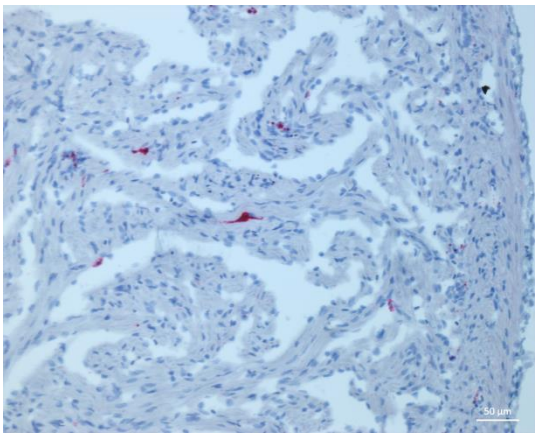
European Union Reference Laboratory for Fish and Crustacean Diseases
NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

Report of the

23rd Annual Workshop of the National Reference Laboratories for Fish Diseases

Kgs. Lyngby, Denmark

May 27th – 28th 2019



ISH staining of PRV-3 in Rainbow trout heart tissue

European sea bass infected with VHS

Organized by the European Union Reference Laboratory for Fish and Crustacean Diseases, National Institute of Aquatic Resources, Technical University of Denmark, Kgs. Lyngby

Contents

Introduction and short summary

The 23rd Annual Workshop of the National Reference Laboratories for Fish Diseases was held 27th – 28th of May, at DTU Aqua, 2800 Kgs. Lyngby, Denmark. This annual workshop was the third to be held at our premises in Kgs. Lyngby.

A total of 58 participants from 32 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 six sessions with a total of 30 presentations, three of which were given by invited speakers;

1) Knut Falk from the National Veterinary Institute in Norway with "ISA: Challenges related to epidemiology, detection, control and documentation of the ISAV situation including questions related to identify the source of new outbreaks"

2) David Stone from CEFAS, England with "Deficiencies in the current assays for the detection and identification of DNA viruses of carp: an assay redesign and evaluation"

3) Agnieszka Pekala-Safinska from Poland with "Interpretation criteria for antimicrobials used in farmed fish – the background of the problem"

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 and crustacean diseases, Niels Jørgen Olesen. 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 2018 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 https://www.eurl-fish-crustacean.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/2019/fiskehelserapporten-2018. An English version will be available later. The two final presentations in Session 1 were an update on the situation of ISA control program in Norway and the findings of viral pathogens in wild brown trout in Czech Republic in 2018.

The second half of the morning was allocated to the Emerging Diseases session. The afternoon of the first day was allocated to further update on important fish diseases in EU and their control, with a focus on the new animal health law which will be implemented in future and implementation of eradication program of VHS and IHN from France. In the second part of the afternoon, a specific session on updates from EURL took place, this year the presentation regarding results of the inter-laboratory proficiency test has been included in the first day in order to ensure that all participants can assist. In the evening, participants were taken on a guided walking tour in the centre of Copenhagen and a social dinner at restaurant "madklubben" was organized.

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 session on updates from the EURL. The programme and application procedures for the annual training courses, which will be provided by the EURL in October 2019, were described. The EURL activities in year 2018 were presented and proposals for the EURL work plan and activities for 2019-2020 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, Jacob Skov, Juliane Sørensen, Argelia Cuenca, Camilla Priess, Niccolò Vendramin and Dagoberto Andres Sepulveda Araneda. Niccoló Vendramin 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, Niccoló Vendramin and Niels Jørgen Olesen, with the help from the rest of the fish disease unit at the National Institute of Aquatic Resources, DTU AQUA and not less with the very important contribution from DTU Aquas 2 excellent secretary Lis Vinther Elmsted and Linda Stuhr Christensen. The meeting next year is tentatively planned to be held 2nd and 3rd of June 2020, and again back to back with the AW for NRL´s for Crustacean Diseases 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.

Niccoló Vendramin and Niels Jørgen Olesen

Programme

Monday May 27th

Annual Workshop of the National Reference Laboratories

08:50 – 9:20	**Registration**
09:20 – 09:40	Welcome and announcements *Niccoló Vendramin and Niels Jørgen Olesen*
SESSION I:	**Update on important fish diseases and their control** *Chair: Olga Haenen and minutes: Jacob Günther Schmidt*
09:40 – 10:10	Overview of the disease situation in Europe *Niccoló Vendramin*
10:10 – 10:30	Overview of disease situation in Norway *Brit Hjeltnes*
10:30 – 10:50	ISA: Challenges related to epidemiology, detection, control and documentation of the ISAV situation including questions related to identify the source of new outbreaks *Knut Falk*
10:50 – 11:10	Monitoring viral pathogens in wild brown trout in Czech republic *Ľubomír Pojezdal*
11:10 – 11:30	***Coffee break***
SESSION II	**Emerging diseases** *Chair: Anna Toffan and minutes: Jacob Skov*
11:30 – 11:50	Piscine orthoreovirus (PRV-3) associated disease in RAS *Niccoló Vendramin*
11:50 – 12:10	Distribution and variability of PRV-3 in Denmark *Juliane Sørensen*
12:10 – 12:30	Update on RMS in Rainbow trout *Jacob Schmidt*
12:30 – 12:50	Emerging parasitic challenges in Rainbow trout farming in Scotland *Eann Munro*
13:00 – 14:00	***Lunch***

SESSION III Update on important fish diseases and their control-2

Chair: Niels Jørgen Olesen and minutes: Juliane Sørensen

14:00 – 14:30	State of play of recent EU aquatic animal health legislative developments and their implementation *Laszlo Kuster*
14:30 – 14:50	Control and eradication program for VHS and IHN in France *Lenaig Louboutin*
14:50 – 15:05	Update on IHN outbreak in Estonia in 2018 *Triin Tedersoo*
15:05 – 15:20	Health management and challenges in modern aquaculture industry *Torsten Boutrup*
15:20 – 15:40	An overview of PMCV in farmed Atlantic salmon in Ireland *Neil Ruane*
15:40 – 16:00	***Coffee break***

SESSION IV: Update from the EURL-1

Chair: Niccoló Vendramin and minutes: Argelia Cuenca

16:00 – 16:20	Results of the Proficiency Test, PT1 and PT2, 2018 *Niccolò Vendramin and Teena Vendel Klinge*
16:20 – 16:40	Experience gained from Inter-laboratory proficiency test for fish viral diseases *Niels Jørgen Olesen*
16:40 – 17:00	EURL Training Courses. Topics and organization of courses 2019 *Niccoló Vendramin and Tine Moesgaard Iburg*
17:00 –	***Bus transport to Hotel Cabinn City***
19:30 –	***BANQUET dinner at restaurant***

Tuesday May 28th

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

Chair: Charlotte Axen and minutes: Camilla Priess

09:00 – 09:20	Susceptibility of Lumpfish (*C.lumpus*) to betanodavirus *Anna Toffan*
09:20 – 09:40	Phage therapy against *Flavobacterium psychrophilum* *Valentina Laura Donati*
09:40 – 10:00	Diagnostic manual for disease diagnostic in European Sea bass and Gilthead seabream. *Snjezana Zrncic*
10:00 – 10:20	Susceptibility of European Sea bass to VHSV and IHNV *Jacob Schmidt*
10:20 – 10:40	Global ornamental fish trade as a risk of AMR development, fish pathogen transfer, and contact zoonosis with focus on CyHV-2 *Olga Haenen*
10:40 – 11:00	***Coffee break*** *Chair: Lenaïg Louboutin and minutes: Dagoberto Sepúlveda*
11:00 – 11:20	Deficiencies in the current assays for the detection and identification of DNA viruses of carp: an assay redesign and evaluation. *David Stone*
11:20 – 11:40	Modelling economical impact of disease in animal husbandry *Carsten Thure Kirkeby*
11:40 – 12:00	Cell susceptibility, genotypes and virulence in rainbow trout of VHSV *Anna Luiza Alencar*
12:00 – 12:20	Full genome sequence analysis of VHSV, in the search of virulence markers *Argelia Cuenca*
12:20 – 12:40	Interpretation criteria for antimicrobials used in farmed fish – the background of the problem *Agnieszka Pekala-Safinska*
12:40 – 13:00	Is production of large smolt in RAS a risk factor for ISA? *Debes Christiansen*
13:00 – 14:00	***Lunch***

SESSION VI: Update from the EURL-2

Chair: Niels Jørgen Olesen and minutes: Niccoló Vendramin

14:00 – 14:15	EURL activities in 2018 *Niels Jørgen Olesen*
14:15 – 14:30	EURL Work Plan for 2019 and ideas and plans for 2020 *Niels Jørgen Olesen*
14:30 – 14:45	Next meeting and end of 23rd Annual Workshop *Niels Jørgen Olesen*
	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 2018

Niccolò Vendramin, Teena Vendel Klinge and Niels Jørgen Olesen

[1]*DTU Aqua National Institute of Aquatic Resources, Kemitorvet 202, Kgs. Lyngby,*

niven@aqua.dtu.dk

INTRODUCTION

This report is based on the data from the questionnaire on Survey and Diagnosis of the listed fish diseases in Europe (S&D) for 2018.

The Questionnaire 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:

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.

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.

Laboratory data from the NRLs and other laboratories, including the numbers of samples examined, and diagnoses of fish diseases made.

A National report describing health and surveillance situation in general. These reports are compiled into one and can be found on the website.

Production data from FEAP and FIGIS:

The data on the European aquaculture production was this year again obtained from the FIGIS online query system which provided data updated to 2017, data provided by FEAP were, at the time of preparing the workshop updated to 2016. It was observed during the preparation of the report, that some discrepancies between the two sources were present. This will be discussed at the Annual Workshop. The report does not include information on the number of fish farms, and therefore these data were obtained directly in the questionnaire.

The total fish production in aquaculture in Europe, including Turkey and Norway, increased slightly from 2016 and is now at 2.396.229 t. Among the EU Member states the production has been almost horizontal in the past 10 years with a total production of 693.725 t., while the 4 non-EU countries Iceland, Faroe Islands, Turkey and Norway produce 1.681.619 t and also experienced a minor increase since 2016.

The Atlantic salmon production, accounts for 1.54 mill ton in 2017, and is by far the largest contingency in Europe. The production of large rainbow trout in sea water accounts now for

103.000 t while the production of portion rainbow trout is of about 300.000 t in 2017 production.Turkey is still the largest contributor of rainbow trout with 106.000 t production. The carp production is still mainly in the Eastern part of Continental Europe, the data from FEAP and FIGIS do not overlap, and it is reported that the production of *Cyprinus carpio* is of about 170.000 t, whereas FEAP data reports approximately 50.000 t. This will be addressed in the workshop. Both the production of sea bream and especially sea bass also increased in the Mediterranean countries with a production of 154.088 t and 178.202 t, respectively. Among other fish species of interest are eel (with 5.938t in 2017 in decline from 2016), sturgeon which is a promising species especially in view of its caviar production has been very stable in the past 10 years (5.662 t) while for the caviar production there are no updated data for 2017.

Turbot production appear in slight increase (11.572 t in 2017 and 10.117 in 2016), the production of other "so called" minor species includes halibut (2.144 t), Arctic charr (6.377 t), sole (1.100 t) and meagre (6.200 t).

The production of cleaner fish as lumpfish and wrasse for lice control is increasing significantly; in 2018, 40 million of lumpfish and 1,6 million of Wrasse were produced in Norway, 2,1 million of Lumpfish juveniles produced in Iceland, and 3,4 million eggs and larvae for export, In Scotland there were 6 sites producing 26 tonnes of lumpfish in 2017 (925,000 fish), and 4 sites producing 4 tonnes of wrasse (58,000 fish).

Number of fish farms in Europe

The total number of authorised/licensed fish farms in Europe was reported to be around 30.049farms, with the largest contingency in Germany with 13.206 farms having a high number of very small 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 13.770 farms with species susceptible to VHS were reported in categorized zones, 12.139 to IHN, 6.519 to ISA and 11.937 farms with cyprinids susceptible to KHV.

76% of the authorised trout farms in Europe are situated in category III zones for VHS and 74% for IHN, with 23% and 24% 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 69% in Category I and 29% 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/EC 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. For **VHS**, 48 new outbreaks were reported in Europe in 2018, the large majority (37) in Germany, importantly a number of confirmed VHSV infection in Belgium (11) and France (1) were subclinical. These ones mostly occurred in put and take lakes.

For **IHN**, 17 new outbreaks were reported. The majority in Finland, as consequence of the epidemics occurred last year. All IHN-positive holding places in Finland have been emptied, disinfected and fallowed. Two-year surveillance program has been started or will start this year in the three zones and one compartment.

For **ISA** Norway reported 14 new sites with ISAV HPRΔ in 2018 and reported 14 in 2017. Unfortunately no report was received from the Faroe Islands. ISA was only reported from Norway.

For KHV, 137 outbreaks were reported in 2018. The vast majority (84) in Germany, 31 in UK. The virus has then been reported in 12 more countries. 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. More and more report BKD (bacterial kidney disease) as an increasing problem- possibly due to increased number of RAS in Europe. In Denmark findings and disease outbreaks linked to PRV-3 in RAS was reported.

In **salmon** farming the major concern is sea lice; after the ectoparasite a number of disease problems cause concerns and includes pancreas disease, heart and skeletal muscle inflammation, cardiomyopathy syndrome), amoebic gill disease and complex gill disease CGD (amoebic gill disease, salmon gill poxvirus, Paranucleospora theridion etc..). Ulcers from moritella and Alivibrio.

In **Cyprinid** it is primarily CEV, *Aeromonas hydrophila*, CyHV-2 has been detected in the Netherlands in cyprinid imported from China

In **seabass** and **seabream** it is primarily VNN/VER, tenacibaculosis, *Vibrio harvey*, *Sparicotyle chrysophrii*. *Aeromonas veronii* and *Lernathropus kroyeri* infection. Of a certain significance is Red Rash syndrome in gilthead sea bream.

Laboratory examinations

There are very large differences between countries on how many samples are tested on cell cultures, ranging from < 100 to several thousands. Private laboratories are also taking a share of the diagnostic in aquaculture. 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.

Questions and comments:

Q: Four countries in Europe are currently producing Tilapia. I would like to ask the whole NRL network: Who is performing TiLV diagnostics at the moment? NRL in Germany and UK, at the EURL we are selecting the test, it will be implemented in future.

Q: You reported the detection of ISAV HPR0 in broodstock in Denmark. Were these sacrificed?

A: Yes, we sacrificed the broodstock and then tested the organs.

Q: You mentioned the VHS detection in healthy fish. In Germany there has been a latent phase of the disease for years now. So after an outbreak you can find it in the endocardium and in the blood vessels in the brain. It will stay forever, it will not go away.

A: Yes, I agree. It was just to point out that normally you diagnose VHS in relation to an outbreak, but especially in put'n'take fisheries fish are bought from many suppliers there is possibility for introducing multiple pathogens in the same unit. Often you have detection without signs and that poses a question about health management. Under the legislation, what do you do about it?

Q: When VHS is found would you say that it is infected?

A: Yes of course.

Update on fish disease situation in Norway 2018

Brit Hjeltnes, Britt Bang Jensen, Geir Bornø, Asle Haukaas and Cecilie S. Walde

Norwegian Veterinary Institute, P.O Box 1263 Sentrum, NO-5811 Bergen, Norway, brit.hjeltnes@vetinst.no

Abstract:

In 2018, Norway produced 1.253000 tons of Atlantic salmon (*Salmo salar*), 64000 tons of rainbow trout (*Oncorhynchus mykiss*) 6000-6500 tons captive wild caught Atlantic cod (*Gadus morhua*), 1900-2000 tons of Atlantic halibut (*Hippoglossus hippoglossus*), 5-600 tons Arctic char (Salvelinus alpinus) and 2-300 tons turbot (*Scophthalmus maxima*).40 million lumpfish (*Cyclopterus lumpus*) and 1,6 million wrass (*Labrus bergylta*)

Cardiomyopathy syndrome (CMS), also known as 'heart rupture,' was diagnosed by NVI on 101 sites in 2017. Considering reported cases from private laboratories (125 cases), this indicates an increase over recent years and CMS now appears to be the overall most important viral disease in Norway

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 2018 although some improvements have been made.

Infection with salmonid alphavirus (SAV) remains one of the most serious virus diseases in sea-farmed salmonids. In total, 163 new sea-farms were registered affected in 2018 which is a reduction compared to 2017. Infectious salmon anaemia (ISA) was diagnosed in 13 farms in 2018 compared to 14 farms in 2017.

Infectious pancreatic necrosis (IPN) was diagnosed in 19 - 23 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.

While AGD (*Paramoeba perurans*) remains an important parasitic infection, the disease was not as severe in 2018 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*) is a decreasing problem due to use of vaccines.

Production losses remain a significant problem in Norwegian aquaculture.

Questions and comments:

Comment from the speaker. Started with comments on the recent major mortality case due to toxic algae bloom. 12.000 tonnes of salmon dead at latest count. Many smallish salmon (below market size). Started around the Norwegian national day 17th of May in northern Norway. An unfortunate combination of weather factors.

Q: Do you think that the algae bloom and resulting fish deaths could lead to a change of mind regarding recirculation also for big fish in Norway? Or do you think people will wait and see if it comes back year by year?

A: There has been a boost in interest in land-based production systems in recent years in Norway - to some extent spurred by Langsand Laks in DK and the build-up of production in Miami. We see projects popping up in several places, but for the industry as a whole; No, RAS is not considered viable. Price is the driver. Land-based RAS is more expensive.

Q: You mentioned Hydrogen sulphite. It is odourless, so how do you diagnose it before you can smell it?

A: NIVA is supplying the Norwegian companies with kits for measuring it.

Comment from audience: It is a very good question. I have been to many farms where they said they have problems with H_2S, but to my mind it is often just poor management. It is a complicate topic which require thorough understanding on the metabolic and physiological situation of each production unit in the farm.

Comment from the speaker: I just wanted to add that there will be a workshop about RAS in relation to the European Aquaculture Society conference in Berlin this year.

Q: How about the PD vaccine. A DNA vaccine has been launched. Is there any evidence from field trials?

A: We hope the new PD vaccine is better, and of course the industry hopes so, but we don't know

ISA: Challenges related to epidemiology, detection, control and documentation of the ISA situation including questions related to identifying the source of new disease outbreaks

Senior researcher Knut Falk

Norwegian Veterinary Institute, Oslo, NORWAY

Knut.Falk@vetinst.no

Abstract

The Norwegian salmon aquaculture industry has at any time 450-500 active ongrowing sea sites, containing on average around 1 million fish. During the last 25 years, the number of annual ISA outbreaks have varied from 2 to 20 cases. Thus, ISA is considered endemic in Norway with the exception of a few ISA-free zones, mainly associated with breeding facilities.

ISA either occurs as single cases, or as small local epidemics caused by horizontal virus transmission. In around 40% of the cases, the source of infection is unknown.

Apart from the ISA-free zones, we do not have a classical ISA eradication programme in Norway. However, we have an ISA control programme established in the early 1990'ties, which in practical terms has the goal of controlling and stopping virus spread when ISA outbreaks occur. This strategy has been relatively successful limiting the potential damage of this disease, in particular, if new cases are detected at early stages, and the appropriate measures are implemented immediately.

Observations by farmers, clinical diagnostic work by fish health services, and classical diagnostic work in laboratories are key factors to be able to detect ISA outbreaks at early stages. While qPCR is a powerful tool to detect ISAV in infected fish, recent observations suggest that qPCR screening for ISAV infection has a low predictive value of a negative test. Thus, ISAV qPCR screening is probably less suitable when it comes to documentation of freedom of infection.

Identification of the source of ISAV infection is currently an important challenge related to ISAV control and eradication programmes. As mentioned above, in around 40% of the Norwegian ISA cases, the source of infection is unknown. Possible sources include new transitions from the prevalent non-virulent HPR0 ISAV type to virulent HPR-deleted virus, infection through smolt, persistence in the local environment, unknown carriers etc. Currently, the possible transition from HPR0 type and accumulation in RAS-based smolt producing facilities are the most discussed possibilities. However, documentation related to these possibilities is scanty.

A major issue now for the Norwegian ISA control programme is the upcoming implementation of EU's new Animal Disease Legislation (ADL). With the implementation of ADL, the legal foundation for the current ISA control programme disappears. The authorities and the industry are thus facing a major challenge of how to control the spread of ISA and prevent a potentially devastating epidemic situation. The ADL only allow classical eradication programmes with a limited duration. This is a problem for Norway both because of the size of the industry, the potential cost, and more importantly because the virus detection methods and testing procedures do not seem to be well suited for this task.

Questions and comments:

Q:We know that viruses persist for years in other animal populations, why can this same thing not be accepted for fish?

A: I don't know. People just tend to say that what we have is horizontal transmission in a very short time-frame, and they are not considering the different possibilities. But we have seen a case where the same virus (presumably) has persisted in the same area. So where has this virus been in the meantime? We don't know

Monitoring viral pathogens in wild brown trout in the Czech Republic

Pojezdal L[1], Adamek M[2], Steinhagen D[2], Reschova S[1], Palikova M[3], Vesely T[1]

[1] *Veterinary Research Institute, Brno, Czech Republic*

[2] *University of Veterinary Medicine, Hannover, Germany*

[3] *Mendel University in Brno, Czech Republic*

pojezdal@vri.cz

Abstract

Brown trout (*Salmo trutta* m. *fario*) is the most important salmonid fish native to the waters of the Czech Republic, with high ecological value and a status of a popular target for sport fishing. Steady decline of local populations of the species over the last few decades is reported and often attributed to habitat degradation, mammalian and avian predators and overfishing. The role of infectious diseases, especially of viral and parasitological origin is also debated, even though specific data of viral presence in wild salmonid populations of Central Europe and the connection between outbreaks in farmed and wild freshwater fish are rarely published.

In the year 2014 an unusually high amount of viral haemorrhagic septicaemia (VHS) and infectious hematopoietic necrosis (INH) outbreaks (12 and 4, respectively) in Czech aquaculture facilities were detected. For this reason a virological and parasitological study of wild and feral salmonids in 8 locations representing main rivers of the Moravia region was approved by the Moravian Fishing Association. In the years 2015 – 2017 a total of 260 fish were caught and examined clinically and pathologically and tested for the presence of VHS virus and IHN virus using real-time RT-PCR, for the presence of infectious pancreatic necrosis virus and piscine orthoreovirus using conventional RT-PCR, for the presence of specific antibodies against VHS and IHN using ELISA and for the presence of the parasite *Tetracapsuloides bryosalmonae* using real-time PCR and immunohistochemistry.

Results of the examinations will be presented, along with the discussion of the estimated impact of these pathogens on the local wild brown trout population.

This study was supported by the Ministry of Agriculture of the Czech Republic MZE-RO0518 and the project PROFISH CZ.02.1.01/0.0/0.0/16_019/0000869 financed by ERDF in the operational programme VVV MŠMT.

Questions and comments:

Q: Serology. Your own ELISA? Coated with what?

A: Based on virus from outbreaks in rainbow trout farms in 2014 and 2016. Confirmed outbreak.

Q: So the serum collection was done at the temperatures shown? One was 4 degrees, and one was higher, but they were all negative even though you have had an outbreak before?

A: Well, the outbreaks were on the farms and we have sampled the rivers connected to the farms – downstream.

Q: I am asking because we have seen in the wild fish if there was an IHN or VHS outbreak in connected farms.

A: Maybe the reason could be the timing. We were not sampling the streams the same year as the farm outbreaks.

Q: The fish you stock are they category I or III.

A: Always category III. I don't think anyone is using category I for that.

Q: In your Brown trout populations do you have this blackening disease as we see in Germany?

A: Yes I heard about it from Dr. Adamek. It was considered in this study, but we have not seen signs like this

SESSION II: Emerging diseases

Chair: Anna Toffan

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 [4], Tine Iburg [1], Espen Rimstad [2], Niels Jørgen Olesen [1]

niven@aqua.dtu.dk

1 DTU AQUA National Institute of Aquatic Resources, Kemitorvet, Bygning 202, 2800 Kgs. Lyngby, Copenhagen,

2 Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, 0454 Oslo, Norway

3 Norwegian Veterinary Institute, 0454 Oslo, Norway

4 Norwegian Veterinary Institute, 5008 Bergen, Norway

Abstract

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

Currently three different subtypes, all associated with disease, are described for this viral species.

- PRV-1 is the causative agent of heart and skeletal muscle inflammation (HSMI) in Atlantic salmon 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. An initial 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 farms.
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 and investigating its virulence and the risk of vertical transmission, was funded and initiated. An overview of the results will be presented.

Questions and comments:

Q Has PRV-3 from Norway been genetically characterized?
A: Yes, we did, further details in the next presentation by Juliane Sørensen .

Distribution and variability of PRV-3 in Denmark

Juliane Sørensen, Niccolò Vendramin, Argelia Cuenca, Tine Iburg, Niels Jørgen Olesen

DTU AQUA National Institute of Aquatic Resources, Kemitorvet, bygning 202, 2800 Kgs. Lyngby, Copenhagen, Denmark.

jusor@aqua.dtu.dk

Abstract

Piscine orthoreovirus (PRV) causes or are associated with emerging diseases in salmonid aquaculture. PRV belongs to the family *Reoviridae*, sub-family *Spinareovirinae*, genus *Orthoreovirus*. It has a double stranded RNA genome consisting of 10 segments. The virion has a double protein capsid with icosahedral symmetry and no envelope.

Three subtypes of PRV have been reported and are denoted as PRV-1, PRV-2, and PRV-3.

PRV-1 causes heart and skeletal muscle inflammation (HSMI) in Atlantic salmon (*Salmo salar*).

PRV-2 was shown to cause erythrocytic inclusion body syndrome (EIBS) in coho salmon (*Oncorhynchus kisutch*) in 2016 in Japan. PRV-3 was detected in 2013 following a thorough investigation of unexplained mortalities in rainbow trout (*Oncorhynchus mykiss*) juveniles farmed in fresh water in Norway

Since 2017 Piscine orthoreovirus subtype 3 (PRV-3) has been associated with severe disease outbreaks in farmed rainbow trout (*Onchorhyncus* mykiss) in Denmark. In order to describe the prevalence of the virus in the country and its significance for Rainbow trout farming, the aquaculture industry supported a surveillance program in 2018. During the project, farms were sampled and tested for presence of PRV-3 RNA by qPCR.

At sampling farms were categorized according to production type (broodstock, juvenile production, on growing) and water recirculation (flow through, RAS type 1, RAS type 3, FREA). Furthermore clinical status of fish stocks at sampling was annotated (presence or absence of disease).

Results show that 80% of the tested production farms were positive for PRV-3, while only 40% of the broodstock farm were positive.

Recirculation of the water maintain the virus on site, being 95% of farms with bio-filters were positive.

To further investigate epidemiology and transmission routes sequencing of positive samples was conducted.

A sample from each positive sampling was sequenced for the S1 and M2 segment. Preliminary results of the S1 segment suggest that PRV-3 was introduced to Denmark 3 times with very little variation in the majority of the isolates from rainbow trout.

Historic samples from 1995 were tested for the presence of PRV-3 RNA in an effort to estimate when the virus was introduced. 11 out of 29 samples were positive, from which 8 were sequenced. Preliminary results show that the samples from 1995 and 2018 are very similar in the S1 segment.

Questions and comments:

Q: Are disease outbreaks restricted to RAS?

A: Yes.

Q: What are the levels of mortality?

A: Quite high occasionally up to 100%.

Q: Do vertical transmission of PRV-3 occur?

A (by Niccolò): It is still debated, but to the best of our knowledge it is not true vertical transmission.

Update on RMS in rainbow trout

Jacob G. Schmidt

DTU Aqua

jacsc@aqua.dtu.dk

Abstract

Red mark syndrome (RMS) is a skin disease affecting rainbow trout. It causes little or no mortality, but gives rise to lesions which have an unappealing aesthetic appearance to customers thus affecting sales. The disease was first recognized in Denmark about a decade ago. Within few years prevalence increased to a point where about a third of Danish trout farmers reported observations of the disease by 2015. Three years later a new questionnaire showed that the situation was more or less status quo. RMS thus continues to pose a problem to Danish rainbow trout farmers.

Due to the growing problems with this disease in Denmark we started investigations on RMS in 2016. We established a cohabitation model, which is still maintained in our high-contained experimental infection facilities at DTU Aqua. We have used this model to learn more about RMS. At the 21st Annual Workshop (AW) in 2017 we presented evidence that the bacterium provisionally called *Midichloria*-like organism (MLO) is the most likely candidate for a causative agent of RMS, and described how the disease typically progresses. At AW22 in 2018 we presented results on the skin immune response in cohabitants during an RMS cohabitation trial. These have recently been published (von Gersdorff Jørgensen & Schmidt *et al.* 2019). We also showed that MLO 16S rDNA could only be detected in cohabitants from around the time that lesions appear, and that MLO had a preference to mucosal sites as the amount of MLO 16S rDNA was around an order of magnitude higher in skin and gills than in investigated internal organs (kidney, spleen, liver and heart).

At AW23 we will present some results obtained with our RMS cohabitation model since AW22. We have investigated:

1. The effect of antibiotics on MLO/RMS.
2. Whether brown trout is a susceptible species to MLO/RMS.
3. Protection following re-infection with MLO/RMS.

Answers to these questions are that 1) antibiotics effectively alleviate RMS symptoms; 2) the answer is up for interpretation, but brown trout are at least less susceptible than rainbow trout; and 3) rainbow trout did not develop symptoms when subjected to a second RMS cohabitation challenge one year after the original challenge. These results will be elaborated at the workshop.

Reference:

von Gersdorff Jørgensen, L., Schmidt, J.G., Chen, D., Kania, P.W., Buchmann, K. and Olesen, N.J. (2019). Skin immune response of rainbow trout (*Oncorhynchus mykiss*) experimentally exposed to the disease Red Mark Syndrome, *Veterinary Immunology and Immunopathology*, vol. 211, pp. 25-34.

Questions and comments:

Q: What samples are taken for detection of the bacterium (MLO)?
A: We have previously shown that we primarily find the bacterium in mucosal tissues skin samples. We typically sample an infected site and a non-infected control site as well as skin scrape from a predefined site for standardized comparison between all fish.
Q: Can the bacterium (MLO) be cultivated on agar or in cell culture?
A: To my knowledge, no one has succeeded in growing the bacterium on agar or in cell culture. We have tried a lot on different agars but only very little in cell culture.
Q: Do you see any secondary infections associated with RMS?
A: Yes, in our cohabitation model, we sometimes have secondary infection with *Flavobacterium* in lesions, but I am not sure about the situation at farms.

. Two *Diplostomum* species associated with eye damage in rainbow trout (*Oncorhynchus mykiss*) farms in Scotland

Eann S. Munro[1*], Harry Hamlin-Wright[2], Rebecca E. McIntosh[1], Mark J. Fordyce[1], Angela Ashby[3] and Richard Hopewell[2]

1 – Marine Scotland Science, Aberdeen, Scotland, UK

2- Dawnfresh Seafoods Ltd., Uddingston, Scotland, UK

3- Fish Vet Group Ltd., Inverness, Scotland, UK

eann.munro@gov.scot

Abstract

In the summer of 2018, aquaculture health specialists observed gross eye damage as a result of significant eye fluke infection pressure at a freshwater rainbow trout (*Oncorhynchus mykiss*) hatchery (site A) operated by Dawnfresh Seafoods Ltd. Eye fluke have previously also been found, typically at lower levels of infection pressure, in farmed fish at a third-party supplying site (site B).

Clinical signs associated with eye fluke infection can vary significantly. Often, and particularly in instances where the infection pressure is high, individual metacercariae within the aqueous humor are visible to the naked eye. The way in which infection manifests as gross eye damage varies significantly and is not strictly related to parasitic burden. In mildly affected eyes, damage can be little more than a slight haziness, which can be diffuse or more particularly associated with the vitreous humor or lens. In the most extreme examples, the entire eye becomes completely opaque, and if the damage is bilateral, these fish are the most likely to be runted and anorexic. Between these two extremes, there can be a myriad of varying degrees of eye opacity and cataract formation.

Tissue material from affected fish on both sites A & B were examined by histopathological examination and the following observations were reported:- In infected fish from site A, metacercariae were identifiable in both the aqueous portion of the eye sampled as well as in close association with the lens. At site B, fluke are typically only recovered from the aqueous portion of the eye sampled, and rarely associated with lens tissue.
The clinical picture and histopathology observations suggested that there were at least two species of eye flukes associated with damage; one species that preferentially targeted the eye lens causing disruption to vision (site A) and the other species associated more with non-specific whole-eye swelling and lower overall impact on vision (site B). In order to determine the species of flukes associated with the cataracts, eye lenses from 10 fish were carefully dissected and pooled into RNAlater (site A) and aqueous eye material collected from 10 fish and pooled into RNAlater (site B). Partial DNA sequencing of the cytochrome *c* oxidase I gene (COI) was performed according to Moszczynska et al. (2009).
Results concluded that two *Diplostomum* spp. were present in the eye samples. *Diplostomum mergi* (linage 3) was associated with the eye lenses dissected from site A and *Diplostomum baeri* (complex sp. 2) was identified was the aqueous eye material.
At site A, several different strategies have been used to try and reduce the risk of eye fluke. These include covering the inlet channel with plastic sheeting with the aim of inhibiting plant growth which would otherwise provide a suitable habitat for *Lymnaea* snails, introducing paddle wheels to the inlet channel in order to disrupt the water flow and infective stage metacercariae as well as standard practices such as draining and disinfecting pond units. It is hard to quantify the effect these measures have had; however, they have not prevented large-scale outbreaks of eye fluke infection

since their introduction. Recently a 30-micron disc filter has been installed and is currently operational. This will filter all of the river water supplying the site during the high-risk months of the year (typically when water temperatures are consistently above 10°C, and particularly during periods of low rainfall in summer). The hope is that the disc filter will remove most of the infective stage metacercaria from the water. At site B, various ground works have been undertaken, including digging out water inlet channels in the hope that snails and their habitats will be disrupted. Again, the true impact of these works is hard to quantify, but they will not altogether remove the risk of infective stage cercariae from the inlet water.

Reference
A. Moszczynska, Sean A. Locke, J. Daniel McLaughlin, David J. Marcoglieses and T. J. Crease (2009). Development of primers for the mitochondrial cytochrome *c* oxidase I gene in digenetic trematodes (Platyhelminthes) illustrates the challenge of barcoding parasitic helminths. Molecular Ecology Resources, 9 (Suppl.1), 75-82.
/microscopic

Questions and comments:

Q: What is the impact of the different infection levels (categories 1 to 4) on the fish?
A: Fish within Cat. 4 and 3 fail and are runted, while Cat. 2 and 1 are able to grow to market size.
Q: Is *Diplostomun baeri* found both in the lens and the vitreous humour of the host eye?
A: Yes, but *D. baeri* is primarily found in the vitreous humour.

SESSION III Update on important fish diseases and their control-2

Chair Niels Jørgen Olesen

State of play of recent EU aquatic animal health legislative developments and their implementation

Laszlo Kuster

European Commission, DG SANTE, Unit G2, Animal Health and Welfare

laszlo.kuster@ec.europa.eu

Abstract

In recent years two significant, overarching EU Regulations were adopted, which are relevant, *inter alia*, for aquaculture, aquatic animal health, for the competent authorities in the EU member states who are managing those and associated official laboratories, as well as for economic operators running aquaculture production businesses. These Regulations will become applicable in the near future, will replace current key EU rules and will affect one way or another, the aquaculture sector and the relevant players in it.

One of these is Regulation (EU) 2016/429, the EU Animal Health Law[1]. This will be applicable from 21 April 2021. Currently several delegated and implementing Commission acts are being prepared with further details necessary for its future implementation. The discussions on these are getting close to their end, while the Commission has not yet officially adopted those acts, with a couple of exceptions. The details in those acts will cover key concepts such as zones, compartments, criteria for freedom, risk-based surveillance, elements for control and eradication of diseases, conditions for movements of live aquaculture animals and products and so on. A selected few of those elements, which are more relevant for diagnosis of diseases and for NRLs and official laboratories, will be shared with the participants.

Details and summaries of Animal Health Law related expert group discussions are publicly available at the following page: https://ec.europa.eu/food/animals/health/expert_group_en .

The other new legislation is Regulation (EU) 2017/625, on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare etc.[2]. Some of its provisions are directly related to EURLs, NRLs, and official laboratories, methods used for sampling, analysis, tests and diagnosis and so on. A selected few of these, which are more relevant for diagnosis of aquatic diseases and for NRLs and official laboratories, will be shared with the participants.

[1] https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1557495911818&uri=CELEX:02016R0429-20160331
[2] https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1557496471588&uri=CELEX:02017R0625-20170407

Questions and comments:

Q : according the new characterization of diseases KHV is placed in category E, so still notifiable but we don't really know what will happen once that the disease is notified (meaning which measures will be taken when we notify the disease).

A: there was a methodology behind the categories of disease, and we have taken the input from the NRLS in consideration to give a score to the different diseases. KHV was delisted at one point, and listed again, as several members states are having additional guaranties for KHV, as they are currently free of KHV

Q: no member state is free of KHV

A: Croatia is free of KHV and Serbia is currently in an eradication program

Q: the other point to discuss is what is notifiable? The disease or the virus

A: I don't know, fine details will come in the delegate acts, disease, virus, clinical signs, etc. all that will be discussed

Comment from EURL: what is notifiable has changed from disease to infection with pathogen, but for some diseases the definition is problematic like ISA and VHS

Q. Which are the consequences of non-exotic disease in category C? Do the member states have the possibility to say that they don't care for category C disease?

A: the members states could apply for a program to be free for the diseases, is optional.

Q: If you don't make a control program, in which category would you be?

A: For the terrestrial is only used infected or non-infected, but in this case you will be to say that you are infected, and accept the level.

Q: One point to further assess is animal welfare. This would not allow to make a proper control program, leaving disease out of control, without funding a sustainable solution, in fish farms there is also a high mortality and that is not acceptable to lose 20% fish in production.

A: you are right, I just wanted to say the scope is in diseases, welfare in general apply to criteria like space allowance, density, and other aspects of animal welfare legislation. But it is contemplated in the disease – welfare balance

Q: I also understand that the eradication programs should be for a defined time. This could be 6 years, is that correct? I don't know any eradication that takes so short time, it takes usually 20 years or more

A: I could imagine that the 6 years mean co-financing, as the financing cycle runs in 7 years periods. But I don't think there is a limitation in the technical controls.

Overview of the French situation regarding regulated fish diseases and operational deployement of the national plan for VHS and IHN eradication and surveillance

L. Louboutin[1], I.Guerry[2], T.Roman[2], F. Almeras[1], M. Baud[1], L. Bigarré[1], J. Cabon[1], L. Pallandre[1], T. Morin[1]

[1]*French Agency for Food, Environmental and Occupational Health & Safety, Ploufragan-Plouzané-Niort Laboratory, Viral Fish Pathology Unit, NRL for regulated fish diseases, Université Bretagne Loire, Plouzané, France*

[2]*Direction Générale de l'Alimentation, Service des actions sanitaires en production primaire, Sous-direction de la santé et de la protection animales, Bureau de la santé animale, Paris, France*

lenaig.louboutin@anses.fr

Abstract

Although our territory contains many zones and compartments free of IHNV and VHSV, these listed Rhabdoviruses have been regularly detected in France since their first description in 1971 and 1989 respectively. In 2018, 3 cases of IHNV but also VHSV were reported. All IHNV cases were identified in Normandie in the context of planned monitoring or following abnormal mortality events. Regarding VHSV, recurrent outbreaks those few past years in Moselle (East of France) triggered important epidemiological investigations, notably with the sampling and analysis of pikes from several ponds of the area where VHSv but also IHNv could be detected. The systematic sequencing of VHSV isolates and their comparison with former French and European isolates highlighted potential commercial links between various fish farms, although located in distant areas. Those various situations raise many interrogations about the origin of those outbreaks. In order to obtain a free status for those diseases, a National Eradication and Surveillance Plan for VHS and IHN, supported by European Union, was started in France last year. In these early phases, it is based on a voluntary and concerted approach by fish farmers located in defined territories to engage a qualification process including clinical visits and sampling for virological analysis, on a period ranging from 2 to 4 years. All the positive sites will be subject to a disinfection protocol integrating fallowing and repopulating with fish imported from category I farms. A total of 38 fish farms were sampled in 2018, with 1990 fish analyzed, and no virus could be detected. The first results as well as difficulties that French administration has to face with will be discussed.

Keywords: *Sanitary situation, France, VHS, IHN, National plan for eradication and surveillance*

Questions and comments:

Q: on pike, is there any consequence if it is found positive for VHSV and IHNV?

A: The positive pond in 2016 was eradicated and the ponds around were tested for the viruses, with negative results

Q: once you get the free country for vhsv and ihn, which surveillance program would apply for maintenance of freedom of disease?

A: The aim is to reduce monitoring each year, if the free status can be obtained

Outbreak of infectious haematopoietic necrosis (IHN) in Estonia 2018

Triin Tedersoo and Olga Piirik

Veterinary and Food Laboratory and Veterinary and Food Board

triin.tedersoo@vetlab.ee

Abstract

Infectious haematopoietic necrosis (IHN) is a notifiable fish disease of rainbow trout and other salmonids, which is caused by the infectious haematopoietic necrosis virus (IHNV), a single-stranded RNA virus belonging to the genus *Novirhabdovirus*, within the family *Rhabdoviridae*.

IHNV is an economically important pathogen causing clinical disease and mortalities in a wide variety of salmonid species, including the main salmonid species produced in aquaculture, Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*).

IHN is listed as non-exotic and a notifiable fish disease in Estonia. Until 2018 there were no known cases of IHN in Estonia.

In mid- May 2018, during the national monitoring plan routine screening, samples for VHS/IHN were taken in the fish farm located in Lääne-Virumaa Vinni parish. From a pooled sample, consisting of eight fish (2 years) organs, IHNV was detected using RT-PCR and sequencing. Fish had minor skin injuries but otherwise there were no visible signs of IHN. After the confirmation from the EURL for Fish and Crustacean Diseases IHN has been officially diagnosed in this fish farm in the end of May. Immediately the local veterinary centre in Lääne-Virumaa county laid down the restrictions to movement on fish, fish eggs, fishery products and animal by-products, transport vehicles, materials and equipment from infected holding. Together with a thorough epidemiological investigation, the determination of the prevalence of the disease was conducted. In addition, an investigation by experts from University of Life Sciences was done in the infected holding. Samples from the closest river Kunda (80 trouts) were investigated and were tested negative to IHN. Additionally samples from nearest aquaculture holding RMK Põlula AQ were taken, which were tested negative to IHN. The expert group opinion, from the University of Life Sciences, could not identify the clear routes of infection, but they assumed the infection occurred with contaminated equipment. All fishes were slaughtered and disinfection of all ponds and equipment was done.

After the first case, the second case was detected in October 2018 around 70 km apart. In October 2018, during the national monitoring plan routine screening, IHN was detected in a fish farm located in Järvamaa Roosna-Alliku. One pooled sample consisting of 10 fish (1-year-old) organs was tested positive using RT-PCR, sequencing and cell culture+ antigen ELISA. The fish had no visible signs of the disease. Additional investigation was carried out, that confirmed IHN infection in the fish farm. Järvamaa Veterinary centre laid down the restrictions to movement on fish, fish eggs, fishery products and animal by-products, transport vehicles, materials and equipment from infected holding. The Veterinary and Food Board laid down the planning activities and did an epidemiological study. No clear clinical symptoms of IHN were reported in the positive fish and there was no mass mortality in the infected farm. The initial origin of the IHNV was not found. The eradication programme is ongoing.

Questions and comments:

Q: do you know who is the egg supplier?

A. They buy the eggs from Denmark. They were also disinfected, so this potential has been ruled out.

Comment from EURL. IHNV is complicated as you can have the subclinical infections. Maybe we should consider to focus more on serology for IHNV, in order to detect the subclinical infections.

Health management and challenges in modern aquaculture industry

Torsten Snogdal Boutrup

Aquapri A/S

torsten.boutrup@aquapri.dk

Abstract

During the last 20 years recirculating aquaculture (RAS) has gained in numbers, and an increasing percentage of cultured fish comes from some type of RAS.

Recirculating aquaculture has some advantages, both with regard to environmental impact and control of fish diseases. On the other hand RAS systems have not proven to be the answer to fish health and –welfare. This is due to the same factors, some fish diseases are hard to control when pathogens are recirculated. And also - though the waste products coming from a RAS system might be less, the environment the farmed fish get very often is of a poorer quality.

This talk will focus on both production diseases and severe diseases - like VHS. What are the challenges with regard to having a good production together with diseases? And how likely is it to get rid of diseases?

Within aquaculture one of the big challenges is; that in the name of RAS general well proven husbandry strategies is sacrificed for production output and keeping fish in the system. Old type traditional farms, like flow through earth pond farms, naturally made good husbandry possible, because it naturally bid into the correct flow of fish and all in all out production.

To be able to pay for the investment in high cost RAS systems, they always need to be running to pay the debt, and often biosecurity is second to get the production running.

The big challenges for the future will be to be very precise on where the cut offs are, for system carrying capacity, combined with economic and fish welfare due to presence or absence of specific fish pathogens/diseases.

Questions and comments:

Q: in the Faroe Islands we are building bigger RAS for the salmon production, and they are quite reluctant to disinfections. What is your recommendation to disinfect in order to increase production?

A: After we did so stringent disinfection, it took three batches of fingerlings to have the RAS running again, as the first two would have deformations. So your farmers are right, it is a balance. With all this cleaning you are also reducing all the beneficial bacterial which are important to have robust fish.

Q – you are working in RAS and biofilters. Are there any technical solutions to design in the future so you don't need to take out the biofilter

A: You can put lot of UV or ozone, but you would lose all the beneficial bacteria. You have to start in the other end, it is better to be sure that nothing is coming in the system. The pathogens come because there are breach in biosecurity system

An overview of piscine myocarditis virus in Ireland

Neil Ruane*, Andrew Tighe*#, Jens Carlsson#

**Marine Institute, Oranmore, Co. Galway, Ireland*

#Area 52 Research Group, School of Biology & Environmental Science, University College Dublin, Ireland

neil.ruane@marine.ie

Abstract

Piscine myocarditis virus (PMCV) is a double stranded RNA virus which has been linked to cardiomyopathy syndrome (CMS) in Atlantic salmon (*Salmo salar* L.). The first recorded outbreak of CMS in Ireland occurred in 2012 and has averaged between 1 and 4 cases per year since then. Analysis of archived broodstock samples since 2006 showed that the first detections of PMCV occurred in 2009, three years before the first clinical case of CMS. Partial sequencing of the open reading frames (ORFs) 1 and 3 showed PMCV to be largely homogenous in Irish samples, showing little genetic diversity. However several amino acid positions within both ORF1 and ORF3 showed consistent variations unique to the Irish PMCV strains when compared with previously published Norwegian strains. The phylogeny generated in the present study suggests that PMCV may have been introduced into Ireland in two waves, both coming from the southern part of PMCV's range in Norway. In addition, over three quarters of the PMCV strains which were sequenced came from fish not exhibiting any clinical signs of CMS.

This work is funded under the Marine Institute Cullen Fellowship Programme (project CF/17/04).

Questions and comments:

Q: looking at the variation in the brood stock is intriguing to see that sometimes you have 100 % and some years 0%, do you know why? And Totivirus do not have a vertebrate host, so how much do you believe that PMCV is a fish virus, and that you are not detecting PMCV as a proxy if other things?

A: We have tested lots of things as positive, so we questioned the specificity of the method. After thorough revision of lab procedures including new PCR protocols, we have re-tested lots of samples with very high ct values, but they may have lots of false positive results.

Q: how many samples should be positive (replicates) to be positive.

A: Cq value in lightcycler is 35, and we found that if we get replicates above 35 they are not consistent.

Q: so did you do a confirmation by rt-pcr?

A: No, we don't get any amplicon

Comment from the speaker: Ct value is consistently below 20 in Clinically affected CMS fish.

Q: we think there is a reservoir of PMCV, so where do we look for it

A: We found it in many fish. We don't think it is only salmon, and we have started to find it in several places more.

Q. You don't think we can have a disease freedom of PMCV?

A: maybe but it is very difficult.

SESSION IV:Update from the EURL Part 1

Chair: Niccoló Vendramin

RESULTS OF THE PROFICIENCY TEST, PT1 AND PT2, 2018

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

EU Reference Laboratory for Fish and Crustacean Diseases,

DTU AQUA National Institute of Aquatic Resources, Kemitorvet, Bygning 202, 2800 Kgs. Lyngby, Copenhagen,

niven@dtu.vet.dk

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). As in previous years, in 2018, 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.

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 tests were sent from the EURL in the end of September 2018.

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

45 laboratories participated in PT1 while 42 participated in PT2. Two laboratories were, due to internal clearance problems, not able to provide the answers before deadline

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.

Résumé and concluding remarks PT1

30% of parcels were delivered by the shipping companies within 1 day after submission, 91% was delivered within 1 week and 96% was delivered within 3 week. The remaining two parcels were never delivered due to border control in the receiving country. The maximum time of shipment was 18 days.

This year EHNV was included in the Proficiency test. 5 out of the 45 countries do not test for Ranavirus. 39 participants provided the correct identification, hereof one laboratory identified correctly the isolate as EHNV by sequencing but submitted the result as Ranavirus and one laboratory did not performed 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 40 out of 45 participants scored 100% success rate and 1 participant scored 90% due to sequencing of the content in ampoule IV (EHNV) and 3 participants scored 80% due to not finding one of the virus. These points will be assessed directly with the single participants that has 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.
42 laboratories participated in PT2. 37 laboratories tested for SAV and 36 correctly identified the virus in Ampoule VIII, 1 laboratory experienced some difficulties due to a non-specific PCR amplification.
40 out of the 42 laboratories correctly identified the ISA virus in ampoule VII, 1 did not test for ISAV and 1 laboratory answered 'No pathogen found'.
40 laboratories correctly identified the KHV in ampoule VI, 1 did not test for KHV and 1 laboratory correctly identified KHV but detected also another virus in this ampoule.
It has been a concern that two laboratories has 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 has 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).

Notably the sequence analysis were highly satisfactory, incorrect identification of the genotype has only occurred in 2 cases in PT1 and 3 cases in PT2, further discussion and instructions on how to fill the spread sheet will be included in the presentation.

Questions and comments:

Comment from the speaker : Should nodavirus be included in the PT? – 11 labs wants it included systematically.

Comment from EURL : This will not happen in 2019, we will further discuss the possibility with the OIE ref.lab for VER/VNN.

Comment from EURL in order to compare results of sequencing it is extremely important to agree on a protocol, and a series of definition for genotypes for the different viruses.

Q: CyHerpesvirus 1 2 3 are species?

A: Correct, but for the requirement for the ringtest it is enough to specify CyHV-3 in case of KHV.

Comment from EURL: all participating laboratories are most welcome to write comments in the right space, so please continue the detailed answers.

Experience from inter-laboratory proficiency tests among European national reference laboratories for detection of viral infections in fish

Niels Jørgen Olesen, Teena Klinge, Niccolò Vendramin

Technical University of Denmark, National Institute of Aquatic Resources, Denmark

njol@aqua.dtu.dk

Abstract

BACKGROUND—With the aim of harmonizing and assuring the quality of the diagnostic methods used by National Reference Laboratories in Europe for surveillance and detection of listed fish diseases, annual inter-laboratory proficiency tests have been conducted by the EURL for Fish Diseases since 1995. The tests are accredited according to the quality assurance standard DS/EN ISO/IEC 17043 and include both qualitative and quantitative assessments of the ability of the participants to detect and identify more than 10 different fish pathogenic viruses.

METHODS—Each annual proficiency test (PT) is divided in two: One for viruses replicating in cell cultures and one for viruses primarily detected by bio molecular methods. All viruses included in a PT are propagated, mixed with equal amount of lactalbumine, lyophilized and sealed under vacuum in glass ampoules. Homogeneity, purity, stability and reduction in viral titres following the lyophilisation process are assessed before shipment. The tests are shipped by courier to approx. 40 laboratories worldwide including all EU Member States- as participation is mandatory for these laboratories. An 8-week deadline for submission of results is given and in a detailed and comprehensive report all laboratories are scored according to their performance. Underperforming laboratories are contacted for assessing the reason and for possible re-testing.

RESULTS— Lyophilization cause significant reduction in viral titres but once sealed no reduction is observed after long time storage under various temperatures. Shipment of live notifiable pathogens ww is challenging. Participants are performing increasingly well over the years- and their score in relation to pathogen identification is in general high. However cell line susceptibility varies a lot between laboratories and within cell line. The same applies for Ct values and sequences among laboratories using similar procedures

CONCLUSIONS— The PTs are the backbone in accreditation and QA of notifiable fish diseases WW and are essential for safe international trade. Significant increases in performance both for pathogen identification and characterization has been observed.

Questions and comments:

No questions.

EURL training courses for 2019

Niccoló Vendramin and Tine Moesgaard Iburg

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

niven@aqua.dtu.dk and timi@aqua.dtu.dk

Abstract

In 2019, 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 7th to Friday the 11th of October

- **Introduction to histopathology in fish and crustacean diseases**
  The course will be held in week 42 from Monday the 14th to Friday the 18th 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:

No questions.

SESSION V:Research update

Chair: Charlotte Axen and Lenaig Louboutin

Experimental infection of Lumpfish (*Cyclopterus lumpus*) with different betanodaviruses

Toffan A.[1], De Salvador M.[2], Scholz F.[3], Pretto T.[1], Buratin A.[1],Toson M.[1], Cuenca A.[4], Vendramin N.[4]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro, Padua, Italy*

[2]*De Salvador Maria, University of Udine, Italy*

[3] *FishVet Group Ireland, Oranmore, Co. Galway, Ireland*

[4] *National Institute of Aquatic Resources, EURL for fish disease Technical University of Denmark, Kongens Lyngby, Denmark*

atoffan@izsvenezie.it

Abstract

This paper reports the results of the first experimental infection of Lumpfish (*Cyclopterus lumpus*) with genetically different betanodaviruses.

Groups of lumpfish were intramuscularly infected with three different viral encephalo-retinopathy viruses (VER) belonging to two different species, namely two cold water viruses belonging to the BFNNV species and one belonging to the widespread RGNNV species. Fish were kept at 12°C and observed daily. All diseased fish were euthanized by overdose of benzocaine. Additionally, healthy fish specimens were collected at fixed time points (7, 4 and 28 days post infection). Brain samples were collected singularly to be analyzed by molecular diagnosis, virus isolation, histology and immunohistochemistry.

Despite reduced survival in the first two weeks due to a behavioural problem (tail biting), no clinical signs referable to VER were observed. On the other hand, all survivors tested positive by molecular testing and virus isolation. Notably, threshold cycles in real time RT-PCR and viral titer appeared to increase over time, supporting viral replication in the brain of infected lumpfish. Histology and IHC evidenced typical vacuoles surrounded with immunoprecipitates in brains and eyes. Lesions were more severe and the viral titre was higher in BFNNV infected lumpfish compared to the RGNNV infected ones.

Results show that lumpfish is susceptible to betanodavirus infection. Further long-term studies are needed to determine whether NNV may cause clinical disease in lumpfish in particular environmental conditions. Differences amongst infected groups are due to different optimal replication temperatures of the VER species used.

In view of the extensive use of cleaner fish in salmon parasites control and of the data generated by this study, the possible VER infection and/or role as carrier of lumpfish should always be taken into consideration.

Questions and comments:

Q: Nodavirus will normally cause mortality in very small fish/larvae - would it make more sense to infect in a younger stage?

A: Good suggestion. This was a very preliminary study and we did not know what to expect, so we went for the “classical” way: standard dose, different viruses, intramuscular, 4 weeks observation

Bacteriophage – coated feed to control *Flavobacterium psychrophilum* infections *in vivo* in rainbow trout fry: a prophylactic approach

Valentina L. Donati[1], Inger Dalsgaard[1], Mathias Middelboe[2] and Lone Madsen[1]

[1]National Institute of Aquatic Resources, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark, [2]Marine Biological Section, University of Copenhagen, DK-3000 Helsingør, Denmark.

valdo@aqua.dtu.dk

Abstract

Due to the rise of antibiotic resistance and the unavailability of a commercial vaccine, alternative environmentally sustainable methods for controlling the spread of *Flavobacterium psychrophilum*, a worldwide-known pathogen in salmonid aquaculture, are of high ecological and economic interest. Bacteriophages, host-specific viruses of bacteria, represent a potential alternative for pathogen control[1,2].

In this study, we investigate the efficiency of a bacteriophage-based prophylactic treatment of rainbow trout (*Oncorhynchus mykiss*), where phages are orally administered through the feed. Rainbow trout fry (1-2 g) are fed with phage-coated feed for 30 days before the exposure with *F. psychrophilum*. Controls fed with conventional feed as well as controls not infected with the bacterium are included in the study. The effects of the prophylactic treatment on fish survival, growth and welfare are quantified and samples from several fish organs are taken over time in order to assess the spread and density of phages. The results on phage distribution and dynamics and their effects on fish survival will be presented and the perspectives outlined.

References

1. Madsen, L., Bertelsen, S. K., Dalsgaard, I. & Middelboe, M. Dispersal and survival of *Flavobacterium psychrophilum* phages in vivo in rainbow trout and in vitro under laboratory conditions: Implications for their use in phage therapy. Appl. Environ. Microbiol. 79, 4853–4861 (2013).
2. Christiansen, R. H., Dalsgaard, I., Middelboe, M., Lauritsen, A. H. & Madsen, L. Detection and quantification of *Flavobacterium psychrophilum*-specific bacteriophages In vivo in rainbow trout upon oral administration: Implications for disease control in aquaculture. Appl. Environ. Microbiol. 80, 7683–7693 (2014).

Questions and comments:

Q: what is the idea? Is it to look at is as a treatment, or for replacing a vaccine?
A: it is a very big project, there a different ideas and different studies that we would like use the phages for, and in this case, it is as prophylactic treatment

Q: possible to find/develop specific phage for the bacteria? Not a widespread, but just for *psychrophilum*?
A: The selection of the bacteria and the phage is the second step. Before this, many people have worked on the isolation and characterization of different phages and different strains of Flavobacterium. The idea is to use one phage that has a broad range – if the phage is too specific, it is not optimal for Flavobacterium, since there are many different strains.

Manual of diagnostic techniques for the main pathogens in Mediterranean marine aquaculture

Snježana Zrnčić

Croatian Veterinary Institute, Laboratory for fish and molluscs diseases, Zagreb, Savska 143, Croatia

zrncic@irb.hr

Abstract

The goal of the EU funded project in the frame of Horizon 2020, entitled Mediterranean Aquaculture Integrated Development (acronym MedAID) is to increase the overall competitiveness and sustainability of the Mediterranean marine fish-farming sector, throughout the whole value chain. An important aspect of the projects is the management of transmissible diseases of farmed fish and, among other tasks, to strengthen diagnostic capacities by harmonizing competences. Partners dealing with health issues within MedAID are endeavouring to provide tools and common strategies for prevention and diagnosis of major diseases by creating an operative and collaborative platform at Mediterranean level. This platform will produce codes of good practice and harmonized standards for integrated health management through the establishment of a network of laboratories capable to get a proper diagnosis in case of known pathogens and support in case of emerging or etiologically unsolved diseases. One of the key elements of the health management strategy is fast, reliable, validated and efficient diagnostic technique capable to detect the health threat timely.

Manual of diagnostic techniques for the main pathogens in Mediterranean marine aquaculture is conceived as an up-to-date guideline providing the standardized methods enabling the harmonized approach to the health challenges due to viral and bacterial pathogens in the farming of sea bass and sea bream. The list of important diseases was agreed during the meetings of the MedAID partners and other fish health experts among which are NRLs meetings, FAO/GFCM meeting and project's thematic meetings.

The Manual is designed in the following chapters:

Chapter "General Sampling Procedures" presents guidelines for sampling on the farm for targeted surveillance to certify the disease freedom, diagnosis in case of mortalities and analysis of mortalities caused by unknown aetiology, instructions for packing and shipping of samples and the guidelines for laboratory receiving the samples.

Chapter "General Requirements for Laboratories" provides detailed information about organisation, equipment and management of the diagnostic laboratory with a specificity of given techniques (bacteriology, virology, molecular methods) which will enable the establishment of a reliable and competent diagnostic unit.

Chapter "Viral diseases" deals with general principles of viral diseases diagnostics and acquaints the reader in detail with all steps in setting up the accurate procedures for detection of VNN.

Chapter "Bacterial diseases" informs about several most important bacterial diseases influencing European sea bass and Gilthead sea bream farming, describing the etiological agent and available validated methods of screening, isolation, identification and confirmation.

Chapter "Diagnostic procedure in the case of mortality caused by unknown aetiology" is streamlining the diagnostic procedures in the case of the outbreak which could not be assigned to the known causative agent.

Finally, the Chapter "Reporting the Results" gives instructions on how to communicate the results of diagnostic procedures to stakeholders.

Questions and comments:

Q: After the end of the project, do you have a more permanent funding?
A: We have a work package that will create a conclusion and recommendation for the time after termination of the project. A conclusion might be that a network should be established, and try to find budget for financial funding for next project.

Susceptibility of Sea Bass (*Dicentrarchus labrax*) to IHN and VHS virus

Jacob G. Schmidt, Argelia N. Cuenca and Niels Jørgen Olesen

DTU Aqua

njol@aqua.dtu.dk

Abstract

Introduction: The susceptibility to infection with viral haemorrhagic septicaemia virus (VHSV) and infectious hematopoietic necrosis virus (IHNV) of sea bass (*Dicentrarchus labrax*) is poorly described. Here we investigate the effect of infection of sea bass juveniles with 1 IHNV and 3 VHSV isolates by intraperitoneal injection and cohabitation.

Experimental set-up: The experiment was conducted in 10 L bowls at 12°C. Each of the 5 groups (negative control, IHNV E, VHSV Ia, VHSV Ie and VHSV IIIb) were in triplicate with 30 fish in each bowl. The fish were ~3-4g. Fifteen of the fish in each bowl were injected with $10^4 TCID_{50}$/fish of virus or with cell supernatant alone and implanted with visible Elastomer tags and the other fifteen left as cohabitants. After 6 weeks the experiment was terminated.

Results: There was almost no morbidity or mortality in the cohabitants for any of the treatments (only a single fish in the VHSV Ie group died). There was no morbidity or mortality in the control and IHNV treatments. In the VHSV III treatment group there was very little mortality, and only one fish died in each bowl. The injected fish in the VHSV Ia and Ie groups showed signs similar to those observed for VHSV infection in rainbow trout, *i.e.* a general darkening and petechial bleedings. Mortality was just below 50% on average for VHSV Ia, whereas mortality was close to 80% for the VHSV Ie group. The VHSV Ie isolate originate from sea bass. The other isolates are from rainbow trout, in which VHSV Ia and IHNV E are highly virulent isolates and VHSV IIIb has medium virulence.

Conclusion: The study showed that juvenile sea bass were not susceptible to IHNV E or VHSV genotype IIIb, but were susceptible to VHSV genotype I by intraperitoneal injection. However, the virus was not transferred to the cohabitants. We therefore conclude that sea bass are relatively resistant to VHSV and IHNV infection.

Questions and comments:

Q: VHSV Ie was the only one isolated from seabass – why do experiment, when this is then a known host for the isolate?

A: If seabass is listed as a susceptible species to VHS, it will have tremendous impact on the industry, because then we have to start a surveillance program in the Mediterranean area, therefor a very strong background for doing so is needed and we good explaination what the risk would be. The risk would be high economic impact to put it on the list, but also a high risk not to put it on the list due to spreading of the disease. This was the background of doing the experiment.

Q: Did you test the cohabitants as well?
A: We tested the cohabitants and we did not find the virus in them

Q: Would it have made sense to test against non-American VHS?
A: This susceptible testing is delicate and difficult point of view, because it can be difficult to argue for why other genotypes were not included, and that would have made sense as well. We took it primarily from a European point of view, but it would have been nice to include more genotypes yes.

Q: Would it have made sense to make the experiment at higher temperature?
A: We were aiming for a better environment for the virus. Winter in black sea, the temperature is low, so it is not so unnatural conditions

Global ornamental fish trade as a risk of AMR development, fish pathogen transfer, and contact zoonosis, with focus on CyHV-2

O. Haenen[1*], D. Ceccarelli[2], I. Roozenburg[1], M. Voorbergen[1], E. van Gelderen[1], N. Tafro[3], Kees Veldman[2], A. van Essen[2], J. Testerink[2], Karel Riepema[2], Tina Zuidema[4], L. Stolker[4], T. Ito[5], and Dik Mevius[2]

[1] Wageningen Bioveterinary Research, Lelystad, Fish Diseases Laboratory, the Netherlands
[2] Wageningen Bioveterinary Research, Lelystad, AMR Laboratory, the Netherlands
[3] The Netherlands food and consumer product safety authority, NVWA, Schiphol, the Netherlands
[4] RIKILT, Wageningen, the Netherlands
[5]Tamaki Laboratory, Research center for fish diseases, NRIA, Japan Fish Res and Educ Agency, Mie, Japan
olga.haenen@wur.nl

Abstract

In global fish trade, yearly, > 1 billion ornamental fish is transported, with >3000 deliveries of tropical ornamental fish into the Netherlands as an important import- and transfer port, from >40 countries worldwide, 50% from SE-Asia. These stressed fish may carry potential zoonotic bacteria, fish virus, and multi-resistant bacteria, and transport water may contain residues of therapeutics, risky to professionals in ornamental fish trade, hobbyists, ornamental fish, and spread to the environment.

In this study, the Veterinary Authority NVWA sent 50 batches of imported freshwater ornamental fish species (36 species/genera from 13 countries), mainly from Asia and S-America, in their original bag, directly from Schiphol Airport to WBVR, for analysis. Transport water was sampled for residues of therapeutics, from 2 fish per batch bacteria were isolated from skin and internal organs, and a liver smear was stained Ziehl Neelsen (ZN), and kidney of goldfish only were tested for CyHV-2. For AMR tests, the fish gut and transport water were sampled, and, after enrichment, purified DNA from the pellet was screened for resistance genes, incl. OXA-48 with conventional PCR, with sequencing. In parallel, AMR bacteria were isolated and tested.

At RIKILT, transport water samples were analyzed (antibiotic residues: tetracyclines, sulfonamides, macrolides, fluoroquinolones, β-lactams, aminoglycosides, nitrofuranes, chloramphenicol and (leuco)malachite green). 49/50 water samples contained one or more antibiotics in concentrations ranging from 0.02 to 10000 µg/kg.

At WBVR, from multi-bacterial cultures, strains of *Aeromonas* spp. (59x) were identified by MALDI-TOF, sensitivities were tested against tetracycline, flumequine, trimethoprim + sulphadizine, neomycine, florfenicol, and nitrofurantoin. From 59 strains of *Aeromonas* spp. 85% were resistant against tetracycline, 52% against flumequine, 31% against trimethoprim + sulphadizine, 34% against neomycine, 9% against florfenicol, and 17% against nitrofurantoin. Concerning potential fish zoonotic bacteria, one fish batch was strongly positive for *Mycobacterium haemophilum*, and one for *Myc*. spp.. *Shewanella*'s were sent to the AMR lab for analysis.

At NRIA, Japan, in a collaborative study, one of eight goldfish samples was found positive for CyHV-2 in cell culture. This isolate appeared highly virulent to the Ryukin goldfish (Ito et al., 2017).

Conclusions: Imported warm water ornamental freshwater fish mostly carried various resistant opportunistic *Aeromonas* spp., and seldom mycobacteria, which are potentially zoonotic. One of eight goldfish samples contained virulent CyHV-2. Often the transport water contained residues of antibiotics, authorized and non-authorized. These fish imports may pose a small to medium risk to man, at direct contact with fish and fish water, to ornamental fish and the environment.

We acknowledge NVWA, who subsidized this project.

References Ceccarelli, D. et al., 2017. Antimicrob. Agents Chemother. Feb 2017, 61, 2: e01013-16. https://doi.org/10.1128/AAC.01013-16 ;
Ito T, et al., 2017. Importation of CyHV-2 infected goldfish into the Netherlands. Dis. Aquat. Org. 126: 51–62. https://doi.org/10.3354/dao03157 .

Questions and comments:

Q: The personnel working with the fish, did you see any disease or antibiotic resistance?
A: We are not very worried based on research, no personnel involved suffered disease

Deficiencies in the current assays for the detection and identification of DNA viruses of carp: an assay redesign and evaluation.

David Stone[1], Peng Jia[2] and Hong Liu[2]

[1]Cefas Weymouth Laboratory, UK
[2]Shenzhen Exit & Entry Inspection and Quarantine Bureau, People's Republic of China.
david.stone@cefas.co.uk

Abstract

Koi herpesvirus disease (KHVD) is highly contagious disease of common carp (*Cyprinus carpio*). The causative agent Cyprinid herpesvirus-3 (CyHV-3) has been listed as notifiable under EU and UK legislation since 2007. Internationally, the disease has been recorded in 18 European countries, with notifications across Asia, and reports in South Africa and Iraq (Boutier et al., 2015).

Carp edema virus disease (CEVD), also known as koi sleepy disease, is caused by a poxvirus associated with outbreaks of clinical disease in koi and common carp *Cyprinus carpio*. Originally characterised in Japan in the 1970s, international trade in koi has led to the spread of CEV but evidence of the disease outside of Japan was not reported until 1996 in the USA. In Europe, the disease was first recognised in 2009 and, as detection and diagnosis have improved, more EU member states have reported CEV associated with disease outbreak

Molecular epidemiological studies have shown significant genetic diversity in the genomes of both viruses, and disease investigations undertaken in the UK and China have identified significant deficiencies in the molecular diagnostic assays currently used to screen for, and then confirm infection with each of these economically important viruses.

In the case of CyHV-3 we are increasingly finding clinical disease associated with CyHV-3 that cannot be confirmed with the recommended real-time PCR assay used for surveillance or the conventional assay used to confirm infections with the virus. In the case of CEV, surveillance studies in the Republic of China using real-time PCR have identified heavy infections in diseased carp that could not be confirmed using the recommended conventional PCR assays, and vice versa.

We have aligned sequences available in the two organisations to redesign the problematic assays and evaluate the new assays; comparing the analytical sensitivity and specificity of the new assays using a broad range of KHV and CEV positive samples.

The results of our initial findings and the evaluation of the new assays will be presented.

Questions and comments:

Q: About the test you designed, what does it mean regarding notification of CyHV-3 positive cases? Because we have received some Koi and according to the results, they were not infected with Koi herpes virus, so we did not notify them.

A: Within the UK, they only act when there are clinical disease, and the clinical disease looks like KHVD. With the variants, we did not have clinical disease.

Q: We have done sybr green to differentiate the variants.

A: You could use sequencing as well.

Comment. The EURL organized a working group discussing the definition of KHV with experts from UK, Germany and Netherlands when we had designed the diagnostic manual for KHV, and we decide that variants were not KHV. According to you, we did not take the right conclusion?

A: According to the phylogenetic tree, I cannot see any difference with the variants.

Question from EURL: We have to address all these questions that you are raising because they are very serious. You are working in Nested PCR, but have you consider developing a qPCR?

A: We have developed a qPCR assay, all European cases have been detected with the new assay, and only the Chinese case was not detected. In a disease situation, you would not need a nested.

Modelling the economic impact of diseases in animal husbandry

Carsten Kirkeby[1,*], Maya Gussmann[1] & Tariq Halasa[1]

[1]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegårdsvej 8, 1870 Frederiksberg, Denmark.*

*ckir@sund.ku.dk

Abstract

The economic effects of diseases in dairy herds are highly important to farmers, and they have been investigated in numerous studies over the years. An important tool to assess these effects is bioeconomic simulation models. Different types of model can be useful, depending on factors such as the detail level and speed of the simulation. In epidemiology, a specific disease is often of interest, and it can be important to assess the probability of reducing the prevalence or even eradication when different actions are implemented. We here present some studies using mechanistic stochastic models to simulate the spread of paratuberculosis and mastitis pathogens within a herd. The models combine population dynamics and production with economic costs and benefits to simulate scenarios of interest. They include the latest research knowledge such as for instance direct and indirect transmission mode, which is crucial to the simulation outputs. These models can also illustrate the dynamics of multiple pathogens that are transmitted between the animals. By exploring possible scenarios for a given herd, the farmer can prioritize control actions, and thereby optimize herd economics. Thus, the models can be useful as decision support tools for the farmers, but also vets and herd health advisors can benefit from simulation studies.

Even though this presentation focuses on a dairy herd population, many parallels can be drawn to other populations such as fish farms, which often also have economic restrictions and can be challenged with disease transmission within populations. Many of the mechanisms, such as direct or indirect transmission of pathogens, can be used in simulations for fish populations.

Questions and comments:

Q: Have these models been based on estimates or real data?

A: It is real data, but also based on literature estimates

Q. You did not include the personal cost on a farm because that would also influence the borderline.

Q: It depends on if you inherit the farm or you just bought it, that is why we did not take it into account.

VHSV: Cell susceptibility, genotypes and virulence in rainbow trout

Se Ryun Kwon[1], Anna Luiza Farias Alencar[2], Argelia Cuenca[2], Niels Jørgen Olesen[2]

1 - Sunmoon University, Department of Aquatic Life Medical Sciences, Asan-si, ChungNam 336-708, Korea

2- Danmarks Tekniske Universitet, Institut for Akvatiske Ressourcer – DTU Aqua, Kemitorvet Building 202, 2800 Kgs. Lyngby, Denmark

srkwon@sunmoon.ac.kr

Abstract

Viral Haemorrhagic Septicaemia Virus (VHSV) stands as one of the most serious fish diseases, with a wide range of susceptible species and varied mortality. In order to assess potential virulence markers, reverse genetics systems for VHSV have been developed over the last decade (Biacchesi *et al.* 2010; Ammayappan *et al.*, 2011; Kim *et al*., 2015) but all of them rely on *in vivo* testing of the generated mutants. These *in vivo* trials conducted in different environmental conditions can lead to a high level of discrepancy when comparing virulence among the same isolate in separate trials due to parameters such as size of the fish, titrer of virus, variation between tanks, temperature and others. We have recently implemented a reverse genetics system at DTU and we would like to have an screening alternative before conducting *in vivo* tests of the generated mutants according to the principles of 3R (replacement, reduction and refinement). *In vivo* correlation of VHSV virulence to *in vitro* virulence in primary cultures of gill epithelial cells has been suggested by Brudeseth *et al*. (2008) and to *in vitro* Mx gene expression by Cano *et al*. (2016). We have tested a wide range of VHSV isolates representing all known genotypes in four different cell lines in order to determine their susceptibility to infection with the various VHSV isolates. Genotypes I and Ia caused high titrers in all three cell lines, whereas Genotype II did not cause CPE in EPC or RTG-2 cell lines. BF2 cell line is susceptible to almost all VHSV isolates except Genotype IVc. The study also showed that VHSV isolated from rainbow trout gives higher titrers in RTG-2 whereas the low virulent isolates from marine fish species give low titrers in RTG-2.

Questions and comments:

Q: How have you identify the culture?. How can you differentiate between RTG2 and BF-2?

A: All cell lines have been barcoded, so we know that they are RTG2 and BF-2. It has been confirmed that it is a rainbow trout cell line.

Q: You can increase the titer in RTG2 cell of the wild type isolates if you do a repetitive passage in RTG2 cell, and then you can see if they get more virulent in rainbow trout.

A. Yes, it could.

Full genome sequence analysis of VHSV, in the search of virulence markers

Argelia Cuenca[1], Farias AL[1], Panzarin V[2], Toffan A[2], Blanchard Y[3], Ryder D[4], Bremont M[5], Morin T[6], Olesen NJ[1]

[1]EURL for Fish and Crustacean Diseases, DTU aqua, Denmak, [2]IZSVe, Padova, Italy, [3]Anses, Ploufragan-Plouzané-Niort Laboratory, GVB Unit, Ploufragan, France, [4]CEFAS, Weymouth, UK, [5]INRA, Paris-Saclay University, Jouy-en-Josas, France, [6]Anses, Ploufragan-Plouzané-Niort Laboratory, PVP Unit, Plouzané, France

arcun@aqua.dtu.dk

Abstract

VHSV is of great concern in the European Union, being the most threatening pathogen for the European trout industry. The identification of VHSV virulence markers is of utmost importance both to predict the in vivo phenotype of viral isolates and to design molecular tests with prognostic value. As part of a European cross-national project, six laboratories from five countries have acted jointly to compile the largest dataset of in vivo virulence data combined with genetic information, with the aim of identifying the molecular markers responsible for VHSV virulence in rainbow trout (*Oncorhynchus mykiss*).

For this work, a panel of 68 VHSV isolates covering all European genotypes was selected and virulence degree (low, moderate, high) was assessed. Simultaneously, full genome sequences were obtained for each isolate and phylogenetic reconstruction carried out.

A high variability in terms of in vivo phenotype was observed, with rainbow trout survival rates ranging between 0 and 100%. VHSV virulence has a strong phylogenetic basis, with almost all isolates highly virulent for rainbow trout clustering within genotype Ia. However, no clear segregation over the tree was found for viral isolates belonging to the moderate and low virulence classes. In fact, for moderately virulent viruses it was not possible to identify virulence determinants, making it difficult to predict there in vivo phenotype.

Funding: *The present work was funded by the Anihwa ERA-Net Consortium (Novimark project, contract G88F13000660001) and the UK Department for Environment, Food and rural Affairs (Defra) (contract* C7577B).

Questions and comments:

Q: You want to reverse genetic, do you want to convert from avirulent or nonpathogenic to pathogenic.

A: Yes. That is what we are doing.

Q: You can find SNPs, but they can be interdependent.

A. The initial idea was to find a pattern, but it is too complicated at the moment. We are trying to re-analyze the data using different parameters to find virulence factors.

All the data presented is about rainbow trout.

Q: Is there any component that makes the virus to replicate more in one species and not in another.

A: We have found some variability in M protein, which has been associated with the virus budding. It can be that the virus can get in, but it cannot get out of the cell.

Interpretation criteria for antimicrobials used in farmed fish – the background of the problem

Agnieszka Pękala-Safińska[1], Ewa Paździor[1], Dariusz Wasyl[2], Kees Veldman[3]

[1]Department of Fish Diseases, [2]Department of Microbiology and Department of Omics Analyses, National Veterinary Research Institute, Pulawy, Poland;
[3]Wageningen Bioveterinary Research, Department of Bacteriology and Epidemiology, Lelystad, Netherlands

A.Pekala@piwet.pulawy.pl

Abstract

Background: Despite the large number of available antibacterial substances, only a few of them have found practical applications in the treatment of bacterial infections in fish. However, the interpretation criteria for pathogenic bacteria for fish are not available. Therefore, the aim of this research is to start establishing interpretation criteria for antimicrobials used for treatment of bacterial infections in farmed fish.

Methods: The following bacteria were used: *Aeromonas hydrophila* (n=93), *Aeromonas sobria* (n=28), *Pseudomonas fluorescens* (n=60), and *Shewanella* spp. (n=59). Susceptibility to quinolones, phenicols, sulfonamides, trimethoprim and tetracyclines were determined by disk-diffusion method. The minimal inhibitory concentrations (MICs) of above agents were also determined with user-defined POLARGEN Sensititre plates (Thermo Fischer Scientific).

Results: Disk-diffusion method indicated the highest proportion of susceptible strains to florfenicol for *A. hydrophila*, *A. sobria*, and *Shewanella* spp. (75%, 78%, and 86% of strains, respectively). Resistance was observed among 19% of *A. hydrophila* strains to oxytetracycline, 77% of *P. fluorescens* to florfenicol, and 96% *Shewanella* spp. to sulfonamides. *A. hydrophila* showed increased MICs for sulfamethoxazole, oxytetracycline and flumequine, *A. sobria* and *Shewanella* spp. only for sulfamethoxazole, and *P. fluorescens* for sulfamethoxazole, flumequine, and florfenicol.

Conclusion: Our study indicates that antimicrobials resistance in ichthyopathology is present. Testing of collection of bacterial strains is a first step towards the development of interpretation criteria for antimicrobials used in combating bacterial infections in farmed fish.

This work was supported by Ministry of Science and Higher Education Donation No. 3173/7.PR/2014/2: "Scientific project financed from financial resources for science in 2014-2018 for the implementation of an international co-funded project", and IMPART project: "Improving Phenotypic Antimicrobial Resistance Testing and setting missing ECOFFs".

Questions and comments:

Comment from the audience it is very useful to have these data together and compile them with other ongoing and finalized project on the same topic.

Is production of large smolt in RAS a risk factor for ISA ?

Senior researcher Debes H. Christiansen

Faroese Food and Veterinary Authority

Debesc@hfs.fo

Abstract

Atlantic salmon farming is of major economic importance and accounts for around 50% of the Faroese export value. Since the Faroese ISA epidemic in the beginning of the millennium, the annual production has increased significantly and reached 86.000 tons in 2014. Although the industry ambitions have been to continue to grow to supply increasing global demands we have for the last couple of years seen a stagnation in production around 85.000 tons with a decrease to 79.000 tons in 2018. Furthermore, whereas Faroese Atlantic salmon production was labelled "best in class" with an average annual mortality post sea transfer of 7.5% from 2006 to 2013 the mortality has since increased and reached 17.2 % in 2018

The major driver for the adverse development of Faroese salmon production is sea lice infestation, which today is one of the costliest challenges facing the global salmon farming industry. Chemotherapeutics such as emamectin benzoate (Clice™) kept sea lice in check for many years. But by 2008 the first signs of the parasite starting to become resistant against Slice emerged. Since, a vast array of methods focusing on solutions for controlling or even eradicating the sea lice have emerged. Today, bath treatment in heated water (mechanical treatments) and cleaner fish are among the main strategies used for controlling sea lice in the Faroes. However, mechanical treatment impose major fish health and welfare issues. Therefore, the production of large smolts (>1 kg) to reduce time in the marine environment form the traditional 18 – 24 months down to 6 – 12 months is another strategy for reducing the impact of sea lice. Thus, the Faroese farming industry has for the last eight years invested massively in new and bigger RAS farms.

One important question is whether production of large smolt in RAS farms is a risk factor for the development and spread of infectious salmon anaemia (ISA). We have documented that the non-virulent subtype of infectious salmon anaemia virus, ISAV-HPR0, is present in all three production compartments of Atlantic salmon i.e. in brood stock farms, in smolt farms and in marine farms. Furthermore, we recently documented that ISAV-HPR0 is the progenitor and reservoir for all virulent ISAV and thus represent a potential risk factor for the emergence of ISA (Christiansen *et al*. 2017 *Journal of Virology*). The specific factors driving this evolution are unknown, but duration of infection and stress seems to be key factors.

Here I discuss the potential risk for ISA in large smolt and the importance of high biosecurity in closed-containment systems of the land-based smolt production facilities.

Questions and comments:

Q. Are you sure that the HPR delta that was found in the sea corresponds to the HPR0 found in freshwater.

A: Yes. They were the same. We have done a full genome sequencing of both isolates and the only differences were the two pathogenic mutations.

Comment from the audience: The stress is because poor management and not because is a RAS. People do not know how to work with RAS, because with RAS, it should be possible to provide the best condition for the fish.

Comment from the speaker: There is more control in RAS when you have the optimal conditions. But at the moment we are facing challenges .

Comment from the audience . About reducing the stress. It is not just keeping the fish in RAS, it is about smoltification. Also, it is necessary to compromise something, so it would be good to stop the system and disinfect the filters.

Q: Stress and time are major players. Do you believe that a high prevalence of HPR0 increases the risk of HPRdeleted?.

A: There are many factors that could increase the risk. But the risk is low otherwise we would have seen more cases. We have to consider that all fish in Faroe island are vaccinated against ISA.

Q: ISA HPR-deleted could be under-detected. Do you know how good the surveillance of ISAV is?

A: It is necessary to test a lot of fish very often to detect HPR0. If it is worth it? I do not think so.

Comment from the audience: The purpose of growing large smolt is to go under fewer treatments against sea lice.

Comment from the audience: If you have a bug in your system you need to clean your filters, but in my opinion, you are going to have weaker fish, it has been shown before.

Q. All fish are vaccinated against ISA. When do you do that?

A. They are 20-30 grams

Q: when do you detect HPR0?

A: Generally is when the fish are bigger, after vaccination. According to a previous talk, there is no difference in the prevalence of HPR0 between vaccinated and unvaccinated

Comment from the audience: It a challenge when we talk about the prevalence of HPR0 because it is a transient infection. When we talk about vaccines, we need to consider that it is a mucosal infection. About the transition from HPR0 to HPR deleted, beside the stress, it could be an interaction with other microbes, there is epidemiology support for that. About the RAS facilities, it is not a good idea to continuously grow fish because the pathogens are also continuously growing.

SESSION VI: Update from the EURL

Chair: Niels Jørgen Olesen

Technical report 2018 of the EURL for Fish and Crustacean Diseases

Niels Jørgen Olesen, Niccoló Vendramin

EURL Fish and Crustacean Diseases, DTU

njol@aqua.dtu.dk

INTRODUCTION The Technical University of Denmark (DTU) was confirmed appointed as the EU Reference Laboratory (EURL) for Fish Diseases in February 2018, and was in June 2018 granted an increase of its scope to Fish and Crustacean Diseases in accordance with the Amendment n° 1 to the grant decision for an action regarding the EU Reference Laboratory for Fish and Crustacean Diseases– SI2.777824 (Ref. Ares (2018)3294875 - 21/06/2018).

The duties of the EURL are described in Council Directive 2006/88/EC of 24 October 2006 (Annex VI). The duties mainly concern the fish and crustacean diseases listed as exotic diseases: Epizootic haematopoietic necrosis (EHN), taura syndrome, and infection with yellow head virus genotype 1; and diseases listed as non-exotic diseases: Infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN), koi herpes virus disease (KHVD) and white spot disease (WSD). This report follows the new format of the work programme adopted for the EURL for 2018, describing activities and sub-activities and the status of on-going projects. In contrast to previous years almost all technical descriptions are given in Annexes making the report shorter, all Annexes can be accessed on the website www.eurl-fish.eu.

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 us door to door with the National Institute of Aquatic Resources (DTU Aqua). It was therefore an appropriate decision to move the entire unit for fish and shellfish diseases and all its functions and duties including the EURL functions from DTU Veterinary to DTU Aqua when the closing of the DTU Veterinary institute became a reality in January 2018.
The transfer to the new institute has given us a number of new opportunities for collaborating with research teams working in the field of aquaculture and fisheries.

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. There were five sessions with a total of 28 presentations. The annual workshop for crustacean diseases was organised by the former EURL at CEFAS in UK and held in Italy before the transfer of the function to DTU.

The annual proficiency test (PT) was divided into PT1 and PT2 with 45 laboratories participating in PT1 while 42 participated in PT2. The tests were sent from the EURL 1st of October 2018.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.

During 2018, 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 2018, Dr. Nikolaj Reducha Andersen had until December 1st the responsibility as the Coordinator of the EURL for Fish Disease. Upon an international call for the position as EURL coordinator for crustacean diseases he successfully obtained this position – 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.

	Technical report
Work Programme for 2018 – Fish diseases part	
1.1. Annual workshop. *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.*	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. There were five sessions with a total of 28 presentations. *The report of the 22nd Annual Workshop is located in **Annex 1***

1.2. Scientific working group. 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.	In 2018, no scientific working group meetings were organized, as we found no acute need for organizing such (e.g. emerging disease situations or scientific questions to solve) *The list of visitors are located in **Annex 2***
1.3. Proficiency test. *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.*	An inter-laboratory proficiency test was provided by the European Union Reference Laboratory (EURL) for Fish and Crustacean Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2). 45 laboratories participated in PT1 while 42 participated in PT2. 2 laboratories were, due to internal clearance problems, not able to provide the answers before deadline. The tests were sent from the EURL 1st of October 2018. *The reports of the Proficiency Test 2018 are located in **Annex 3***
1.4. Novel molecular methods. *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.*	In 2018 the following 2 new diagnostic qPCR methods were introduced in the laboratory: PRV-3 qPCR for surveillance purposes (validation of pooling procedures) BKD qPCR for surveillance purposes.
2.1. Training. *To ensure that employees of the Member State NRLs have the highest scientific and excellent skills in diagnosis of fish diseases.*	Two training courses were successfully organized from October the 8th to 19th, 2018. The two courses prepared were: "Methods for implementation of surveillance procedures for listed fish diseases" with 11 participants and "Introduction to histopathology in fish and crustacean diseases" with 15 participants. The majority of the participants evaluated the courses "very good". *The report of the 2018 training courses is located in **Annex 4*** *A list of Phd and Master Students are located in **Annex 5***

2.2. Webpage. *To provide the Member State NRLs with a fast entrance to information from the EURL.*	The EURL website was constantly updated during 2018 with reports and news from the EURL. The website has been accessed 6098 times; in total 18882 pages of the website has been accessed during 2018. *Link to the website: www.eurl-fish.eu*
2.3. FishRefLabNet. *To ensure that relevant and important information rapidly can get from the EURL directly to the Member State NRLs.*	The e-mail list FishRefLabNet have been continuously updated during 2018 and now contain 145 people with interest in our work. The list now includes all the NRL contacts for the Crustacean Diseases.
2.4. Molecular epidemiology. *To improve knowledge on disease spreading mechanisms within the EU.*	Molecular epidemiological analyses for Piscine orthoreovirus were done. PRV represents a treat to farmed salmonids in Europe. In concrete, we studied the prevalence of PRV-1 for wild salmonids returning to Danish rivers and compared them with isolates in the North Atlantic area. For PRV-3 we studied its possible introduction in Europe in 2017, as well as its characterization, prevalence and molecular characteristics.
2.5. Producing virtual teaching material (e-learning). *To provide the Member State NRLs with "hands on" videos to be used for teaching of staff members.*	In 2018 the EURL created a YouTube channel called "EURL for fish diseases". This channel is used for uploading teaching material regarding proficiency testing and upcoming courses. Currently one video showing how to open proficiency test ampoules has been uploaded. *Link to the YouTube channel here*
2.6. Missions. To ensure *a high standard of diagnostic capabilities of all Member State NRLs.*	A mission to the NRL in Norway was successfully organized in December 2018. *A report of the 2018 Norway Mission is located in **Annex 6***
2.7. International meetings. *To keep the EURL updated on the newest scientific information on emerging and listed exotic and*	EURL employees and members of the fish and crustacean unit at DTU participated in 9 international meetings and conferences and gave 23 oral presentations. The Unit authored 18 publications in Peer reviewed journals.

non-exotic fish diseases, and to disseminate knowledge and scientific data provided by the EURL.	*List of 2018 international meetings and conferences are located in **Annex 7*** *List of 2018 peer reviewed publications is located in **Annex 8***
3.1. Diagnostic manuals. *To have updated diagnostic manuals for all listed fish diseases available for Member State NRLs on the EURL website www.eurl-fish.eu.*	The sampling and diagnostic procedures for detection of VHS, IHN, ISA, KHV, EHN and EUS were kept and updated at our web side. Link to the manuals: http://www.eurl-fish.eu/diagnostic_manuals
3.2. Survey and diagnosis. *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*	The report, based on data from the questionnaire on Survey and Diagnosis of the listed fish diseases in Europe (S&D) for 2017 send from the EURL to all NRLs was prepared and presented at the AW 2018 *The 2017 S&D report is located in **Annex 9***
3.3. Emerging diseases. For the	In 2018, activities on emerging diseases have focused on PRV-3 infection in salmonids. This has also been topic of PhD project of Niccoló Vendramin.

<table>
<tr><td>EURL to have the most updated and highest scientific knowledge of emerging and re-emerging fish diseases in Europa.</td><td>A list of publications in relation to PRV-3 is attached to Annex 12.</td></tr>
<tr><td>4.1. Pathogen library.
For the EURL to have an updated library of fish pathogens relevant for the EURL and Member State NRLs.</td><td>
<table>
<tr><th colspan="3">Member States and countries outside EU</th></tr>
<tr><th>Material received in 2018</th><th>Laboratories</th><th>Units</th></tr>
<tr><td>Diagnostic material for virology</td><td>4</td><td>27</td></tr>
<tr><td>Diagnostic material for PCR</td><td>3</td><td>33</td></tr>
<tr><td>Diagnostic material for bacteriology</td><td></td><td></td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td>Diagnostic material for sequencing</td><td>3</td><td>33</td></tr>
<tr><td>Virus panels or PCR controls</td><td>6</td><td>55</td></tr>
<tr><td>Cell cultures</td><td>1</td><td>2</td></tr>
<tr><td>PRV3 project</td><td>2</td><td>93</td></tr>
</table>
is given Annex 10
In summary the number are given in the table below:</td><td>The EURL received a large number of reagents and pathogens in 2018.
All reagents received at the EURL in 2018</td></tr>
</table>

	Other materials and Proficiency Tests | 5 | 76
4.2. Pathogen characterization. *For the EURL to be able to identify and characterize isolates of listed viral fish pathogens on request from the Member State NRLs.*	In 2018 the EURL has received 2 panels of reference isolates of Infectious Salmon Anemia Virus (ISAV) from Norway and Nodavirus (NNV). The isolates are genetically characterized and will be included in the upcoming proficiency test. *A description of the ISAV and Nodavirus panels is given in **Annex 13**.* We provided support to National Reference Laboratories in the molecular characterization of IHNV isolates occurring in their country. Infection trials were conducted with IHNV from Finland in order to assess and compare the Finnish IHNV virulence to rainbow trout. The survival of fish infected with the Finnish IHNV isolates was equivalent to the survival of the positive controls using a high virulent European IHNV isolate. Isolates from the first outbreak of IHN in Estonia in 2018 were likewise sequenced and characterized.
4.3. www.fishpathogens.eu. *To have an updated database of all serious viral fish pathogens in the EU.*	The code of the database was updated, and a mobile app was developed. As the use of google maps is not any longer free, we changed to freeware software to show maps and distributions of isolates The VHSV database was updated, all isolates cleaned and made open access. Discussions with Norway addressing the possibility of stablishing an ISAV database were started.
4.4. Production and supply of reagents. *For the EURL to be able to quickly provide Member State NRLs with diagnostic reagents.*	The EURL supplied a large number of pathogens and reagents to the NRL´s in EU Member states and related countries. *List of reagents are located in **Annex 11***
5.1. New animal health law. To prepare regulations related to the new animal health law.	The experts of the EURL were involved in giving advice to the content of delegated act, lists of susceptible species and consultancy concerning specific questions raised by the Member states to the Commission.

5.2. Listing susceptible species. *For the EU Member States to have an updated list of susceptible species for the listed fish diseases.*	N. J. Olesen is participating in an ad hoc working group of the OIE assessing the susceptible fish species to the OIE listed fish diseases. The list and the outcome of this work will most likely be inserted in the list of susceptible species given the Animal Health Low and its delegated acts of EU. During this work it was realized that the susceptibility to infection with VHSV and IHNV of the very important aquaculture species Sea Bass (*Dicentrarchus labrax*) is very poorly described, therefore experimental infection trials were conducted on Sea bass juveniles with 1 IHNV and 4 VHSV isolates. The study confirmed that the fish were susceptible to VHS by intraperitoneal injection but the virus was not transferred to co-habitants in the same tanks. It was therefore concluded that sea bass are relatively resistant to VHSV and IHNV infection. The work will be published in 2019 *Report on the infection trial is given in **Annex 14***

Questions and comments:

No comments

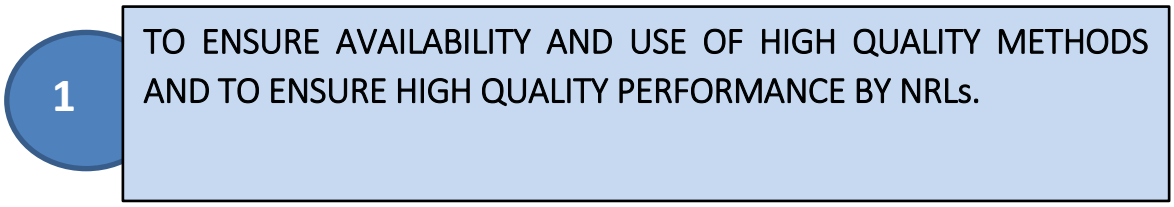
Work programme 2019-2020 of the EURL for Fish and Crustacean Diseases

Niels Jørgen Olesen, Niccoló Vendramin

EURL Fish and Crustacean Diseases, DTU

njol@aqua.dtu.dk

Compared to previous years the work programmes of the EU reference laboratories have become much more detailed specifying objectives, resources, outputs and durations. The work programme for the EURL for Fish and Crustacean Diseases for 2019 and 2020 is given in the following:



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

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 23rd and 24th annual workshop of the National Reference Laboratories (NRLs) for fish diseases in 2019 and 2020, respectively.

Description: These workshops are organised as annual event and all Member State NRLs are strongly recommended to participate in them, as it is an important opportunity to be updated on the newest scientific knowledge of fish pathogens, diagnostics, legislation, epidemiology 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 23rd and 24th annual workshop comprising two full days in May 2019 and 2020. Based on previous experience it is expected that 50 participants will attend the workshop including EU Member States, associated countries and invited speakers. From the EURL team six members will attend the workshop full time. A technical and financial report of the workshops will be produced. The technical reports 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 2019 and 2020. Preparation in February – April and finalizing of the reports in May – August.

Sub-activity 1.3 *(Scientific working groups)*

Objectives: To ensure that fast and reliable scientific advice on specific topics related to listed and emerging diseases and to legislative issues, is provided by organising expert meetings in order to solve arising challenges in EU.

Description: In case of critical fish or crustacean disease related problems within EU Member States, we will organize specific scientific meetings by collating international experts.

Expected Output: We expect to organise four scientific working groups in 2019 and 2020 with the duration of one to two days each. A working group on 1) susceptible fish species to listed diseases in EU, 2) assessing fish and crustacean diseases for possible listing in EU legislation, 3) emerging diseases. The topic of the emerging disease working group will be defined in relation to ad hoc request. From each meeting, a scientific report including recommendations will be delivered to the relevant Member State NRLs and the European Commission and will be available on our website www.eurl-fish.eu.

Duration: Working group 1 and 2 in 2019 and working group 3 in 2020; the timing of working group 4 held will be decided depending on specific need. The meetings will comprise one to two days in Copenhagen and time for organising and reporting.

Sub-activity 1.4 *(Proficiency test fish diseases)*

Objectives: To assess the capabilities of all Member State NRLs to detect pathogens causing fish diseases and to harmonize the procedures used by an inter-laboratory proficiency test.
Description: The EURL is going to prepare Annual Inter-laboratory Proficiency Tests for all Member State NRLs. The tests will include the viral fish 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 a restricted site of 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 Workshops in 2019 and 2020. 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 tests 2019 and 2020. Based on previous experience it is expected that 45 laboratories are participating with a success rate of > 90 percentage for both tests. Underperformances will be addressed by direct communication with the participant. Underperforming laboratories will be considered for mission from the EURL.
Duration: January – December 2019 and 2020. The samples included in the test will be shipped from the EURL in the fall and the final report will be submitted February the following year.

Sub-activity 1.6 (Diagnostic methods)

Objectives: For the EURL to have 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. In 2019 and 2020, the EURL will focus on four techniques; 1) PCR for detection of genomic RNA/DNA from pathogens, 2) In-situ Hybridization (ISH) for pathogen localization in paraffin embedded tissue, 3) Next Generation Sequencing for full genome sequencing and 4) Improved cell culture techniques. In 2020 the EURL will establish a repository of reference viral strains for Infectious salmon anemia virus (ISAV) and implement diagnostic qPCR able to discriminate virulent ISA strains HPRΔ and non-pathogenic ISA strains HPR0. With the ISH technology established in 2019, the main pathogens targeted in 2020 will be VHSV and the emerging pathogen PRV-3. Expected Output: Four new diagnostic methods implemented in the two year period. Four diagnostic molecular methods validated according to the recommendations given by the OIE.
Duration: January – December 2019 and 2020.

2 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

Sub-activity 2.1 *(Training Courses)*

Objectives: To ensure that employees of the Member State NRLs have the highest scientific and excellent skills in diagnosis of fish and crustacean diseases.
Description: The EURL yearly provides two training courses in methods used for diagnosis of fish and crustacean 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. The course contents are also discussed during the annual workshops, where the Member State NRLs are able to provide specific input.
Expected Output: Two training courses of 5 days in 2019 and 2020, with 10-15 participants in each course; more than 90 % of the participants were satisfied with the course based on the 2018 evaluation.
Duration: September – October, 2019 and 2020.

Sub-activity 2.2 *(Website www.eurl-fish-crustacean.eu)*

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 and crustacean diseases. Due to the inclusion of crustacean diseases in the EURL we will 2019 launch a new and updated website. The new website will in the future be located at www.eurl-fish-crustacean.eu and the old one www.eurl-fish.eu will close. The website will be

further developed including a "restricted access area" where reports and information which are specific for targeted stakeholders will be uploaded.
Expected Output: A constantly updated webpage for the Member State NRLs. Establishment of a restricted area and provision of guidelines to all Member States NRLs for access to the restricted area.
Duration: The new website will be up running primo 2019 and maintenance will be from January – December 2019 and 2020.

Sub-activity 2.3 *(EURL Contact Lists)*

Objectives: To ensure that relevant and important information rapidly can get from the EURL directly to the Member State NRLs.

Description: We will aim to have three contact lists. 1) Member State NRLs for fish diseases, 2) Member State NRLs for Crustacean disease and 3) a general list which all interested in the work of the EURL can subscribe to. The EURL use the mailing lists 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 website or other sites for further and detailed information.

Expected Output: The EURL usually prepare and submit around 10-15 notifications per year via the contact lists to ca. 130 subscribers.

Duration: January – December 2019 and 2020.

Sub-activity 2.4 *(Missions to NRLs for fish diseases)*

Objectives: To ensure a high standard of diagnostic capabilities of all Member State NRLs.

Description: Missions are only planned to Member State NRLs for fish diseases, however, we will be able to conduct missions to NRLs for crustacean diseases if it is found necessary. NRLs 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. Two missions per year conducted from the EURL, first draft of the report of each mission provided to the host institution within 1 month from the mission Duration: April and/or November 2019 and 2020.

Sub-activity 2.5 *(International conferences and meetings)*

Objectives: To keep the EURL updated on the newest scientific information on emerging and listed exotic and non-exotic fish and crustacean diseases, and to disseminate knowledge and scientific data provided by the EURL.Description: The EURL staff is able to provide consultancy to Member State NRLs on emerging and listed fish and crustacean 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 4 to 6 international conferences e.g. the 19th International Conference on Diseases of Fish and Shellfish, Porto, Portugal 9th-12th September 2019, OIE

international conference on aquatic animal health, Santiago, Chile 3-4, April, 2019, The 11th International symposium of virus of lower vertebrates and the 5th Nordic RAS Workshop 7-8 October 2019, Berlin.

Duration: January – December 2019 and 2020.

Sub-activity 2.6 *(Confirmatory diagnosis)*

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

Description: Every year the EURL receives strains of 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 serological and genetic characterization, including bioinformatics.

Expected Output: Based on experience from the previous year, the EURL expects to corroborate the diagnosis for five new outbreaks and sequence the isolates yearly

Duration: January – December 2019 and 2020.

Sub-activity 2.7 *(Pathogen characterization)*

Objectives: For the EURL to be able to characterize isolates of listed viral pathogens of aquatic animals as well as emerging pathogen and provide scientific based risk assessment to the scientific community and stakeholders. Description: The EURL every year contributes to characterize relevant pathogens for aquaculture in Europe as an emergency response to avoid unwanted spreading of new pathogens in EU. The EURL describe these strains by pathogenicity testing in-vivo. The experimental trial contribute to establish reference material to be used as positive controls and standards enabling diagnostic validation of new diagnostic methods.

Expected Output: The EURL expect to characterize two pathogens per year. A report of each single infectious trial included in a risk assessment report and/or published in peer review journals. Duration: January – December 2019 – 2020.

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

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

Objectives: To have updated diagnostic manuals for all listed fish diseases available for Member State NRLs on the EURL website.

Description: The diagnostic manual for sampling and detection of listed non-exotic diseases was finally adopted in 2015. However, 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 manuals 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 2019 and 2020.

Sub-activity 3.2 *(Diagnostic manuals crustacean diseases)*

Objectives: To have updated diagnostic manuals for all listed crustacean diseases available for Member State NRLs on the EURL website.

Description: The diagnostic manual for sampling and detection of listed non-exotic diseases was finally adopted in 2015. However, 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 manuals for the viral crustacean diseases White Spot Disease, Taura Syndrome and Yellowhead Disease on the EURL website.

Duration: January – December 2019 and 2020.

Sub-activity 3.3 *(Survey and diagnosis fish diseases)*

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 disease outbreaks from NRLs in all European countries by submitting a questionnaire and disseminating the information gathered in a report and at the Annual Workshop. The data are collated in a "Survey and diagnosis" report, which is made available for the Commission, Member State NRLs and for approved users 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.

Expected Output: A report on "Surveillance and diagnosis of fish diseases in Europe". The report will be presented at the annual workshops and uploaded in the restricted area of the website. The report will be accessible for relevant stakeholders including NRLs and EU commission

Duration: January – June 2019 and 2020.

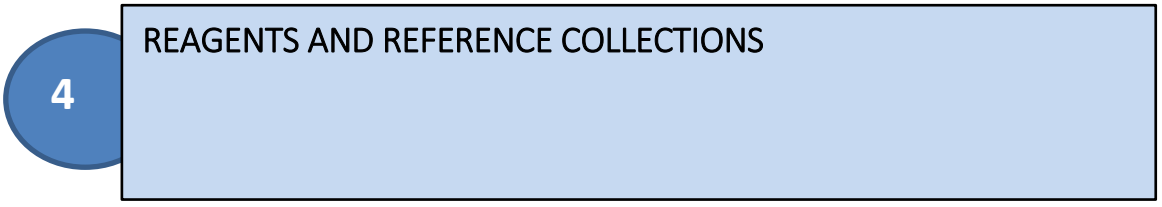
Sub-activity 3.4 *(Risk assessment for emerging diseases)*

Objectives: For the EURL to have the most updated and highest scientific knowledge of emerging and re-emerging fish and crustacean diseases in Europa.

Description: Due to increased international trade of fish and crustaceans, 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 collaboration with specialised experts the EURL foresee to work e.g. with the emerging fish pathogens Infectious Salmon Anemia virus (ISAV), Tilapia Lake Virus (TiLV), Salmonid Alphavirus (SAV) and Piscine Myocarditis Virus (PMCV) in Europe to be able to assess their potential listing as exotic or non-exotic diseases in the future.

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, the European Commission and stakeholders. Scientific knowledge on specific emerging diseases will be disseminated through oral and written presentations in scientific journals (1 publication per year), at annual workshops, conferences (1 oral presentation per conference) etc. The EURL aims to assess diagnostic methods and establish reference material for validating diagnostic methods. Two diseases will be addressed yearly.

Duration: January – December 2019 and 2020.



Sub-activity 4.1 *(The database 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. The more isolates included the stronger the tool for the EURL and Member State NRLs. The database code is, however, more than 10 years old, and an urgent update is needed. This update, together with the addition of new tools to handle full genomes, is already in process and will continue during 2019.

Expected Output: During 2019, around 110 full genome sequences of VHSV will be included in the database, as well as around 30 full genomes of IHNV. Both SAV and Betanodavirus databases will be modified to include full genome data, as well with tools to detect/identify reasserting strains in betanodavirus (2020). In addition, collaboration with groups in Norway will be initiated in order to stablish a new database of infectious salmon anaemia virus (ISAV) isolates (2019-2020).

Duration: January – December 2019 and 2020.

Sub-activity 4.2 *(Pathogen library)*

Objectives: For the EURL to have an updated library of fish and crustacean 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 yearly, furthermore, infected tissue material originated from the infectious trial conducted within the "Pathogen characterization" sub activity (two tissue libraries per year) will be made available upon request to Member State NRLs as positive control material (expected to ship five panel per year).

Duration: January – December 2019 and 2020.

Sub-activity 4.3 *(Production and supply of reagents)*

Objectives: For the EURL to be able to 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 yearly. However, we are able to provide more reagents if there is a need from more Member State NRLs.

Duration: January – December 2019 and 2020.

5 REQUIREMENTS RELATED TO OTHER LEGISLATION

Sub-activity 5.1 *(Scientific advice in relation to aquatic animal health legislation)*

Objectives: For the EU commission and Member States to access scientific based advice on interpretation and implementation of aquatic animal health law.

Description: To harmonize implementation and interpretation of aquatic animal health law across the different Member States.

Expected Output: The EURL expect to receive 10 specific request per year from EU or Member States. First reply within five working days. Final deliver of official reply may change according to the entity of the request.

Duration: January – December 2019 and 2020

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 and crustacean diseases.

Description: With implementation of the new Animal Health Law, there is an acute demand for scientifically assessing the fish and crustacean species susceptible to the listed diseases. Therefore, an increased workload for the EURL will be to assess the listing of susceptible fish and crustacean 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 and crustacean species are susceptible to the listed diseases, to be recommended for adaptation in the new legislation.

Duration: January –March 2019.

Sub-activity 5.3 *(Listing diseases for notification)*

Objectives: For the EU commission and Member states to access scientific based advice on criteria for including or excluding infectious diseases in new Aquatic animal health law.

Description: The EURL provides scientific based advice assessing new putative listed diseases for inclusion or exclusion from the EU legislation. Criteria for including a disease are clear knowledge of aetiological agent, possibility to controlling and limiting the spread of the disease, diseases with severe impact on animal welfare and economy on aquaculture production in EU.

Expected Output: The EURL expect to assess two diseases per year, and provide scientific recommendation for including or exclusion them from the legislation.

Duration: Upon request from the Commission in 2019 and 2020.

QUESTIONS AND ANSWERS

Q- will you publish infection in sea bass –

A- Yes

Q- education to field veterinarians on how to sample

A- it is included in the first training course. It is important to collect the right material but we primarily have to focus on laboratory side

Q- which test to use to KHV after presentation this morning from David Stone?

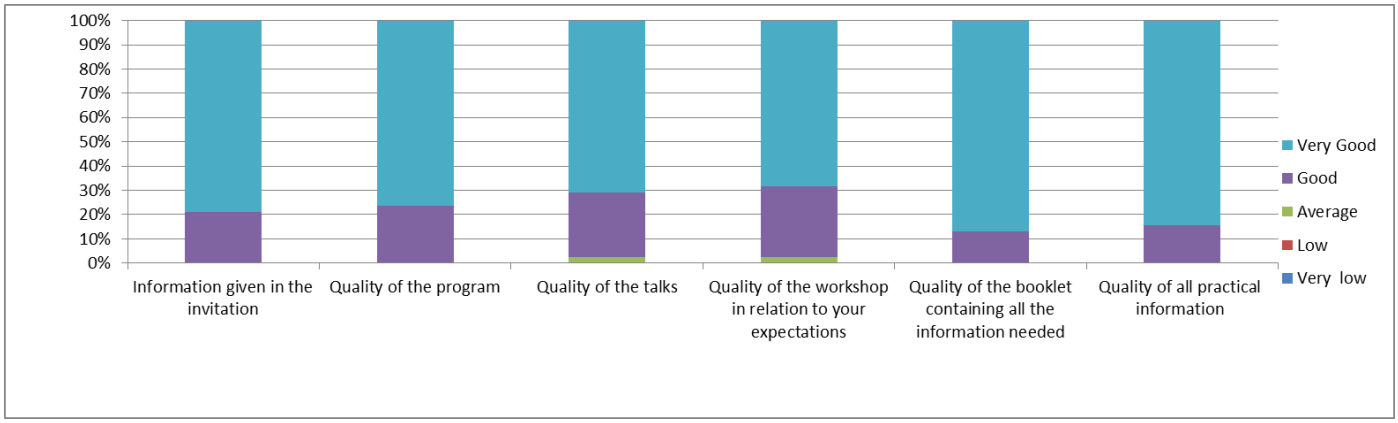
A- point taken, even KHV is now a list E disease it is important to address this question in the near future.

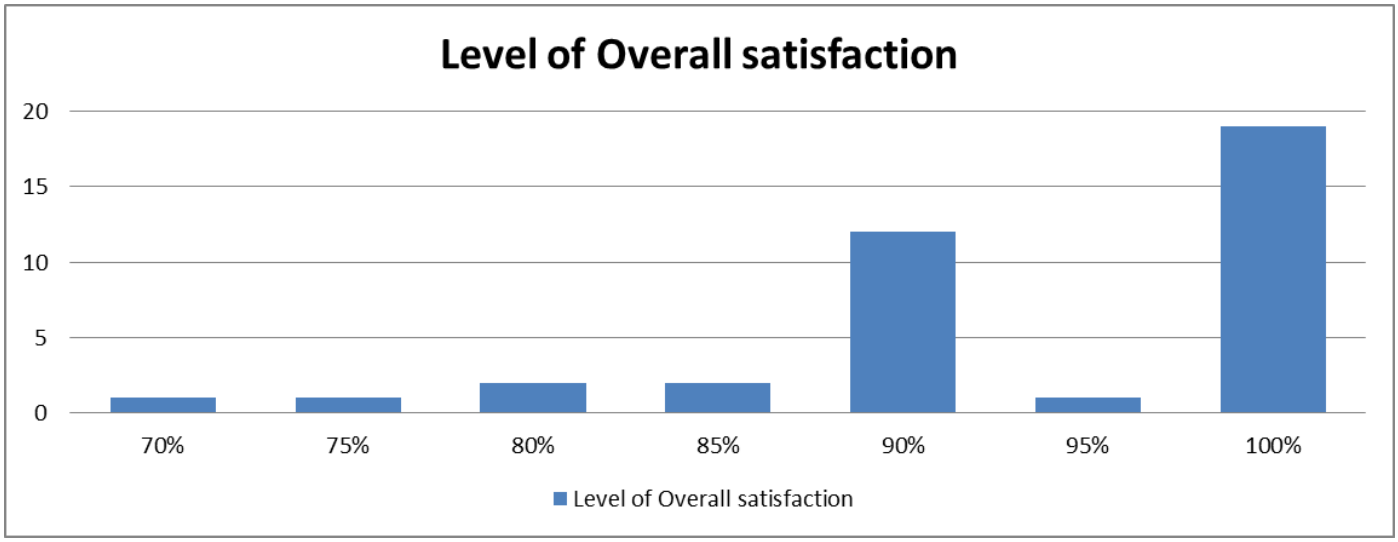
Comment from audience disease definition in farmed and wild fish

It was decided that the 24th AW for NRL for fish diseases will be held on 3-4 june

Workshop evaluation

A questionnaire was delivered to the participants asking to evaluate various aspect of the workshop. An overview of the 38 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 the 2nd -3rd and -4th June 2020. 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