treatments for sea lice pose a risk for the development of CMS.

Here some preliminary results on the Faroese CMS mapping project will be presented.

Questions and comments:

Niccoló Vendramin: *"It seems than we are dealing with an uncultivated virus, the possibility of vertical transmission is an important question. What is the proportion of eggs that you tested?"*

Debes H. Christiansen: *"I don't know, probably a very small proportion of the total, but we did not follow all the females that were stripped, but we tested families instead."*

Torsten Boutrup: *"The important thing is if the virus is inside or outside the egg, it doesn't matter. We agree that the way to do it scientifically is like you did, but the problem is that you need to test 100,000 eggs, and you only need one egg to pass it over to the next generation."*

Debes H. Christiansen: *"Yes, and we need to remember that we tested by PCR, so we don't get necessarily live virus, but RNA."*

Torsten Boutrup: *"You have the ideal condition, as there are lots of virus surrounding the eggs, so if you decontaminate with iodine and later on test".*

Debes H. Christiansen: *"Right, but it is always much more difficult to test a no correlation than a positive effect."*

Economics of diseases in aquaculture –how to quantify losses

Britt Bang Jensen

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Abstract

Quantifying losses in aquaculture is useful as a supporting tool for both fish farmers, decision makers and other stakeholders. Simple models can provide strong evidence of the implications of disease and prevention, and in this presentation, this will be illustrated and exemplified. Description of how to perform a partial budget calculating direct costs of diseases in aquaculture is provided, with relevant examples. The most difficult part of this form of budgeting is to obtain data, and examples of how this can be done is shown. Some suggestions for how to account for uncertainties and variations will also be discussed, and the audience is encouraged to engage in quantifying losses due to diseases.

Examples that are used are based on these publications:

Aunsmo, A., Valle, P.S., Sandberg, M., Midtlyng, P.J., Bruheim, T., 2010. Stochastic modelling of direct costs of pancreas disease (PD) in Norwegian farmed Atlantic salmon (Salmo salar L.). Prev. Vet. Med. 93, 233-241.

Pettersen, J.M., Brynildsrud, O.B., Huseby, R.B., Rich, K.M., Aunsmo, A., Bang Jensen, B., Aldrin, M. 2016. The epidemiological and economic effects from systematic depopulation of Norwegian marine salmon farms infected with pancreas disease virus. Prev. Vet. Med. 132, 113-124.

Questions and comments:

Uwe Fischer: *"In the graphic where you show the uncertainties, why is it not a Gaussian distribution?"*

Britt Bang Jensen: *"Well, you have a minimum, a maximum, and a most likely point (which you ask the experts about) and they have used a triangular, but in principle you could use another distribution".*

Britt Hjeltnes: *"Actually, the price of fish is the easiest of those estimators to get."*

Britt Bang Jensen: *"With this approach you can also understand which of the factors is the most important for the outcome: is it the cost of feed? The losses? The sales price? In this case we know that it is the sales price."*

Merging of the EURLs for fish and crustacean diseases

Nikolaj Reducha Andersen & Niels Jørgen Olesen

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Abstract

In 2017, the EURL for fish diseases was asked for the possibility of taking over the tasks of the EURL for crustacean diseases, by merging the two EURLs into one EURL, with the new name of "EURL for fish and crustacean diseases". Reasons for this were Brexit and the general desire of the European Commission to decrease the number of EURLs in Europe. After negotiations, the EURL for fish diseases accepted the terms, which included the possibility of assigning a full time scientist to implement the functions and duties of the EURL for crustacean diseases from 1 July, 2018. The job vacancy is open for applications until 7 June, 2018. The EURL for fish and crustacean diseases will strive to have a strong collaboration with Cefas, the former location of the EURL for crustacean disease, in order to continue a strong scientific advancement within crustacean diseases in Europe. Visits between DTU and Cefas are being planned this moment. We will give a short update on the status of the merging.

Questions and comments:

No comments

SESSION IV: Results from ongoing research on listed and emerging fish diseases, Part 1

Chair: Brit Hjeltness

40 years of disease diagnostics in Switzerland

Thomas Wahli

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Abstract

The National Fish Disease laboratory (NAFUS) of the University of Bern was set up in 1975 in the light of an epidemic of ulcerative dermal necrosis (UDN) and frequent cases of viral haemorrhagic septicaemia (VHS). In 1978 the diagnostic service was fully active. Since then the data from the diagnostic work of this unit were compiled in a database, which allows to give an overview on the occurrence of specified diseases over a 40 years period.

From 1978 to 2017 a total of over 16'200 cases were investigated at the NAFUS. Samples originated from private and governmental farms, free waters, hobby ponds and aquaria. Over 35% of samples consisted of rainbow trout, which corresponds to the importance of this species in the Swiss aquaculture, while over 15% of cases with brown trout reflect the status of this species as the most important wild fish.

Considering the frequency of diagnoses, parasites are in the first place followed by bacterial infections. While the demonstration of a parasitic infestation does not necessarily mean disease, the presence of bacteria is often the reason for problems particularly in farms, hobby ponds and aquaria. In less than 5% of all cases viruses were diagnosed. However, the impact of viral pathogens causing a notifiable disease is clearly more dramatic for the individual farmer than bacterial or parasitic diseases as stocks have to be culled.

When evaluating the number of cases with viral infections a clear decrease in VHS cases can be observed over the 30 year period. Cases of infectious pancreatic necrosis (IPN) a disease which is also notifiable in Switzerland showed a rather scattered occurrence with several peaks. The number of cases with infectious haematopoietic necrosis (IHN) stayed rather low after its first appearance in 1993. Other viral diseases detected in the diagnostic material were Koi herpes virus disease (KHVD), salmonid alpha virus infection, carp edema virus infection (CEV), cyprinid herpesvirus 2 infection and perchrhabdovirus infections, all in low frequencies.

Among bacterial diseases furunculosis, bacterial kidney disease (BKD) and enteric redmouth disease (ERM) can lead to considerable losses in salmonid aquaculture facilities. All of them have been found in Swiss fish farms. With the exception of ERM the number of cases slightly decreased over time. In contrast, infections by flavobacteria, both externally and systemically, are frequent findings and show an increase in numbers during the last years.

Among parasitic diseases major problems are linked with infections by *Ichthyobodo necator* and *Ichthyophthirius multifiliis* in fish farms and aquaria and by *Tetracapsuloides bryosalmonae*, the causative agent of proliferative kidney disease (PKD) in wild salmonids. While the number of Ich-disease is steadily decreasing, no trend can be seen for *Ichthyobodo* infections nor PKD.

Questions and comments:

Niels Jørgen Olesen: *"What about production and number of fish farms?"*

Thomas Wahli: *"Number of farms is relatively stable in total, few farms are closing and new ones open. A number of new farms open, these are RAS (mostly perch and pike perch)."*

Olga Haenen: *"What about CYHV-2 - which host?"*

Thomas Wahli: *"Goldfish in garden ponds."*

Hege Hellberg: *"Do you have details in the Francisella case in tilapia? Losses?"*

Thomas Wahli: *"Fish showed clinical signs, there were severe losses. We recommended treating but got no response from the farmer."*

Olga Haenen: *We have had one case of Francisella in tilapia with granuloma (we thought tuberculosis and nocardia) but we found Francisella through cooperation with US."*

Thomas Wahli: *"We had the same experience and confirmed Francisella through PCR at the bacteriology institute."*

Results of the 2nd VER Inter-laboratory Proficiency Test

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Abstract

Betanodaviruses, the causative agents of VER, are classified into four different species, based on the phylogenetic analysis of the RNA1 and RNA2 segments: the striped jack nervous necrosis virus (SJNNV), the tiger puffer nervous necrosis virus (TPNNV), the barfin flounder nervous necrosis virus (BFNNV) and the red-spotted grouper nervous necrosis virus (RGNNV). Additionally, due to the segmented nature of their genome, reassortment events amongst different betanodaviruses have been described. In the Mediterranean basin, the presence of the RGNNV and the SJNNV genotypes, as well as of the RGNNV/SJNNV and RGNNV/SJNNV reassortants has been extensively documented. Different betanodaviruses show diverse pathogenicity, host and temperature tropism. Therefore, the capability of detecting all viral species, as well as their correct identification is of utmost importance to provide accurate and reliable laboratory results.

In 2016, the first inter laboratory proficiency test (IPT) for VER molecular diagnostics was organized, with the purpose of assessing the capability of the laboratories working with this pathogen to detect a single genotype (RGNNV) by Real-Time PCR (rPCR) methods. The results of this first exercise were published in the EAFP bulletin (Toffan et al., Bull. Eur. Ass. Fish Pathol., 70, 37(2) 2017).

In 2017-2018 the 2nd VER IPT was organized within the framework of the MedAID European project. The panel comprised 10 vials containing different viral species, including reassortant strains, and different molecular methods (conventional and/or rPCR) could be used for viral detection. Viral species identification was also requested, although it was neither mandatory nor subjected to scoring. The participants were asked to fill a spreadsheet reporting the results, to provide the details related to the diagnostic protocol used to outline the lab's activities connected to VER diagnosis.

The panel was shipped to 32 laboratories located in 18 different countries, most of which European. However, only 29 laboratories out of 32 provided results within the deadline.

Of the 29 respondent laboratories, 15 obtained the maximum score. Ten laboratories produced a percentage of correct answers ranging between 70-90 %, and the remaining 4 laboratories produced less than 50% of correct results. Therefore, the average percentage of correct answers turned out to be 85.5% while the overall agreement (k) was 0.5387 (p = 0.0000).

Only 13 laboratories out of 29 (44,8%) were able to perform the complete and/or partial molecular characterization of the positive samples, and only 2 out of 13 obtained the maximum score.

Meaningful differences were observed among laboratories located in different geographic regions in their capacity of detecting betanodaviruses. Reassortant strains appeared to be the most challenging viruses to detect.

This work has received funding from the European Union's Horizon 2020 research and innovation programme MedAID under grant agreement No 727315

Questions and comments:

Nikolaj Reducha Andersen: *"You should consider using the median, not the mean, to look into correspondence of results."*

Niccoló Vendramin: *"Can you relate poor performance with diagnostic protocol."*

Anna Toffan: *"We don't have statistical support but we have a tendency".*

Real-time RT-PCR specific to Infectious Hematopoietic Necrosis Virus (IHNV): validation and implementation in EU and OIE manuals

M. Baud, A. Cuenca, T. Rundqvist, F. Almeras, L. Pallandre, J. Cabon, T. Morin and L. Louboutin

With the participation of the EURL from Denmark, Reference laboratories of Italy, Norway, Scotland, Germany, Czech Republic, and the Norwegian Veterinary Institute.

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Abstract

Specific viruses highly impacting fish health are regulated in the European Union (Directive 2006/88/EC) and submitted to a surveillance aimed to prevent viral dissemination. Official diagnostic methods used to detect these regulated pathogens were traditionally based on a cell culture phase followed by a characterization step. EU Decision 2015/1554 enriches the panel of official detection tools giving the possibility to use only real-time Reverse-Transcription Polymerase Chain Reaction (RT-qPCR) for detection. In this context, the qualitative RT-qPCR method specific to Infectious Hemorrhagic Necrosis virus (IHNV) developed by Purcell *et al.* (2013) was optimized by the European Union Reference Laboratory (EURL) and the French National Reference Laboratory (NRL)to allow a "one-step" assay starting from organs or culture supernatants.

A first PT was organized by the EURL for fish diseases, with eight participant laboratories: Norway, Germany, Italy, Denmark, Scotland, Croatia, France and Czech Republic. This PT was carried on using Whatman® FTA® cards. Representatives from all IHNV genotypes as well as for VHSV, IPN, Perch rhabdovirus and SAV were included. In general results were consistent among laboratories, but low sensitivity was detected when a different protocol than the provided was used to elute the FTA cards. In addition, contamination problems were also detected in one laboratory.

To obtain an accreditation, an additional PT was realized by the French National Reference lab with six voluntary European diagnostic laboratories. In addition to positive RT-qPCR controls, the French NRL decided to integrate an external exogenous control. In that way, the method included the addition in each sample of a RNA bacteriophage (MS2) in defined quantity, allowing the validation of the extraction step individually (Ninove *et al.* 2011). Analytical and diagnostic specificity and sensitivity were successfully assessed, as well as repeatability. To finalize the validation, interlaboratory proficiency test (PT) allowed evaluating the reproducibility of the method. The mean, standard deviation (SD) and coefficient of variation (CV) were calculated for IHNV and MS2 taking into account the apparatus, enzymes, extraction and PCR conditions used by participants. Excluding RotorGene results, which showed systematically earlier detection signals, CVs inferior to 10% were obtained for both targets.

Based on these results, our RT-qPCR method specific to IHNV seems to be highly reliable and will be rapidly broadcasted to French laboratories involved in the surveillance of this disease.

Questions and comments:

Anna Toffan. *"Will this method be included in the manual?"*

Niels Jørgen Olesen: *"Yes, they will be in the EU and OIE manual."*

Debes Christiansen: *"Why are you using external control instead of fish housekeeping gene?"*

Marine Baud: *"Because we receive very different fish species."*

Niels Lorenzen: *"What is the sensitivity compared to cell culture isolation?"*

Marine Baud: *"We did not compare exactly, very close."*

Argelia Cuenca. *"We did compare and found the same."*

Niels Jørgen Olsen: *"Do you have remarks on external/internal control or not - if they should be included in the manual?"*

Argelia Cuenca: *"I prefer internal control."*

Debes Christiansen: *"I agree".*

Anna Toffan: *"You will have additional costs."*

Argelia Cuenca: *"Without control you cannot compare Ct values, it is very difficult."*

Debes Christiansen: "*They keep track on the quality of the sample you have received."*

Argelia Cuenca: *"We include purification control."*

Viral hemorrhagic septicemia (VHS) and infectious hematopoietic necrosis (IHN): recent advance in molecular characterization

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Abstract

Viral haemorrhagic septicemia (VHS) is a notifiable fish disease in Europe. The aetiological agent is the viral haemorrhagic septicaemia virus (VHSV), a ssRNA virus from the family Rhabdoviridae. VHSV has been isolated from over 80 fish species in freshwater and marine environment in the northern hemisphere, and four main genotypes have been identified, which have a strong geographic differentiation and, at a lesser degree, some host specificity.

Molecular characterization of VHSV are mostly based on the sequence of the glycoprotein (G-gene), as this gene provides higher phylogenetic resolution, especially for sublineages within genotype I. In addition, it has been shown that other genetic regions provide the same overall genetic typing than the G-protein. Although more than 800 full G-gene sequences are public available to date, difficulties remain in stablishing the phylogenetic relationships among the different sublineages within genotype I.

In this study, we present the most comprehensive dataset of VHSV so far, including data from more than 100 full genome sequences. Most of these sequences were obtained in the frame of the NOVIMARK project, including colleges from CEFAS (England), ANSES (France), and IZSVe (Italy). Preliminary results indicate somehow disagreements among genes used to reconstruct the phylogeny, especially in the placement of genotypes Ia, Ib, Ic, and Iu. Indeed, when all data are analyzed together, only two phylogenetic relationships show high support: Ie as the first diverging clade within genotype I, and Ib and Id recovered as sister groups. A closer inspection indicates that phylogenetic incongruence is caused by few clades within the dataset.

In this presentation, we will show preliminary analyses of the VHSV full genome dataset, including potential areas of interest to further molecular characterization of VHSV isolates.

Questions and comments:

Niels Lorenzen: *"When you look at different phylogeny according to G, N and NV, there should be more pressure on the G protein, trying to investigate selective pressure."*

Argelia Cuenca: *"The paper in 2005 try to define which genes that have to be used to classify."*

Niels Lorenzen: *"You will have more resolution with the G gene."*

Argelia Cuenca: *"I agree, what is interesting is to look into discrepancy from the G gene and the others."*

Development of a vaccine against viral nervous necrosis (VNN)/viral encephalopathy and retinopathy (VER)

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Abstract

Infection with betanodavirus currently represents one of the major bottlenecks for the development of Mediterranean aquaculture. Betanodavirus causes the disease Viral Encephalopathy and Retinopathy (VER) which manifest in the nervous system of several marine cultured fish species, including sea bass (*Dicentrarchus labrax*). It causes clinical signs such as abnormal swimming pattern and high mortalities. The virus survives well in the environment and spreads easily making it difficult to prevent infection with biosecurity measures alone. A new experimental recombinant vaccine based on VLPs (Virus Like Particles) produced in yeast has been developed in the recently finalized EC project "TargetFish" (1). Initial vaccination trials suggest ability to induce protection against VER in sea bass and my PhD project focuses on further testing under both lab and field conditions along with analysis of the protective immune response. The work is part of the EC project MedAid (2) and will include collaboration with selected partners from the TargetFish consortium (3).

References:
1. http://targetfish.eu/
2. MedAID (Mediterranean Aquaculture Integrated Development) is a four-year project, funded by the European Union in the frame of Horizon 2020, grant agreement number 727315. The goal of MedAID is to increase the overall competitiveness and sustainability of the Mediterranean marine fish-farming sector, throughout the whole value chain http://www.medaid-h2020.eu/
3. Anna Toffan, Istituto Zooprofilattico Sperimentale delle Venezie *(Italy)*, Ansgar Stratmann, *W42 Industrial biotechnology GmbH (Germany)*, and others.

Questions and comments:

Anna Toffan: *"Which adjuvants did you use?"*

Sofie Hansen & Niels Lorenzen: *"We have two that need to be tested, we will try the purified particles and raw extract of pichia".*

Toni Erkinharju: *"Will you try cohabitation challenge? Infecting a mixed population of vaccinated and non-vaccinated fish?"*

Sofie Hansen: *"That is a good suggestion that should mimic farm conditions."*

Brit Hjeltnes: *"I understand the principle of injection to demonstrate protective immunity, but will you investigate other delivery system?"*

Sofie Hansen: *"Yes, to protect small fish we will try oral delivery and immersion delivery. Possibly also prime-boosting protocol."*

Brit Hjeltnes. *"Why would you like to use oral delivery? Have you seen success in that?*

Sofie Hansen: *"There is one publication which show promising results."*

Anna Toffan: *"Also in Targetfish we had promising results which need to be confirmed and repeated."*

Session IV, Part 2

Chair: Debes H. Christiansen

Update on Red Mark Syndrome (RMS)

Jacob G. Schmidt

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Abstract

Red Mark Syndrome (RMS) is an infectious skin disease of rainbow trout that has spread through Europe since around the turn of the millennium. The disease causes very little mortality, but due to the characteristic skin lesions results in downgrading and rejection of the affected fish. At DTU we have been working with RMS since early 2016, and have established a cohabitation model of infection with RMS, which we have used to learn more about the disease.
Results obtained during the first year of the project were presented at the 21st annual workshop. These included perception of RMS by fish farmers, description of disease progression, and compelling evidence that an MLO (*Midichloria*-like organism) is the causative agent of RMS. A short recap of these results will be presented, followed by results obtained since the last annual workshop.
These include an investigation of the presence of MLO in different tissues over the course of a cohabitation infection. All samples have not yet been processed, but preliminary results will be presented at the workshop. However, initial results indicate that MLO DNA cannot be detected in cohabitants until after at least three weeks, and that very little MLO DNA can be found in internal organs at any time.
We also looked into the immune response in RMS lesions, and found that all three isotypes of antibodies produced by rainbow trout were present in high amounts.

Questions and comments:

Torsten Boutrup: *"When you found the bacteria on the surface and not in the internal organs, did you check the gills? How did they look in comparison to the skin?"*

Jacob G. Schmidt: *"Very variable. I have not done all the samples, but out of the three samples for gills, some scores for gill were high while others were very low. I do not know why, because the scores for the skin were quite consistent."*

Olga Haenen: *"How did you culture the bacteria?"*

Jacob G. Schmidt: *"I did not, and nobody has been able to do that. The only way of having this disease model is by cohabitation and just continue in the fish."*

Olga Haenen: *"How are you going to do the test with the antibiotics?"*

Jacob G. Schmidt: *"During cohabitation experiments. I feed the fish with a preparation of feed with antibiotics. Later on, I see if the group that received antibiotics do not develop the symptoms further and test how much MLO is in the fish."*

Olga Haenen: *"Did you test RTG2 cells?"*

Jacob G. Schmidt: ***"****We tested different cell lines. We could do much more, but we have to decide where to put our effort because there are not many people working on this."*

Francois Lieffrig: *"Did you evaluate the impact of the temperature of the water on the disease?"*

Jacob G. Schmidt: *"We did not check that either. It was in the to-do list, but that experiment would take long time, so we ended up testing fewer parameters than we would have wanted, but it would be very interesting to do that. We did everything at 12 degrees."*

Niels Lorenzen: *"You mentioned that when the fish are transported, clinical signs appear after transportation. Did you try to mimic that stress and put some fish in your car and drive around?"*

Jacob G. Schmidt: *"I stress my fish on regular basis because I take them out of the water and photograph them regularly. I do not see any difference in the way that RMS is developing between my stocks and my experiments. Maybe it is a matter of the amount of oxygen during transportation."*

Toni Erkinharju: *"You mentioned that you had to scale down the immunohistochemistry for IgM, how much did you scale down? 10 fold?"*

Jacob G. Schmidt: *"You should ask Louise. She did those experiments."*

Louse von Gersdorff Jørgensen: *"We did not scale down in this particular case. We used lower concentration of anti-IgM in comparison with other studies."*

An update of Koi herpesvirus (KHV) and KHV disease (KHVD)

Sven M. Bergmann**, Sandro Klafack, Yeonhwa Jin, Lars Schröder and Walter Fuchs

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Abstract

Since the 1990th, KHV possess a threat for aquacultured common carp and koi (*Cyprinus carpio* L.) as well as their hybrids. Due to the nature and characteristics of this aquatic herpesvirus, the diagnosis can be very difficult if latency or persistence is happen in apparently healthy fish after e.g. a silent infection with a low virus concentration or from survivors weeks after a disease outbreak.

EU legislation allows to use molecular techniques like PCR (and nested PCR), qPCR and in-situ hybridization for the detection of the virus.

The focus for diagnostics are the qPCR (Gilad et al. 2004, Bergmann et al. 2010), the PCRs (Bercovier et al. 2005, with sequence analysis in the case of a primary outbreak or detection) and the PAN-CyHV PCR according to Engelsma et al. (2013) where a nested PCR and the sequence analysis of the amplified PCR product is required. The fragment of the PAN-CyHV or its nested PCR shall have a similarity to the three published full genome sequences according to Aoki et al. 2007 to 98%. There is no advice for the fragment size used for sequence comparison. As a rule between 280 and 300 bps are used for this purpose shown in the database. Additionally, for some enzyme kits it seems to be difficult to provide a clear, usable sequence due to the high content of G and C inside the gene fragment.

Over the last years, we developed and tested different types of vaccines to avoid losses of fish and finances for the farms and farmers. Beside inactivated viruses, we also used attenuated viruses but also genetically mutated viruses for vaccination. The results have shown that all vaccine preparations may help to reduce losses for the farmers.

Regarding the confirmative assay for the diagnosis of KHV, we have shown that KHV can be very active and change small or lager parts, especially in generation of its genome. From other herpesviruses, it is known that the enzymes involved in the replication are very conserved. At least for the fragment of the DNA polymerase (ORF 79) it seems not to be actually true. This may be also be valid for the other genome part (thymidine kinase, TK, ORF 55) used for diagnostics. To overcome those discrepancies, we always use the TK nested PCR (CEFAS) and proceed the sequence analysis.

Questions and comments:

Richard Paley: *"Agree with all of that, I definitely recommend sequencing the whole nested PCR product, even better to sequence multiple targets. I am fascinated about reversion, do you think that was evolution or are there mix isolates that have permissive replication at different temperatures?"*

Sven Bergmann: *"We have only cloned the KHVT to do the experiment. All the other European KHV we did not clone, but even for the KHVT there are heavy changes inside the full genome. I am sure that even for KHVT we have different variants inside."*

Richard Paley: *"Is there any particular cut off or it is progressing?"*

Sven Bergmann: *"I think it is progressing. We have 100 passages for all the viruses and we check them every 10 passages with a full genome sequencing. We compared them and we can see that there is development, it is kind of evolution."*

Anna Toffan: *"Do you have any experience testing minor cyprinid species? We have found many positive samples."*

Sven Bergmann: *"From Italy, in the database, there are some fragments with 300 bp sequences. We have done this with 17 other fish species and this is also happening over there."*

Isolation of salmonid alphavirus (SAV6) from wild caught ballan wrasse, *Labrus bergylta* (Ascanius) in Ireland

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Abstract

The use of cleaner fish as a biological control for sea lice in Atlantic salmon aquaculture has increased in recent years. Wild caught wrasse are commonly used in Ireland as cleaner fish and samples of wrasse from each fishing area are screened for potential pathogens prior to their deployment into sea cages. In this study we describe the isolation and genetic characterisation of salmonid alphavirus from ballan wrasse. The virus was isolated from a pooled sample of five ballan wrasse, showing no signs of disease, caught off the coast of Donegal in the north-west of Ireland. Partial sequencing of the E2 and nsP3 genes showed that it was closely related to SAV subtype 6. This represents only the second isolation of this subtype and the first from a wrasse species. Screening of wild wrasse is recommended in order to reduce the potential risk of pathogen transfer between cleaner fish and farmed salmon.

Questions and comments:

Brit Hjeltnes: *"In this wrasse, did you detect any pathology?"*

Neil Ruane: *"No, we noticed that the fish seemed to have many sea lice."*

Brit Hjeltnes: *"What about the virus load in the fish?"*

Neil Ruane: *"We put it in cell culture, it was a pool of 5 fish."*

Brit Hjeltnes: *"It could be very low."*

Neil Ruane: *"Yes, most likely. We could not see CPE until day 14 and they were passed on day 7."*

Brit Hjeltnes: *"Could it just have been vector contamination?"*

Neil Ruane: *"It could be. I do not think that those fish had pancreas disease, they were maybe asymptomatic carriers of the virus. If we had found SAV1 for example, we may think that we picked up the virus from a salmon farm, but because the virus is so different, I do not know what it is doing in the wrasse."*

Brit Hjeltnes: *"It seems that the virus load is very low. If you would go for a screening would you think you would be able to pick up carriers?"*

Neil Ruane: *"Yes, we did by cell culture. However, individual heart samples from the wrasse were also tested by qRT-PCR and they were negatives."*

Debes H. Christiansen: *"What was the decision with these wrasses?"*

Neil Ruane: *"These fish are caught from the wild. The fishermen catch 60 fish and bring them into a small holding pen where we come and sample them. Two weeks later, they get the report. If everything is fine,*

they can use the fish. In this case, they decided not to use them. We did go back months later and sample 60 more fish and we did not find anything. This year we have not detected anything so far."

Niels Lorenzen: *"Is this isolated virus infectious to salmon?"*

Neil Ruane: *"We do not know. There has been only one or two studies that infected salmon with all six subtypes. All of them resulted in PD. The reports suggest that the SAV6 isolates seem to be less severe with a lower progression and the scores of the lesions were lower."*

Edda Bjork Hafstao Armannsdóttir: *"Did you continue passaging the virus?"*

Neil Ruane: *"Yes, It grew very well."*

Toni Erkinharju: *"Do you think that this SAV subtype is restricted to Ballan wrasse or could it be found in another species of wrasse?"*

Neil Ruane: *"Yes, why not, it should be found in other species. We have detected SAV1 in flatfish by qRT-PCR."*

Recent research from Australia and new master programme in tropical aquaculture

Ellen Ariel[1,2], Dean Jerry[2,3], Kelly Condon[1,2,3]

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Abstract

Australia has a strong global brand for the production of high quality aquaculture products. With the global demand for seafood products forecast to expand, Australian aquaculture industries are well positioned for economic growth. The major aquaculture species by value of production in Australia are the cold-water species including salmon, blue fin tuna, edible oysters and the warm water tropical species of pearl oysters and prawns. Additional smaller industries continue to emerge including abalone, yellowtail kingfish and barramundi. Since 2002-03 the gross value of aquaculture production in Australia has increased by 12 per cent to over $1 Billion.

However, since 2015 the culture of four of the five highest value industries have been impacted by the emergence of new diseases or the incursion of exotic pathogens. Whilst the majority of the diseases are caused by viral pathogens, bacterial and parasitic infestations are of significant concern to some industries. Government and industry has responded to the emerging threat of infectious diseases through the establishment of improved biosecurity training and increased focus on disease in research funding.

As part of this response, James Cook University is establishing a Masters degree in Aquatic Animal Health. The rolling out of the degree will be stepwise, with a Graduate Certificate offered in 2019 and a Graduate Diploma and Masters in 2020. Most of the subjects will be offered in intensive 2-week block mode units to enable industry and other stakeholders to “plug and play” with subjects even if students are not intending to study the full degree.

The over-arching principles to achieve sustainable aquaculture will be addressed in both theory and a great proportion with hands-on practical aspects of diagnosis of viruses, bacteria, fungus and parasites of concern to tropical aquaculture. Special requests from stakeholders for a focus on resistance testing and phage therapy as well as both molecular and traditional diagnostic methods and epidemiology have been incorporated into the program. In the later phase of the degree two streams will be offered: *Laboratory Management* with industry placements in accredited laboratories and *Research Methods* with planning, designing and carrying out a minor project of 6 months duration. A final capstone subject on biosecurity, food safety and One Health will pull all the aspects of the degree together in a universal context.

An overview of the Australian aquaculture industry and the pathogens of major economic impact will be discussed and an outline of the new Masters degree in Aquatic Animal Health will be presented.

Questions and comments:

Niels Jørgen Olesen: *"This finding of orthomyxovirus in Tasmania is in fact very close to ISA virus and ISA situation. Why has it never been published and why has the OIE never been notified? It seems that it is a very serious disease as ISA."*

Ellen Ariel: *"Maybe it is because it is not listed."*

Debes H. Christiansen: *"It is also because the farmers are not cooperating."*

Niels Jørgen Olesen: *"Orthomyxoviruses will be an issue in the future and we have to be more aware of this. For instance ISAV is the only recognized orthomyxovirus in fish culture."*

Niels Lorenzen: *"Are you planning to do vaccination against that virus?"*

Ellen Ariel: *"Some yes, they are working on that now in Tasmania. Each company has their own scheme. The governmental laboratories are trying to work on that as well. The farmers are not going to share the information if they find something, because it is going to be an economic advantage."*

Niels Lorenzen: *"In your study plan, it would be good to include a vaccine line. About the bacteriophages that you use to control the pathogenic bacteria. Do you put into the water, are you allowed to do that?"*

Ellen Ariel: *"Yes, it is a probiotics in Australia. We take water sample every day. If one type of bacteria is increasing, we make a cocktail in the lab that controls that specific bacterium. It is possible in the egg stage."*

Epidemiological aspects of the infectious hematopoietic necrosis (IHN) outbreak in Finland

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Abstract

The structure of the Finnish fish farming industry has protected the farms from the most serious diseases in the past. The juvenile production for the on-growing in the sea area is almost entirely situated in the inland area allowing one-way movement and there are strict limitations of moving any live fish or eggs from the sea to the inland area. However, some contagious diseases like IPN virus and bacterial kidney disease appeared also in the continental farms, most probably originating from the sea area.

When IHN virus was detected in a cage farm in the Bothnian bay, the first suspicion of the route of infection was through vector-born transfer or migrating salmon. Quite soon the origin of the sea farm infection was tracked to a state owned continental fish farm selling juveniles widely for on-growing and stocking for fishing ponds and angling sites. In the following sampling effort of all the contacts, a few infected ponds were detected. However, due to the winter period and the small size of most of the contacts not all sites could be sampled yet. Especially the stocking in the wild for the purpose of angling presents a challenge for the sampling.

An additional problem is the definition of the containment areas. Initially the whole water catchment area of the continental farm (36 930 km^2) was set under a restriction. However, this area comprises nine sub-catchments and about a hundred divisions and form one of the main juvenile production areas in Finland. After initial control and sampling of the farms in this area, compartmentalization was applied and the containment zone restricted to the sub-catchment area of the infected farm. The same principle was applied to the following detections of the IHN infection, which were small fishing ponds, two of them in another main water catchment area.

In spite of tedious searching for the entry route of the virus into the continental farm, the origin of the infection could not be verified. Taking in the account the type of the virus, most probably there has been an unknown contact with Russia. There is some export of live fish to Russia, but not from this farm. No connection could be found with the transport vehicles that are used to export fish. Near the farm is an angling site and pond stocked from the farm and frequented by Russian fishing tourists. Failing biosecurity with this connection remains an open question.

The emptying and disinfection of the continental farm, two affected sea cage farms and infected put and take ponds has been a huge effort, including the slaughter and disposal of about 230 t fish. The sampling of farms now considered in high risk will be intensified, while the sampling of the wild fish in ponds and fishing sites that received fish from the continental farm will continue. The surveillance program for regaining the IHN free status for the whole country is planned.

Questions and comments:

Niels Lorenzen: *"Is it correct that you did not see any clinical signs at all?"*

Satu Viljamaa-Dirks: *"Yes, it is interesting because it is a farm. Probably the infection arrived in May. We had several inspections during the summer but there were no positive results for IHNV there. Probably during the summer or in areas outside with the big fish, which we did not test. Afterward, there were elevated mortalities but not clinical signs."*

Niels Lorenzen: "Have you tested the virulence of this isolated in the experimental condition?"

Satu Viljamaa-Dirks: *"Yes, It was sent to DTU. One point is that there could be a high variability in fish species."*

Niels Lorenzen: *"It would be very interesting since different genotypes of IHNV has a high variability in different fish species."*

Niels Jørgen Olesen: *"We received these isolates to test for virulence. Seems there is not a major difference between these isolates and the positive controls. It seems to be a virulent virus.*

SESSION V: Update from the EURL

Chair: Niels Jørgen Olesen

Results of the proficiency test, PT1 and PT2

Niccoló Vendramin[1], Teena Vendel Klinge, Argelia Cuenca and Niels Jørgen Olesen

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Abstract

A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).

PT1 was designed to primarily assess the identification of the fish viruses causing the notifiable diseases: viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), and epizootic haematopoietic necrosis virus (EHNV) or related rana-viruses and in addition the fish pathogenic viruses: other fish rhabdoviruses as pike fry rhabdovirus (PFR),spring viraemia of carp virus (SVCV) and infectious pancreatic necrosis virus (IPNV) by cell culture based methods. PT2 was designed for assessing the ability of participating laboratories to identify the fish pathogens: infectious salmon anaemia virus (ISAV), salmon alphavirus (SAV) and cyprinid herpesvirus 3 (CyHV-3) (otherwise known as koi herpes virus – KHV) by biomolecular methods (PCR based). Also in 2017 Salmonid Alphavirus (SAV) was included in the panel of pathogens to be investigated should include Since SAV is not a listed disease in the European legislation, testing for SAV was done on voluntarily base. The EURL would then take care of calculating the score accordingly.

45 laboratories participated in PT1 while 43 participated in PT2.

The tests were sent from the EURL in the end of September 2017.

Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043. This report covers both the results of PT1 and PT2. Participants were asked to identify the content of each ampoule by the methods used in their laboratory which should be according to the procedures described in Commission Decision 2015-1554.

Participants were asked to download an excel sheet from the EURL web site (http://www.eurl-fish.eu/) to be used for reporting results and to be submitted to the EURL electronically. Additionally, participants were requested to answer a questionnaire regarding the accreditation status of their laboratory.

The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).

45 laboratories participated in PT1 while 44 participated in PT2.

Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043.

Each laboratory was given a code number to ensure discretion. The code number of each participant is supplied to the respective laboratories with this report. Furthermore, the providers of the proficiency test have included comments to the participants if relevant. An uncoded version of the report is sent to the European Commission.

ésumé and concluding remarks PT1

40% of parcels were delivered by the shipping companies within 1 day after submission, 80% was delivered within 1 week and 91% was delivered within 2 week. The remaining four parcels took longer for delivery primarily due to border controls, the maximum time of shipment was 59 days.

This year ECV was included in the Proficiency test. 3 out of the 45 countries do not test for Ranavirus. 38 participants provided the correct identification, 2 laboratory identified correctly the isolate as ECV by sequencing but submitted the result as EHNV and to laboratories did no sequencing.
The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they perform in relation to the other participants. It is up to the individual laboratory to assess if they perform according to their own expectations and standards. We will also take the opportunity to provide a comment to participants regarding submitted results if relevant. Furthermore we encourage all participants to contacts us with any questions concerning the test or any other diagnostic matters.
Overall 39 out of 45 participants scored 100% success rate and 5 participants scored 90% due to sequencing of the content in ampoule IV (ECV) or contamination of ampoule contents. These points will be assessed directly with the single participants that have underperformed.

Résumé and concluding remarks PT2

The EURL have decided to include SAV in the panel of viruses included in PT2 since this was regarded as a proper initiative that strengthen the diagnostic capacities of the NRLs in detecting emerging pathogens.
44 laboratories participated in PT2.
39 laboratories tested for SAV and 37 correctly identified the virus in Ampoule VI, 1 laboratory seems to have switch two of the ampoules and 1 laboratory answered ‘negative’.
41 out of the 44 laboratories correctly identified the ISA virus in ampoule VII. 1 did not test for ISAV and 2 laboratories seem to have switched two of the ampoules.
43 laboratories correctly identified the KHV in ampoule IX and 1 did not test for KHV.
It has been a concern that two laboratories haves identified the correct virus but not in the right ampoule, meaning that some mistake in traceability of the ampoules during the working flow procedure has occurred. These points will be assessed directly with the single participants that have underperformed.
It is an appreciated matter of fact that many laboratories are putting efforts in performing genetic characterization of the isolates through sequence analysis, as it is important that laboratories can discriminate between isolates as e.g. pathogenic ISAV strains with deletions in the HPR region and HPR0 strains, especially after the delisting of ISAV HPR0 (Commission Implementing Directive 2014/22/EU).
From 2018 more focus and acknowledgement of the sequencing work conducted by the participants will be given. The EURL proposes to provide a separate scoring system for the genotyping results, which will be attached to the annexes which display the genotyping results provided by all participants.

Questions and comments:

No comments

EURL training courses for 2018

Nikolaj Reducha Andersen and Tine Moesgaard Iburg

EURL Fish Diseases, National Institute of Aquatic Resources, Technical University of Denmark, Denmark

ngaan@aqua.dtu.dk and timi@aqua.dtu.dk

Abstract

In 2018, the EURL for fish diseases will organize two training courses.

The courses available are:

- **Methods for implementation of surveillance procedures for listed fish diseases**
  The course will be held in week 41 from Monday the 8th to Friday the 12th of October

- **Introduction to histopathology in fish and crustacean diseases**
  The course will be held in week 42 from Monday the 15th to Friday the 19th of October

The content of the training courses and the procedure to register will be described.

More information are available on the EURL website

www.eurl-fish.eu

Questions and comments:

Olga Haenen suggested a course on diseases for specific fish species, e.g. eel diseases, pike perch diseases, perch diseases.

Ewa Lewisch suggested a course in bacterial diseases, maybe more under the subject emerging bacterial diseases. Niels Jørgen Olesen answered that courses will have to focus on notifiable as well as emerging diseases, as this is what EU wants the EURL to pay attention to when it comes to topics for courses. It also has to be remembered that the EURL only has a budget within the field that covers up to two courses for a total of 15 people.

Olga Haenen asked about courses on diseases of zebrafish. Niels Jørgen Olesen answered that a course within this subject would be classified as ”borderline” for the EURL to set up within their function as EURL and with the current working programme.

Niels Jørgen Olesen suggested a course in serology as a potential future course within the framework.

EURL activities in 2017

Niels Jørgen Olesen

EU Reference Laboratory for Fish Diseases, DTU

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Abstract

The National Veterinary Institute, Technical University of Denmark (DTU Vet) is appointed as the European Union Reference Laboratory (EURL) for Fish Diseases, in accordance with the Commission Implementing Regulation (EU) No 135/2013 of 18 February 2013, the notification of grant decision for an action regarding the EURL for Fish Diseases – SI2.725290 and the corresponding grand decision (Ref. Ares(2016)854560 - 18/02/2016) as regards the Union financial aid for the year 2016 and 2017 to the EURL for Fish Diseases.

The duties of the EURL are described in Council Directive 2006/88/EC of 24 October 2006 (Annex VI). The duties mainly concern fish diseases listed as exotic diseases: epizootic haematopoietic necrosis (EHN) and fish diseases listed as non-exotic diseases: infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN) and koi herpes virus disease (KHVD). This report follows the format of the work programme adopted for the EURL for 2016 and 2017, describing activities and sub-activities and the status of on-going projects. The list of functions and duties of the EURL follows this introduction.

In 2017, the National Veterinary Institute moved from central Copenhagen, where it has been placed for more than 100 years, to a new building at DTU Campus in Kgs. Lyngby, 15 km north of the capital. Laboratories and tank facilities, specifically designed for research and surveillance of fish- and shellfish diseases, were designed and built. This new environment, placed door to door with the National Institute of Aquatic Resources (DTU Aqua), gives us new opportunities for collaborations and access to up to date laboratories, experimental facilities, and training and meeting facilities. Significant resources were allocated to this transfer and this is to some extend reflected in this report. We have chosen, however, to give it priority in order to ensure a strong basis for our future activities within the EURL.

The 21st Annual Meeting of the National Reference Laboratories (NRLs) for Fish Diseases was held in Kgs. Lyngby, Denmark, May 30th – May 31st, at the premises of the Veterinary Institute. A total of 63 participants from 35 countries attended over the two days period. There were five sessions with a total of 28 presentations, three of which were given by invited speakers, a working group session and a round table discussion.

Again this year, an inter-laboratory proficiency test was distributed to the NRLs mainly within the EU, however, there were also participants from countries outside EU. The proficiency test consisted of two tests, PT1 and PT2. The PT1 was designed to primarily assess the ability of participating laboratories to identify VHSV, IHNV and EHNV. The PT2 was developed in order to test the proficiency of participating laboratories to identify ISA and KHV. Again in 2017, the identification of SAV was included in PT2 on a voluntary base. The proficiency test is covering all five listed exotic and non-exotic fish diseases. FortyfiveNRLs participated in the proficiency test. A report was submitted medio March 2017. The majority of the laboratories performed well.

An important focus of the EURL is to update the standard operating procedures of the non-exotic and exotic listed diseases. Diagnostic manuals for VHS, IHN, ISA, KHV and EHN are available at the EURL website.

Another important focus area was the development, implementation and validation of diagnostic tools for identification of the listed and emerging diseases and their accreditation. One outcome of these efforts was the publication and acceptance of a new validated conventional RT-PCR for detection of VHSV. Resources were also used to optimize and implement a real-time RT-PCR for detection of PRV-3; an emerging disease in European aquaculture.

During 2017, resources were again used to collate data on surveillance, health categorisation and diagnostics in EU; to identify and characterise selected virus isolates; to type, store and update a library of listed virus isolates; to develop, update and maintain the database containing information on fish pathogens (www.fishpathogens.eu); to supply reference materials to NRLs; to provide training courses in laboratory diagnosis; to update the EURL website (www.eurl-fish.eu) and finally to attend international meetings and conferences.

In 2017, Dr. Nikolaj Reducha Andersen took the responsibility as the Coordinator of the EURL – taking the tasks of organising workshop and training courses, updating our website, conducting in-vivo viral characterisations and strengthens our statistical capabilities. DVM Niccolò Vendramin, during his two year sabbatical leave from the EURL, kept the responsibility of planning, shipping and reporting the proficiency tests and the questionnaires on fish health status in Europe.

The National Veterinary Institute, Technical University of Denmark (DTU Vet) is appointed as the European Union Reference Laboratory (EURL) for Fish Diseases, in accordance with the Commission Implementing Regulation (EU) No 135/2013 of 18 February 2013, the notification of grant decision for an action regarding the EURL for Fish Diseases – SI2.725290 and the corresponding grand decision (Ref. Ares(2016)854560 - 18/02/2016) as regards the Union financial aid for the year 2016 and 2017 to the EURL for Fish Diseases.

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Questions and comments:

Niels Jørgen Olesen suggested 12-14 June 2019 as potential dates for the AW next year.

Olga Haenen asked which laboratories within EU that are doing tilapia lake virus diagnostics? There were no positive answers.

Niels Jørgen Olesen suggested that in connection with new diseases that repository material is send to the EURL.

Work programme of EURL for Fish Diseases

Niels Jørgen Olesen, Nikolaj R. Andersen
EURL Fish, DTU
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DTU-Vet has since 1994 been designated as the EU reference laboratory for fish diseases, and was last approved as such by Grant decision for an action regarding the EU Reference Laboratory for Fish Diseases - SI2.725290 of 18/02/2016 (Ref. Ares(2016)854560). The following proposal for a Work Programme 2018 has been developed based on the function and duties of the Community reference laboratories given in council directive 2006/88/EC annex VI, PART I.

1 TO ENSURE AVAILABILITY AND USE OF HIGH QUALITY METHODS AND TO ENSURE HIGH QUALITY PERFORMANCE BY NRLs

Sub-activity 1.1 ***(Annual workshop)***

Objectives: To ensure knowledge dissemination and sharing between the Member State NRLs on existing and emerging fish diseases and to agree on the future priorities of the EURL, by holding the 22nd annual workshop of the National Reference Laboratories (NRLs) for fish diseases in 2018.
Description: These workshops are organized as annual events and all Member State NRLs are strongly recommended to participate in them as it is an important opportunity to get updated on the newest scientific knowledge of fish pathogens, diagnostics, legislation, etc. Several talks of high scientific standard will be given and discussions at group and plenum level will be facilitated during the two days of the workshop.
Expected Output: Successful preparation and completion of the 22nd annual workshop comprising 2 full days (May 30th and 31st 2018). A technical and financial report of the workshop produced. The technical report will contain abstracts and minutes from all presentations and discussions and will after acceptance be made publicly available through the EURL website.
Duration: The workshop is to be held ultimo May 2018. Preparation in February – April and finalizing of the reports in May – August.

Sub-activity 1.2 ***(Scientific working group)***

Objectives: To ensure that all EU Member States can rely on consultancy from international experts to a broad range of problems that existing or emerging fish disease may be causing in Europe.
Description: In case of critical fish disease related problems within EU Member States, we will organize specific scientific meetings by collating international experts.
Expected Output: Two-three day scientific meeting in Denmark. A scientific report including recommendations will be delivered to the relevant Member State NRL and the European Commission and will be available on our website www.eurl-fish.eu.
Duration: 2-3 day meeting in Copenhagen in 2018 and time for organising and reporting.

Sub-activity 1.3 ***(Proficiency test)***

Objectives: To assess the capabilities of all Member State NRLs to detect fish disease causing pathogens and to harmonize the procedures used by an inter-laboratory proficiency test.
Description: The EURL is going to prepare the Annual Inter-laboratory Proficiency Tests for 2018 for the NRLs. The tests will include the viral fish disease pathogens viral haemorrhagic septicaemia virus (VHSV), infectious haematopoietic necrosis virus (IHNV), epizootic haematopoietic necrosis virus (EHNV), infectious salmon anemia virus (ISAV) and koi herpes virus (KHV), and will also address other common viral pathogens in fish farming (infectious pancreatic necrosis virus (IPNV), spring viraemia of carp virus (SVCV), salmonid alphavirus (SAV), Ranaviruses, etc.). The participation is mandatory for all NRLs in EU. After submission of test results from the NRLs to the EURL, we will collate and analyse information gained from the proficiency test and publish the anonymous data to all participants as a report on our website www.eurl-fish.eu. A non-coded version will be provided to the EU Commission with information on performances and under performances. The results will be presented and discussed at the Annual Workshop 2019. The tests are accredited according to ISO 17043 and are indispensable for maintaining accreditations at the NRLs.
Expected Output: Preparation and shipping the test and subsequently provide a report on the proficiency test 2018.
Duration: January – December. The samples included in the test will be shipped from the EURL in the fall of 2018 and the final report will be submitted February 2019.

Sub-activity 1.4 ***(Novel molecular methods)***

Objectives: For the EURL to have molecular diagnostic methods of the highest scientific standards and to be able to provide these methods to all Member State NRLs.
Description: Novel molecular methods are highly sensitive and specific tools for diagnosis and surveillance of a number of listed pathogens. The EURL will assess and standardize real-time PCR, fluidigm, multiplex (PCR and ELISA) tests for the diagnosis, identification and typing of emerging and the listed non-exotic and exotic fish diseases, e.g. a diagnostic package comprising relevant pathogens for the respective fish species like VHSV, IHNV, ISAV, EHNV, PRV3, IPNV, SAV2, R. salmoninarum (BKD) for rainbow trout VHS, IHN, ISA, SAV1-6, PMCV, PRV1, Pox virus for Atlantic salmon,
Expected Output: Diagnostic molecular methods that are validated according to the recommendations given by the OIE to be used by the relevant Member State NRLs.
Duration: January - December

2 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

Sub-activity 2.1 ***(Training)***

Objectives: To ensure that employees of the Member State NRLs have the highest scientific and excellent skills in diagnosis of fish diseases.
Description: The EURL yearly provides two training courses in methods used for diagnosis of fish diseases. These courses are primarily offered to participants of the Member State NRLs. The content is mainly based on the opinion of the EURL on what is required in the Member State NRLs, e.g. in 2017 we emphasised the

training of the NRLs in blast results reading to be included in the course on diagnostic procedures. However, the course contents are also discussed during the annual workshop, where the Member State NRLs are able to provide specific input.
Expected Output: Two training courses of 3-5 days, with 8-10 participants in each course.
Duration: September – October.

Sub-activity 2.2 ***(Webpage)***

Objectives: To provide the Member State NRLs with a fast entrance to information from the EURL.
Description: The EURL are administrating the webpage, www.eurl-fish.eu, by uploading relevant material such as updated lists of NRLs, annual workshop presentations, training course reports, sampling and diagnostic procedures, newest update on legislation, general news from the community, etc. The website has daily visitors from a great number of countries from around the world and are, therefore, a substantial part of disseminating the work of the EURL for fish diseases.
Expected Output: A constantly updated webpage for the Member State NRLs.
Duration: January - December

Sub-activity 2.3 ***(FishRefLabNet)***

Objectives: To ensure that relevant and important information rapidly can get from the EURL directly to the Member State NRLs.
Description: FishRefLabNet is a mailing list which all interested in the work of the EURL can subscribe to. The EURL use the mailing list for important notifications i.e. meeting calls, training course calls and other relevant information such as information on upcoming conferences, new research findings and relevant reports and publications, emergency situations etc. Often the notifications will include links to the Webpage or other sites for further and detailed information. All Member State NRLs have subscribed to the list, plus a number of fish disease scientists both within and outside Europe.
Expected Output: The EURL usually prepare and submit around 5-10 notifications per year via FishRefLabNet to ca. 80 subscribers.
Duration: January – December.

Sub-activity 2.4 ***(Molecular epidemiology)***

Objectives: To improve knowledge on disease spreading mechanisms within the EU.
Description: To prevent diseases from spreading within the EU, it is important for the EURL to have excellent knowledge on the spreading mechanisms and the actual spreading patterns of the listed and emerging viral fish diseases. A study involving highly important isolates and disease outbreaks, in EU will be conducted. As this/these pathogen(s) are also found outside the EU and are putative threats to EU, data from non-EU countries will also be included in the study. Data from these studies are expected to provide better understanding of fish diseases spreading mechanisms within the EU Member States
Expected Output: Reports, publications and oral presentations generated. from these studies will be given.
Duration: January - December

Sub-activity 2.5 ***(Producing virtual teaching material (e-learning))***

Objectives: To provide the Member State NRLs with “hands on” videos to be used for teaching of staff members.
Description: The training courses offered by the EURL are only available for participation one time per year, generally during the fall and for a limited number of participants. A set of e-learning videos will provide fast and effective learning for NRLs employees when needed.
Expected Output: A series of English instructional videos for conducting proficiency test, fish sampling and other laboratory techniques related to the EURL.
Duration: January - December. The videos will be available via our website during November – December.

Sub-activity 2.6 ***(Missions)***

Objectives: To ensure a high standard of diagnostic capabilities of all Member State NRLs.
Description: Member State countries chosen for a mission are primarily based on performance in the yearly proficiency test. However, if missions to other countries, both EU Member States but also 3rd countries, will be able to provide important scientific knowledge for the EURL to pass on to Member State NRLs, missions to such countries will be conducted. This will ensure EU Member States to be updated with excellent scientific skills and knowledge.
Expected Output: As the decision for appointing target laboratories for missions is based on performances of the proficiency test- no final decision can be taken at this stage- but a preliminary indication would point at 2 missions with one mission to the NRL of France (due to their plan for final eradication of VHS and IHN from France) and one mission to Portugal.
Duration: April and/or November

Sub-activity 2.7 ***(International meetings)***

Objectives: To keep the EURL updated on the newest scientific information on emerging and listed exotic and non-exotic fish diseases, and to disseminate knowledge and scientific data provided by the EURL.
Description: The EURL staffs is able to provide consultancy to Member State NRLs on emerging and listed fish diseases, and attending conferences are an important way of the EURL to keep the excellence of this function. Conference participation therefore ensures up-to-date knowledge within the EURL.
Expected Output: The EURL expect to participate in 2 to 3 international conferences e.g. Eighth International Symposium on Aquatic Animal Health, Prince Edward Island, 2-6 September 2018. https://isaah2018.com/. EAFP UK & Ireland Branches Third Meeting ''Connecting Academia with Industry for improved Aquatic Animal Health" Galway 11-12 September 2018.
Duration: January – December

3 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO THE EUROPEAN COMMISSION AND OTHER ORGANISATIONS

Sub-activity 3.1 (***Diagnostic manuals***)

Objectives: To have updated diagnostic manuals for all listed fish diseases available for Member State NRLs on the EURL website www.eurl-fish.eu.
Description: The diagnostic manual for sampling and detection of listed non-exotic diseases was finally adopted in 2015. But as the diagnostic procedures for identification and surveillance of the listed diseases is rapidly evolving new procedures will be assessed and validated for inclusion in the first revision of the diagnostic manuals.
Expected Output: Updated sampling and diagnostic manual for the viral fish diseases viral haemorrhagic septicaemia (VHS), infectious hematopoietic necrosis (IHN), infectious salmon anaemia (ISA), koi herpes virus (KHV) and epizootic haematopoietic necrosis (EHN) on the EURL website.
Duration: January - December

Sub-activity 3.2 ***(Survey and diagnosis)***

Objectives: As part of our duties given in given in C.D. 2006/88/EC Annex VI, Part I.5 (f) to "collate and forward information on exotic and endemic diseases, that are potentially emerging in Community aquaculture" data on emerging and endemic fish diseases and fish health surveillance in Europe will be collated in order to ensure that the EU Commission, the Member State NRLs and the EU in general are

updated on the fish diseases situation in aquaculture and natural fish populations in Europe. Description: The EURL collect data on emerging and endemic fish diseases outbreaks from NRLs in all European countries by submitting a questionnaire in January 2018 and disseminating the information gathered in a report and at the 2018 annual workshop. The data are collated in a "Survey and diagnosis" report, which is made available for the Commission, Member State NRLs and for public use on our website. This report includes information on the presence of all the listed non-exotic fish diseases given in Council Directive 2006/88/EC Annex IV Part 2, on emerging diseases, and on all surveillance programmes on fish diseases conducted in EU. This is the only comprehensive overview on fish diseases and fish health management in Europe that is published.
Expected Output: A report on "Surveillance and diagnosis of fish diseases in Europe 2017", will be published on the EURL website www.eurl-fish.eu and presented at the annual workshop in 2018.
Duration: January – June.

Sub-activity 3.3 ***(Emerging diseases)***

Objectives: For the EURL to have the most updated and highest scientific knowledge of emerging and re-emerging fish diseases in Europa.
Description: Due to increased international trade of fish, focus will be given to emerging diseases and rapid response to Member State NRLs and EU in case of outbreaks. An assessment of risk for contracting and spreading specific emerging and re-emerging diseases in EU will be conducted in 2018. In collaboration with specialised experts the EURL foresee to work e.g. with the emerging viral fish pathogens piscine orthoreovirus3 (PRV3) in rainbow trout in Europe to be able to assess their potential listing as exotic or non-exotic diseases in the future. Diseases like
Expected Output: The EURL will have relevant and updated scientific knowledge on emerging fish diseases in EU and be able to provide immediately consultancy to all Member State NRLs and the European Commission and stakeholders. Scientific knowledge on specific emerging diseases like PRV3 will be disseminated through oral and written presentations in scientific journals, at Annual workshops, conferences etc.
Duration: January - December

4 REAGENTS AND REFERENCE COLLECTIONS

Sub-activity 4.1 ***(Pathogen library)***

Objectives: For the EURL to have an updated library of fish pathogens relevant for the EURL and Member State NRLs.
Description: The EURL are going to update and maintain a library of isolates of the viral fish pathogens infectious salmon anaemia virus (ISAV), viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), koi herpes virus (KHV), enzootic hematopoietic necrosis virus (EHNV) and other relevant putative emerging fish pathogens.
Expected Output: The library will be updated with around 10 to 20 pathogen isolates in 2018.
Duration: January - December

Sub-activity 4.2 ***(Pathogen characterization)***

Objectives: For the EURL to be able to identify and characterize isolates of listed viral fish pathogens on request from the Member State NRLs.

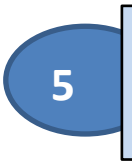
Description: The EURL every year receive strains of fish pathogens for corroboration of diagnostic results in the EU Member States. Regularly these strains must be characterized properly as an emergency response to avoid unwanted spreading of new pathogens in EU. The EURL describe theses strains by pathogenicity testing in-vivo and in-vitro and by serological and genetic characterization.
Expected Output: The EURL expect to describe and characterize around 5-10 fish pathogen strains in 2018.
Duration: January - December

Sub-activity 4.3 ***(www.fishpathogens.eu)***

Objectives: To have an updated database of all serious viral fish pathogens in the EU.
Description: The database www.fishpathogens.eu is a valuable tool for all Member State NRLs for virus characterisation and molecular epidemiology of listed and non-listed fish pathogens viruses. The more isolates included the stronger the tool for the EURL and Member State NRLs.
Expected Output: The EURL expect to update the database with salmonid alphavirus (SAV) in 2018, and to expand the existing database with more isolates (30-50 isolates).
Duration: January - December

Sub-activity 4.4 ***(Production and supply of reagents)***

Objectives: For the EURL to be able to quickly provide Member State NRLs with diagnostic reagents.
Description: Diagnostic reagents (i.e. polyclonal antibodies raised in rabbit, monoclonal antibodies from stored hybridoma cells or in situ hybridization (ISH probes) will be produced according to demand form the Member State NRLs.
Expected Output: The EURL expect request of diagnostic reagents from around 15 Member State NRLs in 2018. However, we are able to provide more reagents if there is a need from more Member State NRLs.
Duration: January - December



REQUIREMENTS RELATED TO OTHER LEGISLATION

Sub-activity 5.1 ***(New animal health law)***

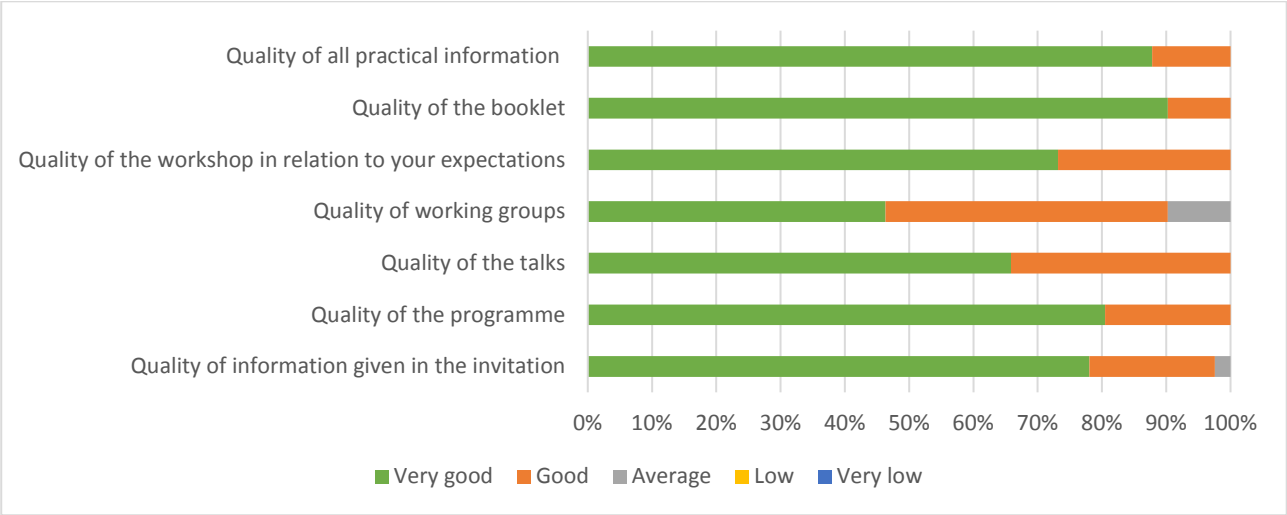
Objectives: To prepare regulations related to the new animal health law.
Description: The EURL expect to assist the EU Commission in preparing regulations for the new animal health law in 2018.
Expected Output: Depending on requests from the EU Commission.
Duration: January - December

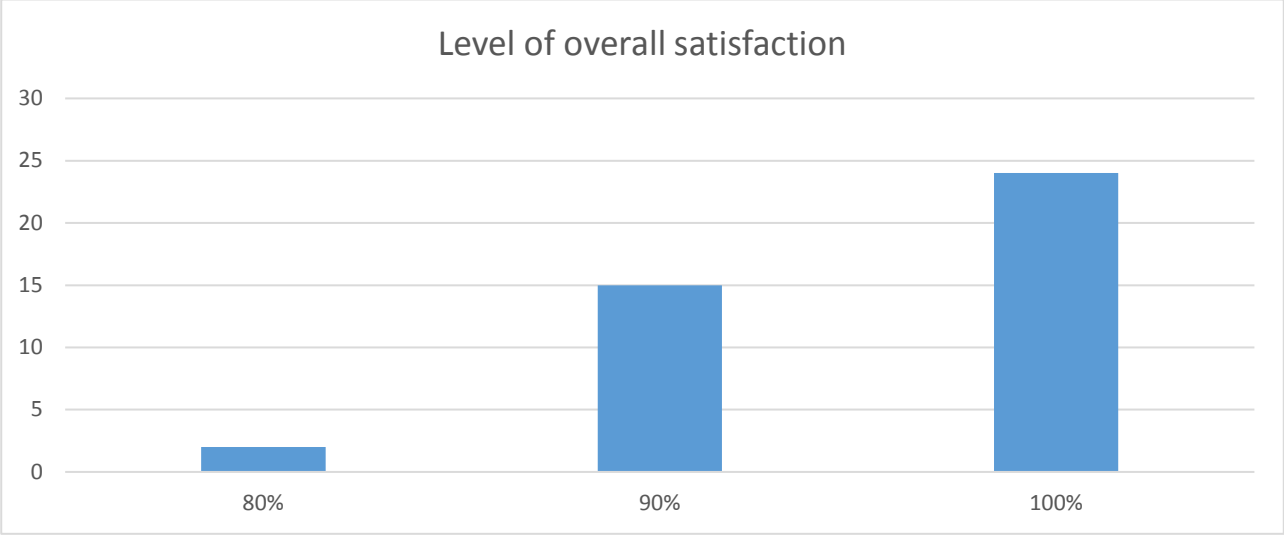
Sub-activity 5.2 ***(Listing susceptible species)***

Objectives: For the EU Member States to have an updated list of susceptible species for the listed fish diseases.
Description: With implementation of the new Animal Health Law there is an acute demand for scientifically assessing the fish species susceptible to the listed diseases. Therefore, an increased workload for the EURL in 2018 will be to assess the listing of susceptible fish species, e.g. assess susceptibility of cleaner fish (wrasse and lumpfish), sea bass and sea bream to VHS and IHN, etc.
Expected Output: Provide a report with a list of which fish species are susceptible to the listed fish diseases, to be recommended for adaptation in the new legislation.
Duration: January -March

Workshop evaluation

A questionnaire was delivered to the participants asking to evaluate various aspect of the workshop. An overview of the 41 questionnaires retrieved is shown below. Specific comments are going to be considered for the next annual workshop organization.





Greetings and conclusions of the meeting

The next meeting will be held at the 12-14 June 2019. It will be organized at our facilities here in Kgs. Lyngby. Thanks a lot to the people arranging the meeting as well as those of you who helped running the meeting by being chair, presenter and/or participant.

We are looking forward to seeing you all next year!

With kind regards,

The EURL fish team