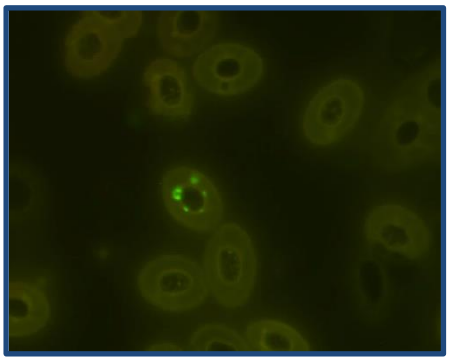
Report:

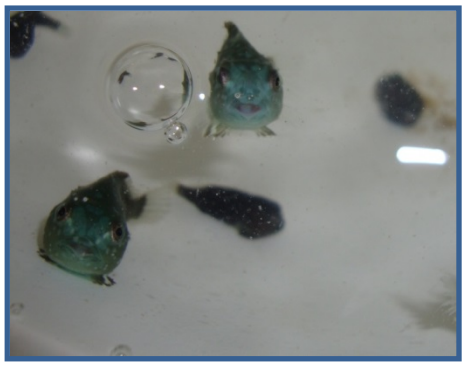
20th Annual Workshop of the National Reference Laboratories for Fish Diseases

Copenhagen, Denmark
May 31st – June 1st 2016

Red mark syndrome in Rainbow trout



Atlantic Salmon Red blood cells with intracytoplasmatic inclusions



Lumpfish (*Cyclopterus lumpus*)

Organised by the European Union Reference Laboratory for Fish Diseases

National Veterinary Institute, Technical University of Denmark

Contents

INTRODUCTION AND SHORT SUMMARY

The 20th Annual Workshop of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark,31st May and 1st of June at the Auditorium of DTU Veterinary Institute, Bülowsvej 27, 1870 Frederiksberg C., Denmark
A total of 65 participants from 33 countries attended over the two days period; unfortunately one participant cancelled his participation right before the meeting leading to minor re-arrangements of the program. There were five sessions with a total of 29 presentations, 3 of which were given by invited speakers, a working group session and a round table discussion. The scientific program of the Annual Workshop was wide and covered many different topics of current interest.

The meeting was opened with the traditional session "Update on important fish diseases in Europe and their control", where participants from the Member States had the opportunity to present new findings from their respective countries.
Initially, an overview of the disease situation and surveillance in Europe 2015 was provided on the basis of the results obtained from the Survey & Diagnosis questionnaire, this year a new questionnaire has been used in order to make data and results more accessible, a report compiling all information will be made available on the EURL website http://www.eurl-fish.eu/Activities/survey_and_diagnosis.
Then the fish disease situation in Norway was presented; a detailed report in Norwegian is available at: http://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2016/fiskehelserapporten-2015 with the English version at: http://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2016/fish-health-report-2015
The results of a survey on the impact of fish disease in the Mediterranean basin, achieved thanks to the collaboration with a panel of acknowledged fish pathologist experts, were presented.
The results of experimental trial conducted in the experimental facilities of DTU VET with newly described PRVom in rainbow trout were presented.
The last talk of the first session described results of survey conducted in Iceland for PRV in wild and farmed salmon.

The second half of the morning was allocated to an interactive activity, introduced for the first time during the Annual Workshop in 2014. Briefly, each participant was asked to consider the relevant infectious diseases for the 2 most important fish species farmed in his country. In this perspective, all participants received on beforehand 4 tables listing the most renowned pathogens for the most important farmed fish species in Europe. Before the WS each participant had the opportunity to interact with different stakeholders and assess impact on production, economy and legislative consequences for the different infectious diseases in 2015.
During the first part of this working group session, the activity was implemented at a country level, meaning that each participant was asked to rank the disease characterized by the higher impact in 2015. After this first level of investigation, representatives of different macro-areas in Europe were grouped. The regions were Northern Europe, gathering the main Salmon producing countries, Eastern Europe focusing mainly on cyprinids and subsequently rainbow trout, Western Europe producing mainly rainbow trout and cyprinids and finally Southern Europe producing mainly the marine species European sea bass and gilthead sea bream and then rainbow trout. Experts had the possibility to discuss and describe the impact of each disease focusing on the 3 most important parameters. The first topic considered was the impact of a given disease on production, meaning the severity of losses in terms of

mortalities, reduction of growth, etc. Then impact on the economy, indicating the cost of prevention (i.e. vaccination and biosecurity measures), treatment, reduced value of the product was considered. Finally consequences due to trade restrictions, national plans for control/eradication, suspension time after antibiotic treatment etc. Each group had to finalize its task by selecting and describing the most important diseases.
During the final part of the session a representative of each area described the agreed findings to the whole assembly.

The second session of the WS was dedicated to Emerging diseases.
Firstly, the invited speaker from France presented a review of sturgeon viruses and the diagnostic challenges posed by these viruses.
This was followed by two combined presentations describing the detection of two viral pathogens (one Ranavirus and VHSV of genotype IV) in lumpfish in Iceland and the experimental activities conducted that the facilities of EURL at DTU Vet to assess the risk for farmed salmonids and lumpfish of this new VHSV.
The following presentation addressed the outbreak of nodavirus in Gilthead seabream, for many years this fish species has been considered resistant to the virus but re-assortant strains have shown to affect especially larval stage of this host.
This session was closed by presenting the results of an experimental trial conducted in Atlantic Salmon looking into the susceptibility of this host for European strain of IHNV and interaction with infection with PRV.

The third session, on control and surveillance of relevant pathogens in the EU, started by a presentation describing epidemiological investigation and management of VHSV detected in Atlantic salmon in France in 2015.
This was followed by a presentation of the "diagnostic manuals" finally adopted and fully implemented March 2016 and available at http://www.eurl-fish.eu/Diagnostic_Manuals
The third presentation provided interesting overview of the OIE aquatic animal commission.
Finally an overview of the structure of the new aquatic animal health law with focus on criteria for disease listing was given.

In the evening a banquet dinner was held at Restaurant ***"Spise Loppen"*** in Christiania.

The second and last day was opened by the session on scientific research update; this fourth session faced several different topics covering molecular characterization of pathogens, development of new diagnostic techniques including serology, conventional PCR and Real Time PCR, cell cultures; and characterization and description of new viral fish pathogens belonging to poxvirus group.
The session started with a presentation addressing the use of serology in finfish diagnosis, first a systematic review was presented and subsequently the specific use of serology in KHV diagnosis was presented.
The session continued with a presentation describing if and how molecular characterization and epidemiology has to be combined while addressing the tracing of viral fish disease.
Afterwards the development and validation of a conventional RT-PCR protocol for detection of VHSV that overcome pitfalls of already available methods have been presented. This work has been developed within an OIE twinning project between South Korea and Denmark.
After the coffee break, the topic of infection with poxvirus in farmed fish was presented. First the infection in salmon describing different type of diagnostic cases was presented by invited speaker from Norwegian Veterinary Institute.
Subsequently the outcome of investigations of Carp Edema Virus in cyprinids and networking activities across Europe in regards to this virus were presented by Dutch NRL representative
The session continued presenting the use of salmon embryonic cell lines in detection of fish rhabdovirus.
The presentation were finalised by the description of novel paramyxovirus in UK.
The participant from Faroe Islands cancelled his participation and thereby his presentation was withdrawn from the program.
The section finished with round table discussion where participants representing Scottish, French, Norwegian, Danish and Czech laboratories showed their results on the process of validation of qPCR protocol for surveillance of IHNV.

The annual meeting ended with the traditional update from the EURL. The results of the two proficiency tests sent out in 2015, PT1 and PT2, were presented.
The program and application system for the annual training courses that will be provided by the EURL in October 2016 was described. The planned EURL activities in year 2016-2017 were presented and proposals for the EURL work plan for 2017 were discussed.

Minutes from the meeting were taken by Drs. Jacob Günther Schmidt, Nikolaj Gedsted Andersen, Tine Moesgaard Iburg, Lone Madsen and Niccolò Vendramin. Niccoló Vendramin has assembled a draft of the report which has been sent to all the presenters for correcting in order to avoid misunderstandings. Niccolò Vendramin has finalised the report thereafter.

We would once again like to thank all the presenters for their great contribution without which the meeting would not have been a success. The workshop and meeting was organised by a team consisting of Teena Vendel Klinge, Niccolò Vendramin and Niels Jørgen Olesen, with the help from the rest of the fish disease section at the National Veterinary Institute, DTU.

The meeting next year is tentatively planned to be at the end of May 2017, more details will follow.

We wish to thank all of you for participating and look forward to seeing you next year!

Copenhagen 08/08/2016

PROGRAM

Tuesday May 31st

Annual Workshop of the National Reference Laboratories

8:45 – 9:15	**Registration and welcome address**
9:15 – 09:30	*Welcome Address and announcements*
	Niccolò Vendramin and Niels Jørgen Olesen
SESSION I:	**Update on important fish diseases in Europe and their control**
	Chair: Olga Haenen– and Minutes: s Tine Moesgaard Iburg
9:30 – 9:50	Overview of disease situation in Europe Niels Jørgen Olesen
9:50 – 10:10	Update on the disease situation in Norway Brit Hjeltnes
10:10 – 10:25	Update on the disease situation in aquatic organisms in the Mediterranean Niccolò Vendramin
10:25 – 10:45	PRVom infects and causes heart pathology in Rainbow trout and Atlantic salmon Niccoló Vendramin
10:45 – 11:05	Survey for PRV in Atlantic salmon in Iceland Heiða Sigurðardóttir
11:05 – 11:25	***Coffee break***
11:25-13:00	Working groups: Perception of the impact of infectious diseases in Europe.
13:00-13:45	***Lunch***

SESSION II: Emerging Diseases

Chair: Thomas Wahli–and Minutes: Jacob Smith

13:45 – 14:15	Risks associated with sturgeon viruses Laurent Bigarré
14:15-14:35	Detection of Ranavirus and VHSV Genotype IV in lumpfish in Iceland Sigríður Guðmundsdóttir
14:35 – 14:55	Risk assessment of new VHSV from Lumpfish for Rainbow Trout, Atlantic Salmon and Lumpfish Niccoló Vendramin
14:55– 15:25	Emergence of Nodavirus infection in Gilthead sea bream. Anna Toffan
15:25-15:45	Susceptibility of Atlantic Salmon to IHNV E-genotype and interference with PRV infection Niccoló Vendramin
15:45-16:05	*Coffee Break*

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

Chair Brit Hjeltnes – and Minutes: Niccoló Vendramin

16:05 - 16:20	Epidemiological investigation and management of a case of VHS detected in Atlantic salmon in a French fish farming in 2015 Lénaïg LOUBOUTIN
16:20 – 16:40	Diagnostic Manual for NON EXOTIC and EXOTIC fish diseases Niels Jørgen Olesen
16:40 - 17:00	An update on the work programme of the OIE aquatic animals commission Edmund Peeler
17:00- 17:20	New aquatic animal health law Knut Roenningen

Wednesday 1st of June

SESSION IV Scientific research update

Chair Richard Paley– and Minutes: Lone Madsen

9:00 – 9:20	Serology in finfish for diagnosis, surveillance, and research: a systematic review Edmund Peeler
9:20 – 9:35	Serology for KHV diagnostics Richard Paley
9:35 - 10:05	Molecular phylogeography of viral fish diseases Valentina Panzarin

10:05 – 10:20	Development and validation of a conventional RT-PCR for detection of VHSV Niels Jørgen Olesen
10:20 - 10:40	*Coffee break*
	Chair: Uwe Fisher– and Minutes: Nicolaj Andersen
10:40 – 11:10	Pox virus infection in fish with emphasis on Gill infection in Atlantic salmon Ole Bendik Dahle
11:10 - 11:25	Carp Edema Virus in Europe, current status Olga Haenen
11:25 – 11:40	Usefulness of embryonic Salmon cells (CHSE & SSE) for IHNV diagnosis Oskar Shachner
11:40 – 11:55	Characterization of novel carp paramyxovirus in UK Richard Paley
11:55- 12:15	~~Re-emergence of a ISAV-HPR-deleted in the Faroe Islands~~ ~~Debes Christiansen~~
12:15-12:50	ROUND TABLE DISCUSSION. Validation of q PCR method for surveillance of IHNV in Europe
12:50-13:00	***Group picture***
13:00 – 13:45	***Lunch***
SESSION V:	***Update from the EURL***
13:45 – 14:00	Results of the proficiency test, PT1 and PT2, 2015 Niccolò Vendramin
14:00 14:15	EURL Training courses. Topics and organization for courses 2016 Tine Iburg and Ole Bendik Dahle
14:15 – 14:30	EURL activities in 2015 Niels Jørgen Olesen
14:30 – 14:45	EURL workplan for 2016 and 2017; Ideas and plans ? Niels Jørgen Olesen
14:45 – 15:00	Next meeting and end of 20th Annual Workshop
15:00-15:30	Coffee, cake and goodbyes

Welcome

Niels Jørgen Olesen and Niccolò Vendramin wished everyone welcome to the 20th Annual Workshop. 65 participants consisting of scientists from 33 countries as well as Ph.D. students are attending the meeting.
Unfortunately one delegates had to cancel right before the Workshop; they will be informed through the report. After information on technical and practical issues Niccolò Vendramin described briefly the content of the folder distributed to all participants and on some practicalities for reimbursement.

SESSION I:

UPDATE ON IMPORTANT FISH DISEASES IN EUROPE AND THEIR CONTROL

Chairman Dr. Olga Haenen

OVERVIEW OF THE DISEASE SITUATION AND SURVEILLANCE IN EUROPE IN 2015

N. J. Olesen[1] and Niccolò Vendramin [1]

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Abstract:
The Questionnaire on Surveillance and Diagnosis (S&D) which is collated annually is the only comprehensive overview of the disease situation in aquaculture 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 S&D have changed significantly since last year. The reason for this is that we have realised that it is not possible to obtain an exact knowledge on the de facto prevalence, spreading and severity of the listed non-exotic fish diseases in Europe. The questionnaire, however, still comprise 3 parts:

1. General data on aquaculture fish production: Number of fish farms, and the health categorization according to Council Directive 2006/88/EC, and information on national surveillance programmes.
2. Epidemiological data on the disease situation in each Member State with focus on the listed diseases (information on number of out breaks and increase or decrease in number of infected farms and severity of outbreaks) but also including other diseases of interest.
3. Laboratory data from the NRLs and other laboratories, including the numbers of samples examined, and diagnoses of fish diseases made.

Production data from FEAP
The data on the European aquaculture production was this year obtained from the "European aquaculture production report 2005-2014" Prepared by the FEAP secretariat August 2015. We validated the data against the FIGIS database and concluded that they were almost identical except for the common carp production estimated by FEAP to be only 1/3 of the production data we obtained from FIGIS. The report does not include information on the number of fish farms, and therefore these data were obtained directly in the questionnaire. The report only provides data from back to 2014. The total fish production in aquaculture in Europe increased again in 2014 after a decrease in 2013. The increase however is almost only due to increases in non-EU Member states. Among the Member states the production has been almost horizontal in the past 10 years. In 2014 the Atlantic salmon production, account for 1,55 mill ton against 1.43 mill ton in 2013, and is by far the largest contingency in Europe. The rainbow trout production is again below 400 000 t after steady increases in the previous years. The decrease is primarily due to reduced production of table size rainbow trout. After several years of increased production Turkey have experienced a significant reduction in 2014. The carp production is still mainly in the Eastern part of Continental Europe and is very stable with 57.000 t produced in all. Both the production of sea bream and especially sea bass also increased in the Mediterranean countries with a production of 146.000 t and 148.000 t, respectively. Among other fish species of interest are pike-perch (increase to 573t), eel (increase to 6.507 t from 4.017t in 2013), sturgeon (increase to 2.795t), turbot (decrease from 12.676t to 9.891t in 2013 and increase again to 10.787 in 2014), and halibut (1.600 t) the cod production have almost collapsed from 22.729t in 2009 to 3.310t in 2013. The production of cleaner fish as lumpfish for lice control is increasing significantly but the total production is not that easy to retrieve due to the many species involved in this industry. Pike-perch have still not yet obtained the expected impact, a large farm in Denmark started in 2015 might give promises of an increase of this production in future, the sturgeon production is still on growing and more attention regarding health management will be given to this species- (see program for the 20th Annual Workshop).

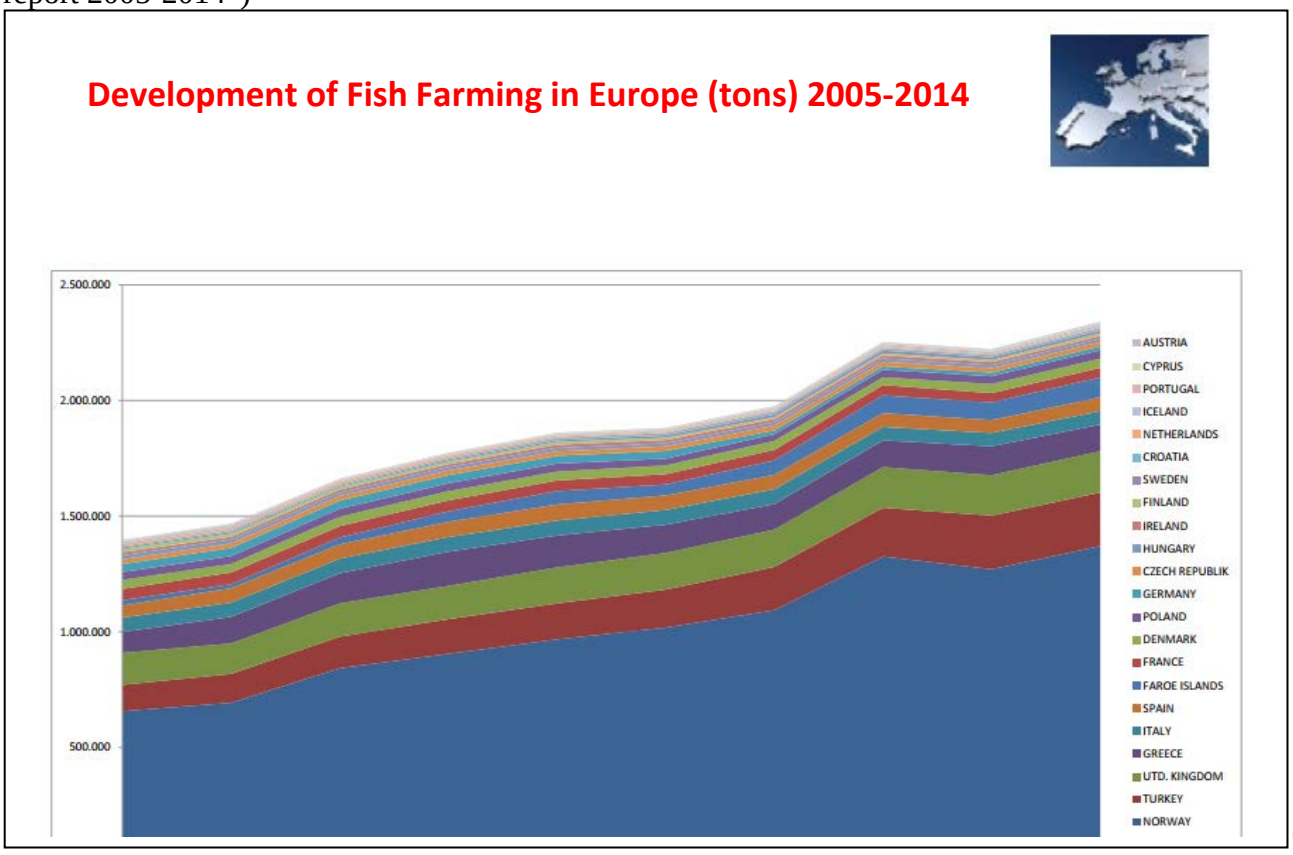
Health categorization of fish farms:
Many countries provided very clear and correct answers and almost all Member States did reply to the questionnaire when compared to the previous year's providing a rather complete overview of the status of fish health categorization in Europe. There was a significant increase in the reported number of farms

in categorized zones and compartments (From 8.505 in 2012 to 14.508 in 2015 for VHS and from 7.360 in 2012 to 12.130 in 2015 for KHV!) this was especially due to Germany who successfully included almost all their farms in categorized zones.
76% of the authorised trout farms in Europe are situated in category III zones for VHS and 74% for IHN, with 21% and 23% respectively in Category 1. For both diseases the remaining 3% 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 64% in Category I and 35% 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 nor categorized.
There are still several different views on how categorisation shall be performed, e.g. should VHS free marine rainbow trout farms be placed in Category III or I? considering the risk of infection with VHSV from the marine environment.
Commission Decision 2015-1554 provide the guidelines for obtaining disease-free health statuses with regard to ISA and to contain infection with HPR deleted ISAV, saying that detection of Isavirus HPR0 will not compromise the health status of a fish farm. 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 has now been adopted and includes all aquatic animals; in this connection the categorisation system might be simplified and be made more transparent.
Outbreaks and severity of listed diseases in Europe
Concerning the epidemiological data on the non-exotic diseases a moderate increase in the number of VHS infected farms were observed in Austria, Belgium, Bavaria, Rhineland-Palatine, Saxony, and Thuringia, whereas a decrease was observed in Czech Republic, no reports of changed severity of VHS was given except for pike infections in Austria. VHSV is regularly isolated from wild marine fish species, but it was anyway a big surprise and worrying that it appeared in wild caught lumpsuckers in Iceland, in brood fish that were caught for breeding of lumpsuckers as cleaner fish in the salmon industry. The isolate belong to VHSV genotype IV in a putative new subgroup.
For IHN an increase in number of infected farms were observed in Italy, Bavaria, and Baden-Württemberg. In Italy the severity of IHN seem to increase while a decrease in impact of IHN was observed in Baden-Württemberg.
For ISA Norway reported an increase in number of affected farms. The reason may be that infected smolts from one hatchery were transferred to three sea locations before the infection was detected. ISA was not seen outside Norway in 2015.
Concerning KHV Germany reported increases in number of infected farms in Bavaria, Lower Saxony, Rhineland-Palatina, and Saxony-Anhaltinia and a decrease Saxony. No other information on KHV in Europe were given.
Other fish diseases problems in EU
A whole range of other disease problems 2015 were reported:

- In rainbow trout the major concerns are flavobacteriosis (RTFS), enteric redmouth, and infectious pancreatic necrosis but also, lactococcosis, bacterial kidney disease, proliferative kidney disease, ichthyophtariasis, and furunculosis (especially in brown trout).
- In salmon farming it is sea lice, pancreatic disease, heart and skeletal muscle inflammation, cardiomyopathy syndrome, amoebic gill disease, and moritella.
- In cleaner fish it is primarily vibriosis and A. salmonicida infection giving problems
- In seabass and seabream it is primarily VER, Photobacteriosis, and Vibriosis (Annex 3)

In northern European countries the most common problems in the salmon production are thus sea lice, PD, and AGD, in addition several countries reported finding of Winter Ulcer Disease in salmon caused by Moritella viscosa. In continental Europe it is primarily bacterial diseases like ERM and Aeromonas infections, AGD and RTFS – but parasite infestations as Ich is still a very serious problem especially in

view of the foreseen prohibition of use of formalin, while problems in the Mediterranean countries are the same as in continental except for Lactoccocosis wich is more common in Southern Europe and Nodavirus infection in mariculture which definitely plays an important role and as a bottleneck for especially the seabass production.
There are very large differences between countries on how many samples are tested on cell cultures, ranging from < 100 to several thousands. Annex 5 provide the total number of laboratory examinations conducted in Europe in 2015 on VHSV, IHNV, ISAV, KHV, SVCV, CEV, IPNV, SAV, and Nodavirus, respectively.
Total production of fish in aquaculture in Europe 2005 to 2014 (”European aquaculture production report 2005-2014”)



Total production of fish in aquaculture in Europe 2005 to 2014 (”European aquaculture production report 2005-2014”)

Questions and Comments

Edmund Peeler: According to OIE guidelines HPR0 detection is still reportable

UPDATE ON FISH DISEASE SITUATION IN NORWAY 2015

Brit Hjeltnes, Cecilie S. Walde and Britt Bang Jensen .

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Abstract:

In 2015, Norway produced 1.234200 tons of Atlantic salmon (*Salmo salar*), 71600 tons of rainbow trout (*Oncorhynchus mykiss*) 40000 tons of Atlantic cod (*Gadus morhua*), 1700 tons of Atlantic halibut (*Hippoglossus hippoglossus*) and 700 - 900 tons of other species.
Salmon lice (*Lepeophtheirus salmonis*) infestation represents one of the most significant challenges to Norwegian aquaculture and increased resistance to anti sea lice chemicals is an increasing 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.
The main viral problem is pancreas disease, infection with salmonid alphavirus (SAV). The number of reported cases (137) is about the same level as last year (142).
15 cases of infectious salmon anaemia (ISA) were registered in 2015. Most of these cases are associated with a local epidemic in Northern Norway.
IPN was diagnosed in a total of 30 sites and the decline in the number of outbreaks seen in the recent years, continues. Selective breeding and virus eradication programs carried out by the industry; appear to contribute to this significant reduction of disease outbreaks.
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 (135) and private laboratories (52) indicate a similar situation in 2015 as in 2014.
Cardiomyopathy syndrome (CMS), also known as 'heart rupture,' was diagnosed by NVI on 105 sites. Considering reported cases from private laboratories (24 cases), this indicates an increase over recent years.
Bacterial ulcers remain a significant fish health and welfare problem especially in Northern Norway.
The amoeba *Paramoeba perurans* was detected on several sites throughout the year. Despite this, Amoebic gill disease, *Paramoeba perurans* (AGD) was reported to be less severe than last year.
Gill disease remains a significant problem in all fish stages but especially for salmon in sea water.
Production losses remain a significant problem in Norwegian aquaculture.

Questions and comments

On September 20-22 2016, the conference on aquatic epidemiology AquaEpi 1- 2016 will be held in Oslo Norway Further information available at https://www.berg-hansen.no/eventportal/?E=1453&A=55839&Att=0&WebNo=1&Sec=cbblpkckkZktcXiK#

Niccoló Vendramin : what is the prevalence of PRV in freshwater and saltwater, farmed and wild fish?.
Brit Hjeltnes: according to published data, the virus is widespread in wild fish and most likely very widespread in farms even though there is often no clinical outbreaks
Niccoló. Vendramin: what are the health demands for cleaner fish?
Brit Hjeltnes: there are no official demands at the moment, but farmers have fairly high demands.
Niels Jørgen Olesen: The unexplained background mortality is very high in the salmon production. Ideas to reduce mortality in general in fish farms?
Brit Hjeltnes: Be able to identify the problems, e.g. be better at engaging the people working in the farms to identify problems.

UPDATE ON FISH DISEASE SITUATION IN THE MEDITERRANEAN BASIN

Vendramin N.[1]

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Abstract:

The Mediterranean basin represents an interesting area for aquaculture. The production in the area is quite composite, over than historically established salmonid (rainbow trout, brook trout and charr) and carp farming, Mariculture (sea cages aquaculture) has developed in the last 20 years.

This work started in 2012 has reached a significant milestone during autumn 2015, where a specific workshop on challenges posed to the mariculture sector has been organised during the 17th – 2015 Las Palmas, Gran Canaria, Spain. The output of the workshop has been published and made publicly available (Fish health in Mediterranean Aquaculture, past mistakes and future challenges N. Vendramin, S. Zrncic, F. Padrós, D. Oraic, A. Le Breton, C. Zarza & N. J. Olesen. EAFP bullettin volume 36(1),2016)

The aim of this initiative is to set up a platform that can link authorities and stakeholders aiming to target the main sanitary issues in the basin and focus future research activities on these topics.
A simple questionnaire asking to rank the three most important diseases for marine and fresh water sector was delivered to a panel of experts.
Contributions from 17 experts were obtained about disease situation in the Mediterranean basin for 2015. Data will be presented and discussed showing comparison with previous years focusing both on important known diseases and emerging pathogens.
Data and presentation will be uploaded on the website of the EURL for fish diseases at the following link: http://www.eurl-fish.eu/Activities/annual-meetings

Questions and Comments:

Angela Trent: About the vaccine to be used for prevention of lactococcosis, is there any help to understand the vaccination strategy?
Niccoló Vendramin: There is room for improvement of the implementation of the strategy. There are definitely good examples should be put forward. The financial crisis makes things more difficult.

SURVEY FOR PRV IN ATLANTIC SALMON IN ICELAND

Heiða Sigurðardóttir

Institute For Experimental Pathology, University of Iceland, KELDUR

Abstract:

Heart and skeletal muscle inflammation (HSMI) was first described in Norway in 1999 and later in Scotland and elsewhere. The disease affects Atlantic salmon and has been identified in hundreds of farms in Norway. It generally appears 5-9 months after smolts are transferred to sea. The causative agent of HSMI is thought to be Piscine Reovirus (PRV). Interestingly, only a part of the fish that carry the virus shows symptoms and the high rate of symptomless positive fish in farms raises questions about the relationship of the virus and the disease. PRV is common in Norway in farmed salmon and the RNA from this virus has been found in several fish species both at sea and in freshwater.

HSMI has never been suspected in Iceland and Icelandic material has not been screened for PRV prior to the survey reported here, that was conducted in 2014. In that survey, RT-qPCR tests, recently developed by Norwegian scientists, were run on samples from wild salmon caught in various rivers, from salmon in land based tanks as well as from net-pens. PRV was detected in all cultured salmon and 22% of the wild salmon.

These results enhanced interest in screening more samples from various groups of salmon. In 2015 another research study was started. Samples were taken from juveniles bred from wild salmon for releasing into rivers and from juveniles bred from cultured salmon for releasing into net-pens. The latter were followed up and samples taken 8 months after the smolts were transferred to sea. Further screening for PRV will be done in salmon after 18 months at sea and also in wild salmon returning to the rivers for spawning after one year at sea.

Questions and Comments:

Britt Hjeltnes: How do you refer the detection of the PRV in regards to export of eggs?
Heiða Sigurðardóttir : we have not seen any clinics so far

Niels Jørgen Olesen: Is there any policy for releasing positive PRV fish in the wild?
Heiða Sigurðardóttir: No

Sven Bergmann: Is there an explanation for the difference in CT-values that you observe at different time points?
Heiða Sigurðardóttir We will have more information once the project move forwards

PRVom INFECTS AND CAUSES HEART PATHOLOGY IN RAINBOW TROUT AND ATLANTIC SALMON

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Abstract:

During autumn 2013, abnormal mortality was observed in some hatcheries producing rainbow trout in western Norway. The fish, 30-100g, showed signs of a novel disease characterized by circulation failure, pale viscera, anaemia and liquid (ascites) in the abdominal cavity. Haematocrit analysis confirmed severe anaemia. The histopathological examination revealed inflammation of the heart and red muscle and liver necrosis. Moderate to high mortalities have been observed.
Extended microbiological examination performed at the National Veterinary Institute (NVI) in Oslo ruled out known pathogens, while pyrosequecing analysis detected the viral genome of new aetiological agent called virus Y. Further studies have shown the virus belongs to the orthoreovirus family and is distantly related to PRV (piscine orthoreovirus) that most likely is involved in Heart and Skeletal Muscle Inflammation (HSMI) in Atlantic salmon.

A cooperative project between DTU-VET in Copenhagen, NVI and NMBU in OSLO was initiated in order to:
- · Assess whether this new virus represent a new high pathogenic pathogen for farmed Rainbow trout in Europe
- · Investigate disease pathogenesis.

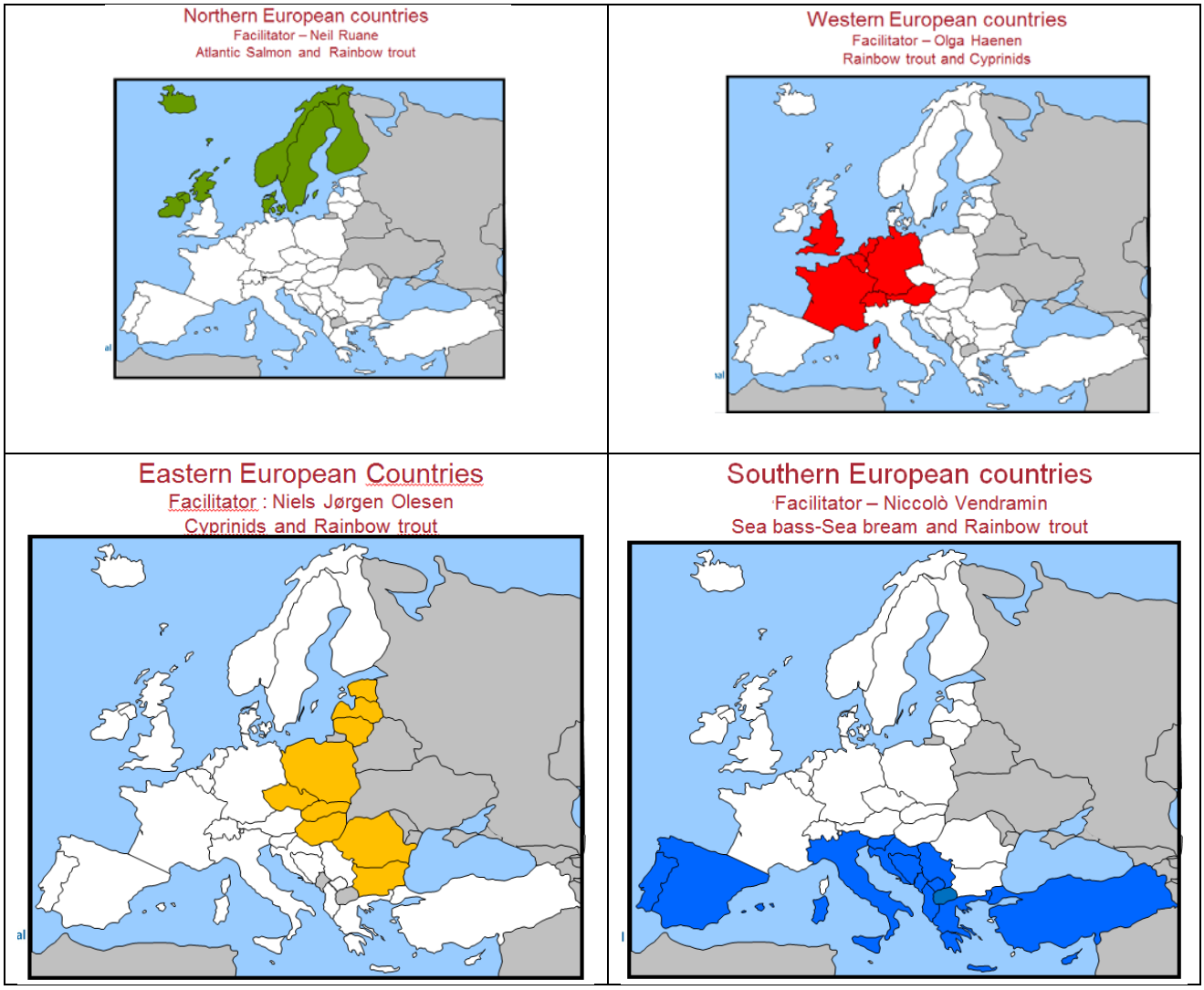
In cohabitation trials, naive Rainbow trout were exposed to shedders injected IP with blood pellets from infected fish.
Sampling was carried out at defined time points for 14 weeks.
The virus load in various organs was assessed by RT-qPCR analysis in shedders and cohabitants revealing a fast and active transfer of virus from shedders to cohabitants.
During the second half of the trial it was possible to observe a certain clearance process of the virus which occurred early in the shedders and later in the cohabitants.
Very little mortality and few clinical signs were observed.

Question and comments

Sven Bergmann. What is your opinion on a CT level of 45 as negative. Unsure if the fish really clears the virus. A limit should be stated like “no ct” above a certain ct value (e.g. above 40 or 42).
Niccoló Vendramin: it is an arbitrary value assigned to negative samples just to plot the graph.

WORKING GROUP:
Perception of the impact of infectious diseases in Europe

For the third time, this activity, introduced in 2014 was conducted during the WS.
In order to integrate data provided through the questionnaire on Survey and Diagnosis in Europe with direct inputs from the NRL representatives, an interactive activity was organized.
The countries in Europe were clustered into macro-regions and for each region the 2 most important farmed finfish species selected.



As first level of investigation, participants were asked to fill a table including a list of relevant diseases for each fish species with a view to the sanitary status in their home countries. For each disease all were asked to give a score on 3 different parameters characterizing the impact of the disease:

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

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

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

In order to allow the groups to discuss more the material was send already 10 days before the WS to each member state representative.

After this the participants were grouped according to the country clusters previously presented. A facilitator was allocated to each group. Based on discussions each group were asked to agree on a score (from 1-10) for each of the 3 parameters per disease and fish species. Gaps in the information were also highlighted for some of the diseases.

The following criterion was established to compare diseases being characterized by different patterns, how much of the expected economical revenue is lost because of a single specific disease?

Each group was asked to select a representative to describe the outcome of the work for the full audience.

The output of this work was as follows.

In the countries clustered under **Northern Europe** the fish species addressed were Atlantic salmon and rainbow trout.

For the Nordic countries, Dr Torsten Boutrup was in charge of describing the output of the description.

Concerning Atlantic salmon the most important diseases is considered to be caused by Sea lice. This parasitic infestation is considered to be the bottleneck for future development of the salmon production; the economic impact of this disease is mainly related to increased resistance to treatment.

Secondly viral infection by SAV,salmonid alphavirus causing PD, pancreas disease, in Atlantic salmon is considered to have high impact on production and economy. Due to national legislation in some areas of Norway there is a special focus for this disease also under the legislative aspects.

Finally the third disease to be considered for salmon production in Europe is AGD- amoebic gill disease. This pathogen is considered to have significant consequences on production and economy.

Concerning Rainbow trout production the infectious disease considered having the highest impact on production and economy is Enteric Red Mouth –ERM caused by *Yersinia ruckerii*. The second most important disease is parasitic infestation caused by *Ichtiophtirius multifiliis*, with high impact on production and economy.

Among viral pathogens both IPNV and SAV are considered to have a certain impact, whereas most of the countries in this group are free of VHS and IHN and thereby their impact is related to the legislative consequences of maintaining a disease-free status.

In **Western Europe** the main species were Rainbow trout and Cyprinids.

Angela Trent from the English fish health inspectorate had the opportunity to present the output of the discussion. A first remark was that, within this region, the perception of the infectious disease is heterogeneous and differs from country to country.

For Rainbow trout, the impact at farm level for the two listed rhabdoviruses IHNV and VHSV are considered to have the highest impact on production and economy; furthermore their relevance is amplified by the legislative consequences. Furthermore also IPNV is considered to have high impact on production and economy.

Looking into bacterial diseases, the two most important diseases in 2015 were Rainbow trout Fry Syndrome-RTFS caused by *Flavobacterium psychrophilum* and ERM having a significant impact both

on production and on economy, these diseases generally imply antibiotic treatment for their management and concerns on antibiotic resistance is increasing
Finally parasites were considered to have low to moderate impact on Rainbow trout production.
For the cyprinid species, the disease considered having the highest impact on production economy and legislation is KHVD. Secondly it is raising the impact of another cyprinid virus – Carp Edema Virus reported by 5 countries in the region and finally the third disease ranked according to impact on production and economy is bacterial infection causes by *Aeromonas* spp.

In **Eastern Europe** the main species addressed were Cyprinids and Rainbow trout.
Ľubomír Pojezdal, from NRL in Czech Republic was in charge of presenting the output of the discussions. The situation appeared to be pretty stable and consistent with the description of the previous year.
Concerning the cyprinid production KHV is considered to cause the highest impact, whereas infections with the other two cyprinid herpesviruses (CyHV-1 and CyHV-2) are not observed often. SVC is still considered as a relevant problem. Some countries detected new cases of CEV and reported them as an emerging disease. Infection with *Aeromonas* spp, *is considered to have significant impact on production and economy*.
Concerning rainbow trout production the two most important diseases are still considered to be the viral diseases VHS and IHN; these still cause relevant and significant losses both in terms of production, economy and legislative consequences. Among bacterial diseases Furunculosis caused by *Aeromonas salmonicida*, RTFS and ERM are still an important issues. In regards to parasites information are sparse.

In **Southern Europe** the farmed species targeted by this working group were the two important marine species European seabass and gilthead seabream, and rainbow trout for freshwater production.
Dr Prapas from NRL in Greece was in charge of describing the discussion output for southern European countries.
For seabass and seabream, VER-VNN is the most important disease due to high impact on production and economy. Secondly gill infestation with gill flukes (specifically *Sparicotyle chrysophrii*) due to high mortality that can be caused by the outbreak and the impact of economy. Vibriosis including classical vibriosis caused by *V. anguillarum* and atypical vibriosis caused by *V. harveyi* is at the third place because of its high impact on economy, in general all bacterial diseases mentioned in the questionnaire were considered to have a certain impact on production and economy.
For rainbow trout, the listed diseases VHS and IHN are considered to pose the highest impact on economy, production and legislative consequences. Among bacterial diseases RTFS and Lactococcosis are considered having the highest impact on production and economy for 2015.
It was appreciated this year that the knowledge exchange between diagnostic laboratories and other stakeholders as farmers, private consultants and veterinarians is improved and it is expected that this cooperation will continue in order to be able to describe a more precise and detailed picture of the fish diseases situation in European aquaculture year after year as this data collection aims to depict the impact of infectious fish diseases in a more holistic view, not focusing only on legislative obligations.

SESSION II: EMERGING DISEASES

Chairman: Dr. Thomas Wahli

RISKS ASSOCIATED WITH STURGEON VIRUSES

Laurent Bigarré

ANSES, Plouzané, France

Abstract:

Sturgeon farming has become increasingly important worldwide for the production of caviar and fish flesh, as well as for restoration programs set up to save endangered wild populations. Viruses of the *Herpesviridae* and *Iridoviridae* families are the major threats for the farms.
To date, two alloherpesviruses, Acipenser herpesviruses 1 and 2 (AciHV1 and AciHV2), have been described, but their prevalence has been poorly studied. AciHV2 has been associated with mortalities in the USA and Russia.
Various iridoviruses have been described in the north-American continent. One virus provoked in 2009 an outbreak on pallid sturgeon in an American hatchery and was identified as Frog iridovirus3 (FV3), a well-known ranavirus [3]. Nearly 20 years before, a different iridovirus was found in the integument of white sturgeon (*A. transmontanus*), causing fatal disease to benign infections [2]. This virus was named white sturgeon iridovirus (WSIV). Since this discovery, other viruses related to WSIV were found in the USA and Canada, and named according to their host or origin: Missouri river white sturgeon iridovirus (MRSIV), British Columbia white sturgeon virus (BCWSV), Namao virus (NV) and others. Recently, related, though distinct, viruses were found by PCR in Europe, infecting at least four different sturgeon species [1].
No full-length-sequence has been obtained yet for any of these WSIV-related viruses. Their genetic relationships are unclear, despite the alignment of the partial sequence of the major capsid protein gene. Are they distinct species or strains of a unique one ? Strikingly, the available sequences of MCP are very different from other members of the *Iridoviridae* and more related to members of the *Mimiviridae*. This taxonomic issue reinforces the need of obtaining at least one complete genome.
For WSIV-related viruses, generic cPCR tools have been proposed recently and are particularly useful for diagnostics because most of these viruses cannot be produced in cell culture, impeding the development of serological tools. Efforts for developing genotype-specific real-time PCR were also engaged with a potential to be used in order to screen for healthy broodstock. Preliminary studies suggest that the prevalence of WSIV-related is high. Therefore, the associated risk is considered has high in European farms.
Several factors increase the risks of viral diseases in sturgeon farms: frequent international transfers of living contaminated material, mix of species in the sites, hybrids, lack of diagnostics tools and poor knowledge on the biology and virulence of the large DNA viruses. More efforts in research must be engaged in the next decade to control these complex diseases.

1. Bigarré L, Lesne M, Lautraite A, Chesneau V, Leroux A, Jamin M, Boitard PM, Toffan A, Prearo M, Labrut S, Daniel P (2016) Molecular identification of iridoviruses infecting various sturgeon species in Europe. J Fish Dis in press
2. Hedrick RP, Groff JM, McDowell T, Wingfield WH (1990) An iridovirus infection of the integument of the white sturgeon Acipenser transmontanus. Dis Aquat Organ 8:39-44
3. Waltzek TB, Miller DL, Gray MJ, Drecktrah B, Briggler JT, MacConnell B, Hudson C, Hopper L, Friary J, Yun SC, Malm KV, Weber ES, Hedrick RP (2014) New disease records for hatchery-reared sturgeon. I. Expansion of frog virus 3 host range into Scaphirhynchus albus. Dis Aquat Organ 111:219-227

Questions and Comments:

Sven Bergmann: There is a fantastic PhD thesis from Poland which is covering the whole Eastern part of Europe in regards to Sturgeon viruses unfortunately available only in Polish. I will ask for sending the thesis to you at the university of Szczecin Prof. Kempter..

DETECTION OF RANAVIRUS AND VHSV GENOTYPE IV IN LUMPFISH IN ICELAND

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Abstract:

Culture of lumpfish (*Cyclopterus lumpus*) for exportation of juveniles as cleaner fish was begun in Iceland in 2014. The use of wild brood fish requires screening for notifiable viral diseases using cell culture. All batches screened in 2014 tested negative but in 2015 two viruses were isolated from different batches of wild lumpfish intended for breeding.

The first one was recovered from lumpfish caught off the south coast of Iceland and identified as a new iridovirus isolate. A sequence of the MCP gene was obtained and when blasted in NCBI database showed high homology with *Ranavirus* isolates from cod (*Gadus morhua)* and turbot (*Psetta maxima*) isolated in Denmark in the latter half of the last century, but they were also closely related to known pathogenic ranaviruses such as European catfish iridovirus (ECV), European sheatfish iridovirus (ESV) and epizootic haematopoietic necrosis virus (EHNV).

The second isolate turned out to be a new isolate of VHSV, genotype IV. That brood fish was caught in July in Breidafjördur, a bay in west Iceland and immediately brought to a land based farm. When sampled the fish looked healthy, with neither external nor internal macroscopic signs of disease. When the samples were being processed in September two groups of lumpfish on the farm started to show clinical signs of a disease. One group had been spawned in 2014 and destined to become brood fish and the other group consisted of fingerlings that had been spawned early in 2015. VHSV was isolated from these groups as well and it was inferred that there had been a transmission from the brood fish since all the above mentioned groups were kept in tanks in close vicinity to each other. Immediately, samples were sent to the EURL lab in Denmark for verification and subsequently OIE was notified of the outbreak in accordance with the European Community Council Directive 2006/88/EC. A notification to member states was issued on October 23rd, 2015. Measures taken were stamping out and disinfection of the facilities. This is the first time that a notifiable fish virus has been detected in Iceland.

Isolates from all three groups were identified as VHSV using conventional PCR targeting the G gene. Sequencing of the G gene and comparison to a panel of 30 known isolates shows that the new Icelandic isolate does not match with any of the available sub-clades IVa, IVb and IVc. Further analysis is planned, including the sequence of other genes (i.e. N-Gene and NV-Gene) to provide a broader panel of information before final classification of the isolate can be made

Questions and Comments:

Eann Munro: We have also recently isolated a very similar Ranavirus from lumpfish in Scotland, quite possible the same virus and the fish may have originated from Iceland. We have sequenced multiple genes and will share reults in due course. Did the VHS cause mortality in your lumpfish?

Sigridur Gudmundsdottir: adult fish were brought into facilities and broodstock to produce juveniles and the new broodstock. The juveniles showed signs of sickness, but there was also other infections, so not sure if it was due to the VHS.

Torsten Boutrup: 1. Is it possible to disinfect eggs. 2. Are there attempts to culture brood stock, so we can close the circle?

Sigridur Gudmundsdottir: 1. Yes, disinfection of eggs is possible. 2. Yes. And hopefully they will succeed, because screening all these wild fish is demanding.

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RISK ASSESSMENT OF NEW VHSV FROM LUMPFISH FOR RAINBOW TROUT, ATLANTIC SALMON AND LUMPFISH

Niccoló Vendramin[1] , Sigríður Guðmundsdóttir[2], Teena Vendel Klinge[1], Christina Flink Desler[1],

Niels Jørgen Olesen[1]

National Veterinary Institute, Technical University of Denmark, Denmark

Abstract:
In 2015 a VHS outbreak occurred in Iceland. The virus detection was confirmed from the EURL and phylogenetic analysis revealed that the virus belong to a new genotype IV.
In vivo experiments to characterize the pathogenicity of this virus in farmed salmonids rainbow trout and atlantic salmon and lumpfish (cyclopterus lumpus) were carried out at the experimental facilities of DTU-VET.

Infection trials.
Risk assessment for farmed salmonids
Infection trials in Rainbow trout and Atlantic salmon were performed to test the pathogenicity of the Icelandic VHSV strains in these species. The fish were infected by immersion (bath) or by intraperitoneal (i.p.) injection.

Virus isolates:

1) The virus isolate Icelandic strain 15-19852 was passaged once in BF-2 cells and titrated on the same cell type according to standard procedures
2) Positive controls: The VHSV strain DK-3592B, highly pathogenic for Rainbow trout was used as a positive control by immersion only for Rainbow trout, and both by immersion and IP in AS. Furthermore VHSV strain isolated from Atlantic salmon genotype IVa isolated from Port Angles WA was used as reference for VHS genotype IV both by immersion and IP for RT and AS.
3) As a negative control, EMEM with tris-buffer and 10% newborn calf serum (dilution medium) was likewise used by immersion only.

Trials: All groups of both RT and AS, both experimental, positive and negative control were tested in triplicate 31 fish in each tank.

Pathogenicity in cleanerfish
Infection trials in lumpfish were performed to test the pathogenicity of the Icelandic VHSV strains in this species. In order to assess the pathogenicity of this trial for lumpfish triplicate of 30 fish of lumpfish will be infected by:

1) Bath infection: 6 hours both for salmon and trout. Negative controls will be mock infected with naïve cell culture supernatant
2) IntraPeritoneal IP Injection fish will be anesthetized with benzocaine and injected with 0,1 ml VHSV. Negative controls fish will be anesthetized as well and IP injected with naïve cell culture supernatant.

Data will be presented and discussed

Questions and Comments:

Torsten Boutrup: Mortality in the salmon must be reported. If they died from the experimental infection you should be able to find it. So I think the inability to isolate it from the dead salmon is a “tank thing”. That is why I would use triplicates. Maybe the salmon are not doing so well in the white bowls.
Niccoló Vendramin: Correct, both biomolecular and virological examination are negative. For the little mortality observed in the salmon, we may also consider the effect of salinity in small fish.
Sven Bergmann: Is the isolate behaving like normal marine isolates as we know them from the Atlantic or the Baltic Sea?
Niccoló Vendramin: From the results we have, we have to believe it is a lumpfish pathogen. Yes, some salmon died, the infectious dose was rather high. If we compare it to genotype IVa from Atlantic salmon it is a completely different picture.

Edmund Peeler: You of course have to be cautious in your conclusions, but I guess you could say that Atlantic salmon are not susceptible to this lumpsucker strain of VHS, and therefore from a risk assessment and management point of view, what would your conclusions or recommendations be?
Niccoló Vendramin: I agree, for recommendation on cleanerfish health management we will have a working group on this topic in autumn involving different stakeholders.
Edmund Peeler:how about the high viral load in the very beginning, because the seeders should have the maximum excretion later. Did you test the salmon before?
Niccoló Vendramin: Yes, of course. They were negative.
Niels Jørgen Olesen: Almost all shedders were dead by day 5, so high viral load in the tanks very early.
Torsten Boutrup: Did you test the water for virus?
Niccoló Vendramin: Yes, after 7 days. Ct was around 40.
Sven Bergmann: Do you plan to include pure mRNA to see replication in the Atlantic salmon? The spleen is a very nice organ.
Niels Jørgen Olesen: We are really at detection limit, so I am not sure it is possible.

EMERGENCE OF NODAVIRUS INFECTION IN GILTHEAD SEA BREAM

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Abstract:

Viral Encephalopathy and Retinopathy, also known as Viral Nervous Necrosis (VNN), is one of the most devastating infectious diseases for marine aquaculture worldwide. A large number of economically relevant fish species have proven to be susceptible to the disease, such as the European sea bass (*Dicentrarchus labrax*), Asian sea bass (*Lates calcarifer)*, groupers (*Epinephelus spp*.) striped jack *(Pseudocaranx dentex*), Senegalese sole (*Solea senegalensis*), turbot (*Scophthalmus maximus*), Atlantic halibut (*Hippoglossus hippoglossus*) and cod (*Gadus morhua).* The occurrence of disease outbreaks in these species has been widely documented both in farmed and wild fish. On the other hand, Gilthead sea bream (*Sparus aurata*) has been generally considered resistant to the disease. Indeed, sea bream is frequently reared in close proximity of VNN infected European sea bass but shows neither mortality nor clinical signs. Experimental infections by intramuscular injection highlighted that only young sea bream developed clinical signs and mortality, while adult fish never showed any disease sign despite testing positive by PCR and virus isolation. Notably, these experimental trials were performed with RGNNV, the most commonly VNN species detected in European sea bass as well as in the Mediterranean basin.

Unexpectedly, in the last two years an increasing number of sea bream hatcheries in Europe have experienced disease and mortality in sea bream larvae. Affected fish displayed symptoms and lesions clearly associated with VNN and tested positive for betanodavirus. The viral isolates were characterized as reassortant strains RGNNV/SJNNV. A subsequent genetic characterization of all sea bream VNN isolates inventoried at IZSVe revealed that the majority of these strains are actually RGNNV/SJNNV. Remarkably, anamnestic data related to these isolates indicated an increase in mortalities of all RGNNV/SJNNV infected sea bream larvae. Taken together, all these information strongly suggest that RGNNV/SJNNV possess a particular tropism to sea bream, as already observed in the case of Senegalese sole.

In light of the increasing number of VNN detections in clinically affected *S. aurata* larvae, it is of paramount importance to strengthen the awareness in scientist and fish farmers of the emergence of RGNNV/SJNNV nodavirus in this species. On the other hand, it is essential to improve the knowledge on the disease pathogenesis and epidemiology in this new host, as well as the prevention strategies.

Questions and Comments:

Brit Hjeltnes: I have a question about temperature. 19 °C wouldn't that be a rather low temperature to observe clinics?

Anna Toffan: The influence of temperature on the development of the disease in sea bream must be further investigated. However, this re-assortant strain display RNA1 from warm water RGNNV genotype, the betanodavirus species showing the widest tolerance to temperatures.

Edmund Peeler: The virus is obviously wide spread geographically and with respect to species. Do you really think that vaccination is going to be the most effective management strategy?
Anna Toffan: Yes, I believe vaccination could help – at least to reduce the prevalence of the disease. Because right now it is not clear if wild fish are infecting farmed fish or vice versa or both.
Brit Hjeltnes: Vaccination needs to be done at a very very young stage.
Anna Toffan: Yes, but we can also try to vaccinate the brood stock. Or develop bath vaccines. Some authors have shown it could work.
Niccoló Vendramin: It seems that the industry in the south is learning from the Norwegian salmon industry. It is more and more common that old inland farms are used as nurseries. Small fish are kept here where there is high biosecurity until they are 20 grams and vaccinated and then moved to sea cages.

SUSCEPTIBILITY OF ATLANTIC SALMON TO IHNV E-GENOTYPE AND INTERFERENCE WITH PRV INFECTION

N. Vendramin, A. Alencar, S.S. Mikkelsen, T. Rundqvist, N.J. Olesen

National Veterinary Institute, Technical University of Denmark, Denmark

Abstract:

Infectious haematopoietic necrosis (IHN) is a serious infectious disease that affects salmonids. The disease is endemic in certain areas of Europe and so far affecting mostly rainbow trout production.
The aetiological agent of IHN in Europe, novirhabdovirus- IHN virus (IHNV) belongs to genotype E.
It has recently been demonstrated in experimental trial that Atlantic salmon is susceptible to different degrees, to representative of all genotypes circulating in north America (U,L,M) (Kurath et al., 2016)

In this study the susceptibility of Atlantic salmon to one IHNV strain belonging to E genotype was tested, in order to assess the risk for this species in Europe.

Furthermore, due to wide spread of Piscine orthoreovirus PRV in farmed atlantic salmon population in Europe, the interaction in vivo of these two pathogen was investigated in double infection model, aiming to describe how infection with PRV may interfere with the susceptibility of Atlantic salmon to IHNV.
Disease development and morbidity monitoring where combined with time point random sampling, quantifying the two pathogens in target organs.

The interplay of the two viruses in Atlantic salmon and the potential interference of PRV with a high virulent IHNV (Infectious Hematopoetic Necrosis Virus) strain has been investigated in vivo in order to assess the risks and the outcome of an outbreak of IHNV in PRV infected fish.

- G. Kurath, J R Winton, O B Dale, M K Purcell, K Falk and R A Busch. Atlantic salmon, Salmo salar L. are broadly susceptible to isolates representing the North American genogroups of infectious hematopoietic necrosis virus Journal of Fish Diseases 2016, 39, 55–67

Questions and Comments:
Torsten Boutrup: How long after the PRV infection was the IHNV infection?
Niccoló Vendramin: 4 weeks. So we were at the peak of the PRV infection.
Torsten Boutrup: If you compare to what Niels Lorenzen has done with VHSV and IHNV vaccination and so on, do you think it is strange that you don't see any mortality in the super-infection PRV-IHNV?
Niccoló Vendramin: No, but my experience is that if you take VHSV trials in rainbow trout and you take IPNV infected fish you see very little or no mortality.
Uwe Fischer: If PRV infection is chronic and the fish do not die, then innate immunity is kept at a certain level?
Niccoló Vendramin: Yes, this is a good theory we have to test for this.
Brit Hjeltnes: well, maybe that PRV infection has some interaction but Salmon do get infected with whole range of pathogens
Torsten Boutrup: Adding onto what Brit said, you have to look much more into the area of interplay between different pathogens. Important to understand why infections often act differently than what we expect them to.
Olga Haenen: I agree. In eels for example, you see certain types of coinfection more often than others. There is still a lot to discover.

SESSION III:

UPDATE ON IMPORTANT FISH DISEASES IN EUROPE AND THEIR CONTROL

Chairman: Dr. Brit Hjeltnes

Epidemiological investigation and management of a case of VHS detected in Atlantic salmon in a French fish farming in 2015

L.Louboutin[1], J.Cabon[1], M.Baud[1], L.Bigarré[1], C.Pau[2], T.Roman[3], A. Le Breton[4], T. Morin[1]

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[2] DDCSPP des Hautes-Pyrénées – Tarbes, France

[3] DGAL Direction Générale de l'Alimentation – PARIS, France

[4] Alain Le Breton - Grenade sur Garonne, France

Abstract:

Even if an eradication plan will be submitted soon to the EU for VHS and IHN, France is currently not totally free of these regulated diseases but our territory contains several free disease zones and compartments, defined as category I in accordance to decision 2006/88 EC. To achieve and maintain this status, aquaculture establishments have to undergo official control procedures including laboratory analysis. In 2015, a VSHv positive result was obtained in a French farm situated in a disease free zone under official surveillance. Control measures were rapidly taken to avoid spreading of the disease to other aquatic animals and farms. NRL expertise was required to confirm primary diagnostic obtained by a departmental laboratory. PCR and further sequencing were performed to identify the incriminated isolate. Results showed a very high sequence similarity between this isolate and a French isolate from 1971 routinely used by French accredited laboratories as positive control for cell culture. Considering risk associated to a potential VHSv spread to other farms, sentinel program was initiated for a duration of 6 weeks in strictly controlled conditions and was coupled with sample analysis at two different times (in the middle and at the end of the procedure). No VHSv could be detected, by cell culture neither by PCR. This presentation aims to give information about the chronology of events and especially the results of the epidemiological survey carried out in collaboration with the EU-RL, which helped to quickly return to a controlled situation.

Questions and Comments:

Ed Peeler: What is the source of infection?
Lenaig Louboutin. We can't exclude a laboratory contamination, and we are in favor of this hypothesis.

Eann Munro: do the farmers get economical compensation?
Lenaig Louboutin: no compensation was given

Thomas Wahli: where the fish come from originally?
Lenaig Louboutin: imported eggs from other farm from disease free zone

DIAGNOSTIC MANUAL FOR LISTED NON-EXOTIC FISH DISEASES

N. J. Olesen

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Abstract:
The **Commission Decision 2015-1554**, implementing Directive 2006/88/EC as regards requirements for surveillance and diagnostic methods is now adopted and implemented.

The annexes in CD 2015-1554 are divided into 2 parts, Annex I on SURVEILLANCE AND CONTROL METHODS and Annex II on DIAGNOSTIC METHODS AND DETAILED PROCEDURES. Both annexes concern the non-exotic listed diseases Viral haemorrhagic septicaemia (VHS), Infectious hematopoietic necrosis (IHN), Koi herpes virus (KHV) disease and Infectious salmon anaemia (ISA) and in addition the mollusc and crustacean diseases Infections with *Marteilia refringens*, Infections with *Bonamia ostreae* and White spot disease (WSD).

- The major changes compared to the previous Decisions on VHS, IHN and ISA are
- Risk based surveillance
- Reduced number of samples to collect in order to achieve disease free status
- Possibility for conducting surveillance for the 4 diseases by RT-qPCR alone (before cell cultivation was mandatory for VHS and IHN),
- Surveillance and notification only for ISAV-deleted not for ISAV HPR0.
- New updated technics are included and others are removed.
- Entire KHVD chapters were created from the beginning.

The major issues for each of the 4 fish diseases will be presented and discussed. As these decisions are mandatory to follow in contrast to the OIE Diagnostic Manual all NRL´s shall be aware of them. The new manuals for each disease will be presented and can be found at www.eurl-fish.eu .

Questions and Comments:
Vlasta Jencic: For carp sample preparation, which tissues to be included?
Niels Jørgen Olesen: for surveillance gill and kidney are primarily investigated.

Sven Bergmann: complain for number of fish (150 fish with 2 fish pools = 75 PCRs) for KHV detection compared to VHSV/IHNV were only 15 PCRs are necessary. The request is to reduce the number of fish to 30 (= 15 PCRs) like OIE is recommending (KHVD in the manual).
Niels Jørgen Olesen: The two year program comprise 2 x 75 fish per year as for VHS and IHN, while the 4-year programme only comprise 30 fish/sampling. The reason for not pooling more than 2 fish is due to your validation that max 2 fish in a pool is acceptable in order to keep a satisfactory sensitivity. The design of these Diagnostic manuals has been made searching for the largest consensus among Member States.

Anna Toffan: which kind of blood sample is validated to test KHV
Sven Bergmann: I would suggest to use the leucocyte. 10^7 leucocytes to test from 0.2 – 0.5 ml.

AN UPDATE ON THE WORK PROGRAMME OF THE OIE AQUATIC ANIMALS COMMISSION

Dr Edmund Peeler

Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, UK

Abstract:

The role of the OIE's Specialist Commissions is to use current scientific information to study problems of epidemiology and the prevention and control of animal diseases, to develop and revise OIE's international standards and to address scientific and technical issues raised by Members Countries (MC). The Aquatic Animals Commission (AAC) meets twice a year and its report is circulated to MC for comments. The report contains revised code and manual chapters first circulated for information, and then following revision, for adoption (at the next OIE General Assembly). Specific areas of work through expert ad hoc working groups. An important focus in recent years has been on strain splitting concluding with the recognition of two strains of infectious salmon anaemia (deleted and non-deleted). Both strains are listed (thus notifiable) and this allows MC to self-declare freedom from one strain but not the other. Strain splitting work will continue with consideration of phenotype and distribution of different viral haemorrhagic septicaemia genotypes. Another continuing strand of work is the assessment of host susceptibility. An ad hoc group will be formed to systematically review the literature for listed finfish diseases and determine which species fully or partially meet criteria for susceptibility (replication, viability, pathology, location). A new chapter of disinfection has recently been circulated for comment by MC. More work on biosecurity, including compartmentalisation, is planned partly in response to recommendations from a recent OIE international conference on aquatic animal health. Following on from guidance on test validation (new code chapter) an ad hoc group will revise the structure of the disease specific manual chapters, and provide more detailed guidance to experts on the assessment of tests (including validation) against purpose. This is intended to address inconsistencies between chapters. The emergence of aquatic animal diseases means that the AAC frequently review cases for the listing of new pathogens.

Questions and Comments:

Angela Trent: What are OIE expectations in regard to the guidelines provided to member countries?
Ed Peeler: **N**otification is most important guideline to follow.

Brit Hjeltnes: What is your opinion on notification system?
Ed Peeler: there is room for improvement that can be done on both emerging diseases and listed diseases.

THE NEW ANIMAL HEALTH LAW – IMPACT ON THE AQUATIC ANIMAL HEALTH LEGISLATION

Knut Roenningen

Abstract:

The new animal health law (AHL) was adopted by the European Parliament and the Council earlier this year and published in the *Official Journal* (Volume 59) 31 March. The AHL will apply from 1 April 2021 and from that date replace most of the current Union legislation on animal health, including Directive 2006/88/EC.

As AHL is a regulation, the provisions given both in the law itself and the delegated and implementing acts to be adopted by virtue of empowerment given to the Commission, will be directly binding for all parties involved, including operators, veterinarian and the Member States. With regard to aquatic animal health, the main principles in Directive 2006/88/EC are maintained in the AHL. However, some new elements have been introduced, which also will have impact on the aquatic animal health, and the most important ones are the following:

- Enhanced tools for controlling diseases in populations of wild animals. Such tools will include specific transport requirements, record keeping obligations and requirements for health certification.

- New criteria for listing and categorisation of diseases. All current listed diseases have to be assessed in accordance with the new criteria before the final list is adopted. The two categories "exotic" and "non-exotic" diseases will in addition be replaced by five new categories, giving more flexibility with regard to obligations for surveillance, movement restrictions and control measures.

- More responsibility for disease prevention and control put on the operators such as farmers, transporters and persons responsible for slaughtering and processing plants.

The main supplementary legislation in terms of delegated and implementing acts will now within a period of three years both be drafted and adopted by the Commission. The best guarantee for a result of this process is strong involvement from the Member States.

Questions and Comments:

Olga Haenen: How about mandatory visits in fish farms?
Knut Roenningen: there is possibility for mandatory visit, saying that every farm needs to have a certain kind of inspections periodically
Niels Jørgen Olesen: the new listing system will open up for a number of diseases, we may have 5-10 diseases to consider?
Knut Roenningen: at the moment we have to take also into consideration resources that can be allocated to this activity, but there could be more flexibility
Niels Jørgen Olesen: what about health categorization?
Knut Roenningen: The actual Category 3 most likely will disappear and category 4 will remain.

SESSION IV: Scientific research update

Chairman: Dr. Richard Paley and Dr. Uwe Fischer

SEROLOGY IN FINFISH FOR DIAGNOSIS, SURVEILLANCE, AND RESEARCH: A SYSTEMATIC REVIEW

Diana Jaramillo [a], Edmund J. Peeler[b], Emilie Laurin[a], Ian A. Gardner[a] and

Richard J. Whittington[c]

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Abstract:
Serological tests have been under-used for finfish diseases, when compared with their use in terrestrial animal health. A number of authors have concluded that the non-specific immune response in fish made serology unreliable and inferior to the direct measurement of agent analytes. We conducted a systematic review of peer-reviewed publications that reported on the development, validation, or application of serological tests for finfish diseases. A total of 168 articles met the screening criteria; most of them were focused on salmonid pathogens (e.g. *Aeromonas* spp. and viral haemorrhagic septicaemia virus). Before the1980s, most publications reported the use of agglutination tests but more recently our review indicates that ELISA has become the dominant serological test. The main application of serological tests has been in the assessment of vaccine efficacy, with few applications for surveillance or demonstration of freedom from disease, despite the advantages of serological tests over direct detection at the population level. Non-lethal sampling, low cost and post-infection persistence of antibodies should make serological assays the test of choice in surveillance for may pathogens. Arguably, their adoption for disease diagnosis at the population level has been constrained by poor characterisation and validation. The number of publications in our review reporting diagnostic sensitivity and specificity of serological tests in finfish was small (n= 7). Foreseeing a wider use of serological tests in the future for diagnostic end-purposes, we offer recommendations for mitigating deficiencies in the development and evaluation of serological tests, including optimization, control of non-specific reactions, informed cut-off points, diagnostic accuracy, and serological-baseline studies. Achieving this agenda will pave the way for the greater international recognition of serological testing in programmes to support aquatic animal health.

Questions and Comments:

Niccoló Vendramin: Can access to reference sera for ELISA be a limitation?

Ed Peeler: we can address this technical question in the next talk by my colleague Richard Paley, that for sure this can be a limitation.

Niels Jørgen Olesen: can you provide practical examples of serological tests in surveillance?

Ed Peeler: KHV is a good example. Here serology will corroborate findings together with other detection methods, like direct detection.

Brit Hjeltnes: ISA is another example in for Norway.

Ole Bendik Dale: commented that he had presented serology of ISA at the 1999 EAFP Conference.

Eann Munro Salmon farmers in Scotland use serology for detection of SAV.

Brit Hjeltnes in Norway it has been discussed to use serology for surveillance of PD.

Angela Trent Serology should be taken forwards as a surveillance tool

SEROLOGY FOR KHV DIAGNOSTICS

R Paley, G Wood and K Way

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Abstract:

Serology is a valued and well used tool for diagnostics and surveillance in human and terrestrial animal health but is much under-utilised in fish health. The exception to this is surveillance for the cyprinid herpesviruses for which poor growth and isolation of the viruses in cell culture mandates a different approach. Whilst the OIE recommended PCR tests are appropriate for the diagnosis and confirmation of clinical disease, they are far from suitable for surveillance and establishing freedom from disease. Serological analysis is an appropriate tool to complement these PCR tests.

A number of ELISA-based antibody assays have been developed to detect antibodies to KHV in carp serum (Adkinson *et al*., 2005; St-Hilaire *et al*., 2009). Although the ELISA has only a moderate diagnostic sensitivity the performance characteristics showed that the assay was effective at the population level with a positive cut-off set to give a high specificity. The ELISA requires similar sample sizes, is cheaper than PCR and has the added value of being a non lethal test.

KHV serological tools do however require improvement particularly in relation to cross reaction in the KHV ELISA test with antibodies raised in the fish after infection with other herpes viruses (eg carp pox). More specific assays are currently being developed collaboratively utilising alternative antigens to the whole virus such as synthetic peptides and recombinant proteins (J. Wasa, Institute of Aquaculture, Stirling and J. Kattlun, University of Veterinary Medicine, Vienna). Similarly recently developed alternative platforms for robust and rapid analysis of substances based on electrochemistry biosensors (Vantix Research Diagnostics) are being assessed. The biosensor system reputedly offers benefits of increased sensitivity, significantly more rapid analysis and ability to work in complex fluids with minimal sample preparation (whole blood). Successful improvement of the KHV antibody ELISA will also provide an opportunity to fully validate an assay for detection of fish antibody for recognition by the OIE and it is hoped that extensive validation of an improved assay will provide evidence to persuade the OIE that antibody detection assays are suitable for surveillance of certain aquatic diseases.

Questions and Comments:

Niccolo Vendramin What is the general consensus about using serology for surveillance?

Richard Paley This is disease dependent, depending on the persistence of each individual pathogen.

Angela Trent Serology can be a useful tool for policy making.

Torsten Boutrup it depends on what you want to use it for and suggested that serological methods can be used by farmers

Angela Trent Farmers that want to know the status of their stock can use serology.

PHYLOGENY AND TRANSMISSION PATTERNS OF FISH RHABDOVIRUSES IN EUROPE

Valentina Panzarin

Istituto Zooprofilattico Sperimentale delle Venezie, Department of Comparative Biomedical Sciences, Legnaro, Padova, Italy

Abstract:

Viral haemorrhagic septicaemia virus (VHSV) and infectious hematopoietic necrosis virus (IHNV) are the causative agents of two notifiable diseases severely affecting European trout farming. Despite the application of EU directive 88/2006 and the implementation of eradication programs, nowadays disease outbreaks still occur in several countries, causing serious economic losses in the trout industry. In this framework, epidemiological investigations are of crucial importance to better understand VHSV and IHNV ecology and provide useful information for the development of control strategies to prevent viral spread. More specifically, molecular epidemiology integrates sequence data with traditional epidemiological information and has demonstrated to be a powerful tool to i) identify the existence of epidemiological connections between different trout farms ii) recognize the origin of a disease outbreak and iii) track viral spread.
The Emida-Era-Net MolTraq Project (Molecular Tracing of Viral Pathogens in Aquaculture) is a multidisciplinary trans-European research project, whose aim is to generate and use spatio-temporal, epidemiological and genetic data to identify the most important factors responsible for the spread of diseases in aquaculture. In this presentation, we will provide three examples on how descriptive molecular data and traditional epidemiological information were blended within the aims of the MolTraq project, to study the etiological determinants of salmonid rhabdoviruses distribution in different European countries. In detail, a descriptive phylogenetic and evolutionary study of IHNV and VHSV circulating in Italy in the past twenty years was carried out, and different scenarios for viral spread among farms were proposed by combining genetic and epidemiological information. Similarly, a dataset of approximately 300 Danish isolates collected between 1978-2009 was molecularly characterized, and models for viral spread were inferred to understand the diffusive dynamics of the disease over the time period under investigation, as well as the effectiveness of the eradication programs adopted in Denmark. Finally, the largest-ever VHSV dataset including viruses originating from different European countries and collected since 1971 was phylogenetically analyzed. The study shed light on the phylogenetic radiation of the VHSV-Ia sublineage onto different sub-clades and described the country-specific distribution of each Ia subclade as well as the individual route of spread.

Questions and Comments:
Richard Paley: Google imaging might be a very useful way to educate farmers when it comes to notifiable diseases and how to avoid them.

DEVELOPMENT OF A NOVEL ONE-STEP RT-PCR FOR DETECTION OF VIRAL HAEMORRHAGIC SEPTICAEMIA

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**Presenting author*

Abstract:

Viral haemorrhagic septicemia (VHS) is the most serious viral disease in salmonids and olive flounder farms. Various diagnostic methods for detection of VHS virus have been described in the OIE Diagnostic Manual. Among them, the conventional RT-PCR method is typically used for detecting VHSV and sometimes also genotyping into I to IV. However, we found a low sensitivity for detection of Korean VHSV IVa isolates using these OIE primers. In addition, the RT-PCR often showed non-specific reaction with fish cell lines. Thus, we needed to improve the specificity and sensitivity of the conventional RT-PCR given in the OIE Diagnostic Manual in order to detect all VHSV genotypes and to remove the non-specific reactions due to fish cell lines. In this study, we selected the candidate primers from 5 regions in VHSV N gene, and a highly sensitive primer set was selected among these. The reaction conditions of the selected primer set were established and no non-specific reactions in fish, fish cell lines or with heterologous viruses were observed. The sensitivity of new RT-PCR was tested in parallel with cell cultivation, RT-qPCR, and conventional OIE VN RT-PCR. It was concluded that the sensitivity for all genotypes were at the same level when using cell culture, qPCR and the conventional new RT-PCR. While it was lower for the OIE VN RT-PCR. The novel RT-PCR was following tested on 80 VHSV isolates representing a worldwide collection of all known genotype and subtypes. In this study clear and unique amplicons were observed for all 80 isolates. In conclusion a new conventional RT-PCR have been developed and validated according to all steps given in the guidelines of the OIE diagnostic Manual, except for a reproducibility study that will be conducted between 5-6 laboratories in an inter-laboratory proficiency test which is under preparation and we thereby recommend that this new 3F2R RT-PCR shall replace the current method given in the OIE manual for detection of VHSV.

Questions and Comments:

Richard Paley Are mixed bases at some of the places in the primers?

Niels Jørgen Olesen no.

Sven Bergmann were titrations done on isolates or RNA.?

Niels Jørgen Olesen First titrations were done on isolates and then RNA afterwards.

POX VIRUS INFECTION IN FISH WITH EMPHASIS ON GILL INFECTION IN ATLANTIC SALMON

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Abstract:

Poxviruses are large DNAviruses causing disease in many animal species, including reptiles, birds, and mammals. Poxvirus-like particles have been detected in diseased farmed koi carp, ayu, and Atlantic salmon. From Atlantic salmon the first genome sequence of a fish poxvirus: Salmon Gill Poxvirus (SGPV) was recently described. Phylogenetically SPGV is the deepest known representative of the *Chordopoxvirinae*. So far SPGV has not been cultured and quantitative PCR and immunohistochemistry have been used to determine aspects of salmon gill poxvirus disease. The gill was the main target organ where immature and mature poxvirus particles were detected. The particles were detected in detaching, apoptotic respiratory epithelial cells preceding clinical disease in the form of lethargy, respiratory distress, and mortality. In moribund salmon, blocking of gas exchange would likely be caused by the adherence of respiratory lamellae
and epithelial proliferation obstructing respiratory surfaces. The virus was not found in healthy salmon or in control fish with gill disease without apoptotic cells. However, transmission of cultured virus and fulfillment of Kochs' postulates remains to be demonstrated. PCR of archival gill tissue confirmed virus infection in 14
cases of gill disease with apoptosis in Norway starting from 1995.

Ongoing diagnostic work show that SPGV associated gill disease in farmed Atlantic salmon occurs over a wide geographic area and comprises both fresh-water and sea-water sites. In addition to the overt cases dominated by SPGV infection and apoptosis we have now found SPGV in several instances of complex gill disease. Some of these cases will be presented.

In complex gill disease it can be difficult to detect the changes that raise suspicion of SPGV involvement. As many of these cases may have one or several other easily detectable agents, SPGV may go unnoticed unless qPCR on gill material is done. So far, it appears that SPGV is not ubiquitous, but present in some, but not all gill disease outbreaks. At present we thus recommend to use the SPGV qPCR on all cases of gill disease in Atlantic salmon to obtain more information on the distribution and significance of this virus.

Questions and Comments:
Torsten Boutrup: Do you think that the disease is associated with eggs and it is possible to disinfect eggs?
Ole Bendik Dale: We don't think the eggs are the infection route. We have a good chance of getting rid of it with the right diagnostics.
Eann Munro: Are there virus other places than the gills?
Ole Bendik Dale: No CT in the organs in the dying fish. Lot of virus is found in the skin of moribund fish.
Olga Haenen: Have you tested rainbow trout?
Ole Bendik Dale: No, rainbow trout are not affected at this time. The virus has coevolution with the host.
Niels Jørgen Olesen: Have you been looking after virus in the other organs (skin)?
Ole Bendik Dale: I am not sure it the virus is coming from the skin or just attaching to the skin.
Q (?): Do you always run PCR in case of gill disease?
Ole Bendik Dale: No, but I think it should be standard. We do it when we see apoptosis, but this is also missed sometimes.

Carp Edema Virus in Europe, current status

Olga Haenen[1], Keith Way[2], Niccoló Vendramin[3], David Stone[2], Michal Reichert[4], Marek Matras[4], Charlotte Axén[5], Sven Bergmann[6], Laurent Bigarré[7], Lénaïg Louboutin[7], Mikolaj Adamek[8], Verena Jung-Schroers[8], Tomáš Veselý[9], Bartolomeo Gorgoglione[10], Oskar Schachner[11], Mansour El Matbouli[10], Veronika Piačková[12], Valentina Panzarin[13], Anna Toffan[13], Miriam Abbadi[13], Vlasta Jencic[14], Susie Sommer Mikkelsen[3], Niels Jørgen Olesen[3]

1) NRL Fish Dis, CVI, NL; 2) NRL Fish Dis, CEFAS, England; 3) EURL Fish Dis, DTUVet, Denmark; 4) NRL Fish Dis, PIWET, Poland; 5) NRL Fish Dis, SVA, Sweden; 6) NRL Fish Dis, FLI, Germany; 7)NRL Fish Dis, ANSES, France; 8) University of Vet Med, Hannover, Germany; 9)NRL Fish Dis, VRI, Czech Rep; 10) University of Vet Med Vienna. Austria; 11)NRL Fish Dis, Uni Vet Med, Vienna, Austria; 12) University of South Bohemia, Czech Rep; 13) NRL Fish Dis, IZSVE Italy; 14) NRL Fish Dis, NVI, Slovenia

Abstract:

Carp Edema Virus (CEV), the etiological agent of Koi Sleepy Disease (KSD) was originally described in Japan in the 1970's as a viral edema of juvenile color *Cyprinus carpio* koi and later morphologically identified as a poxvirus. The virus causes severe damage to gill lamellae, leading to hypoxia and lethargy, which manifests as sleepy behavior, and mortality can reach 80-100% (Ono et al. 1986, Lewisch et al. 2015). KSD is known to be widespread across Japan where koi are cultured: the disease has been managed rather than eradicated. International trade in koi has undoubtedly led to global spread of CEV/KSD, but the occurrence of disease outbreaks has often not been recognized and consequently, rarely reported. In the USA, CEV has been detected in association with disease outbreaks in koi at import sites and in hobby ponds since 1996 (Hesami et al., 2015). In Europe, outbreaks of KSD and PCR detections of CEV-like virus were reported from 2009. At a CEV workshop in Copenhagen in January 2015, delegates from Austria, Czech Republic, France, Germany, Italy, The Netherlands and the UK reported multiple KSD outbreaks in imported koi and also outbreaks in common carp (http://www.eurl-fish.eu/Reports). After the workshop information was received on KSD/CEV cases in Poland. At the EAFP Conference at Las Palmas, Sept 2015, CEV lectures were presented in the Novel cyprinid virus workshop (Haenen et al., 2016), and a CEV discussion lunch meeting was held (http://www.eurl-fish.eu/Reports).

Knowledge gaps identified, to be addressed to assess the true impact of the disease on European carp populations include: 1) the mechanisms of virus survival outside the host 2) are there susceptible aquatic species other than carp (carriers)? 3) does the virus exist as a low-level persistent infection and are there aquatic vectors? 4) what is the prevalence in carp populations? 5) what are the important environmental factors responsible for triggering CEV/KSD outbreaks?
More tools are needed to provide early warning of emerging pathogenic viruses and resolve diseases of unknown etiology. Possible improvements include: 1) a better characterization of known viruses and more use of next generation sequencing, particularly to allow effective design of generic PCR primers that detect a range of DNA viruses; 2) development of qPCR assays for sample screening; 3) better mechanisms in Europe (with support from the EU) for collaboration and networking to tackle serious or potentially serious epizootics.
A **CEV review and alert paper** giving the emergence of CEV in Europe and its significance to European aquaculture is currently in preparation (Keith Way et al., in prep.).

In this lecture, an update is given on data since Sept 2015 on CEV related to:

- **New detections of CEV in Europe**, as far as reported by the authors.
- **Isolation and replication of CEV**: this has been attempted at various laboratories in Europe, without success so far in continuous cell cultures, but with chances of success in primary cell cultures. An overview will be presented on which cells have been tried at which temperatures.
- **Diagnostic methods:** qPCRs were developed and partly validated.
- **Genetic characterization of CEV strains:** Data from infection trials at ANSES (France) and PIWET (Poland) in cooperation with CEFAS (UK) will be briefly presented.
- **New infection experiments:** Data from ANSES (France), PIWET (Poland), VRI (Czech Republic) will be shown.

- We invite you to actively add to the discussion, to be able to have an overview on CEV in Europe, and to formulate further steps to manage CEV and KSD in Europe.

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Questions and Comments:

No questions or comments

Usefulness of embryonic Salmon cells (CHSE-214 and SSE-30) for IHNV diagnosis

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Abstract:
Over the years, especially in recent times, Chinook salmon embryo (CHSE-214, Fryer et al., 1965) and Sockey salmon embryo (SSE-30, McCain, 1970) cells proved to be most convenient and reliable for the detection of various viruses, in particular IHNV. As the destructive effect of IHNV is prolongated in these robust cell lines, both were most conveniently applicable in the immune-fluorescence test.
Being more frugal and persistent, they continuously exhibited IHNV-susceptibility similar to other epithelial fish cell lines. Once in a proficiency test, CHSE cells were the most susceptible one for IHNV.
Although in their enzymatic profile CHSE cells resembled to epithelial cells like FHM, their metabolic activity and nutritional requirement seemed to be lower. Unlike those they were growing in diluted culture medium as well as in normal MEM-10, without displaying higher susceptibility; in contrast, under nutrient-poor conditions virus replication was slightly more prolongated. Inversely, glucose supplementation most frequently did not restrict susceptibility as in FHM cells.
The more slowly proliferating SSE cells were overmatching CHSE cells in persistence and both were overmatching other epitheloid cell lines in resistance to toxic fish organ compounds and bacterial products. Usually *Mycoplasma* spp. infection enhanced their adherence forces without restricting their growth and susceptibility to IHNV. CHSE cell passages seemed to mediate IHNV infectivity to refractory RTG-2 and BF-2-cells, possibly with the aid of *Mycoplasma* spp.

Questions and Comments:

Niels Jørgen Olesen In the EU manual we suggest the use of BF-2, EPC, FHM or RTG-2 cell line, what is your recommendations in regards to CHSE?

Oskar Schachner: These cells appear to be rather resistant to environmental contaminants and susceptible to IHNV, so they are a useful tool for diagnostics and surveillance for this virus.

CHARACTERISATION OF A NOVEL PARAMYXOVIRUS FROM CARP

R. Paley, C. Sharp, C. Joiner, I. Cejas, R. Van Aerle, K. Bateman, A. Rice, P. Dixon, and D. Stone

Cefas, Weymouth, UK

Abstract:
Over a number of years diagnostic testing of UK and imported cyprinids has occasionally resulted in the isolation of viruses showing a characteristic syncytial cytopathic effect (CPE) in cell culture that is not caused by the known and expected viruses of carp (22 isolations since 1996). Initial characterisation by electron microscopy indicated large (200 nm) pleomorphic, spherical, enveloped virions that are released from cells by budding. The isolates replicate slowly (average of 17 days to develop full CPE) on carp cell lines producing low titres (3.4 to 5.7 log TCID50/ml). Bromodeoxyuridine and chloroform treatment indicated RNA genome and presence of a lipid envelope respectively. Together these results indicate a possible myxo-like virus, but the isolates were not able to haemagglutinate or haemadsorb carp or salmon erythrocytes. Furthermore no amplification was seen using generic primer sets designed to amplify orthomyxovirus and paramyxovirus polymerase genes.
Viral nucleic acid was subjected to next generation sequencing by Roche 454, and Illumina MiSeq and HiSeq platforms, with de novo assembly using CLC genomics, Blast (Altschul et al., 1990) comparison against sequence databases and assessment of assignments using Megan (Huson et al., 2007). Despite recovering over 1 million and 10 million sequence reads for isolates in each sequencing platform respectively no significant similarities were identified. Isolations from carp in Germany, of viruses with similar CPE, morphology under electron microscopy and biochemical properties have been previously described (Body et al., 2000; Granzow et al., 2014; Neukirch & Kunz, 2001) and were provisionally ascribed to the family *Paramyxoviridae* but without successful full characterisation or molecular assignment.
Recently however the HiSeq generated raw sequence reads from one of the virus isolates were re-assembled using a very recently published new assembly program, IVA (Hunt et al., 2015). Surprisingly a 16 kb putative viral sequence was generated and subsequently annotated using a selection of bioinformatics tools including FGENESV0 (http://linux1.softberry.com), Prokka (Seemann, 2014) and Blast. Based on the single segment genome architecture encompassing at least eight putative genes and the average nucleotide identity over putative nucleoprotein, fusion and polymerase genes of 21%, the virus was classified as a putative Paramyxovirus. Failure to group with any of the known paramyxoviruses (including Atlantic Salmon paramyxovirus) in phylogenetic analyses indicates this isolate represents a new species and quite likely a new genus within the family *Paramyxoviridae*.

Questions and Comments:

Olga Haenen: This can maybe explain carp mortality in other countries. Will you look into samples with an already identified virus?
Richard Paley We don't see the virus very often. We can look back into old samples. We won't look into samples with already identified viruses.

ROUND TABLE DISCUSSION

VALIDATION OF REAL-TIME RT-PCR METHODs FOR SURVEILLANCE OF IHNV IN EUROPE

Niels Jørgen Olesen[*] Lenaig Louboutin, Ľubomír Pojezdal, Torfinn Moldal,

Susie Sommer Mikkelsen, Eann Munro**

**Introducing author*

***Chair person of the round table*

Abstract:

As several NRLs have undertaken validation of real time RT-PCR methods for surveillance and diagnosis of IHNV in Europe it would be beneficial to coordinate the work and to provide recommendations for the other NRL´s on how to implement and validate a real-time RT-PCR.
Most NRLs are implementing the Purcell et al. RT-qPCR with several utilizing the G24 IHNV panel from Gael Kurath, Seattle for the validation. A problem with the Purcell et al. PCR is however the 2 step procedure increasing the risk of contamination. Therefore a one-step procedure would be more appropriate in a guideline. A one-step procedure from modified Purcell et al. PCR has been examined by Ľubomír Pojezdal, VRI, CZ. at the EURL and preliminary results indicated that it worked well. Lenaig Louboutin et al. from ANSES have since last year already gone through validation of an IHNV detection method by RT-qPCR, and tried to adapt the Purcell described method to work only in one-step procedure, and she will summarize their result. The same applies for Dr. Eann Munro from Marine Scotland who will share his experience with us. Dr. Torfinn Moldal from VI, Norway has used a modified version of Liu *et al*. (2008) RT-qPCR and apparently obtained better results. The round-table discussion aims to propose the optimal validation procedures for future use. In addition organizing a proficiency test in order to assess the reproducibility of a modified one-step procedure will be discussed.

SESSION V: Update from the EURL

RESULTS OF THE PROFICIENCY TEST, PT1 AND PT2, 2015

Niccoló Vendramin[1], Teena Vendel Klinge, Susie Sommer Mikkelsen and Niels Jørgen Olesen

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Abstract:
A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2). PT1 was designed to primarily assess the identification of the fish viruses: viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), epizootic haematopoietic necrosis virus (EHNV), spring viraemia of carp virus (SVCV), and infectious pancreatic necrosis virus (IPNV) by cell culture based methods. PT2 was structured with the aim of assessing the ability of participating laboratories to identify the fish pathogens: infectious salmon anaemia virus (ISAV) and Cyprinid herpesvirus 3 (CyHV-3) (otherwise known as koi herpes virus – KHV) by biomolecular methods (PCR based). For 2015 the EURL decided that the panel of pathogens to be investigated should be expanded to include Salmonid Alphavirus (SAV). Since SAV is not a listed disease in the European legislation, all participants were free to decide if they would be testing for SAV or not. Each participant was asked to declare whether they would test or not. The EURL would then take care of calculating the score accordingly.
44 laboratories participated in PT1 while 43 participated in PT2.
The tests were sent from the EURL in the beginning of October 2015.
Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043. This report covers both the results of PT1 and PT2. Participants were asked to identify the content of each ampoule by the methods used in their laboratory which should be according to the procedures described in Commission Decision 2015-1554.

Participants were asked to download an excel sheet from the EURL web site (http://www.eurl-fish.eu/) to be used for reporting results and to be submitted to the EURL electronically. Additionally, participants were requested to answer a questionnaire regarding the accreditation status of their laboratory.
The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).
The number of National Reference Laboratories (NRLs) participating in PT1 and PT2 was 43.
Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043.
Each laboratory was given a code number to ensure discretion. The code number of each participant is supplied to the respective laboratories with this report. Furthermore, the providers of the proficiency test have included comments to the participants if relevant. An uncoded version of the report is sent to the European Commission.

Résumé and concluding remarks PT1
49% of parcels were delivered by the shipping companies within 1 day after submission and 80% was delivered within 1 week. It was, however, unfortunate that six parcels were more than 2 weeks on the way and one of these was 35 days on the way before delivered to the laboratory primarily due to border controls.
This year ECV was included. 43 participants were able to identify Ranavirus of these laboratories 38 correctly identified 'Ranavirus' or 'not EHNV'. 40 laboratories performed sequencing and among these 35 identified 'ESV/ECV' correctly.
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.
It was unexpected and quite unfortunate that the PFR/ tenchRV like virus – S64- showed up in Ampoule V.
The presence of this rhabdovirus was confirmed in the ampoules V in addition to the expected VHSV strain DK-5151 + IHNV strain 32/87. The virus identified was Tench Rhabdovirus S64. The scoring system has been adjusted on the background of the finding from the participants and the final confirmation conducted at the EURL. This issue has been taken seriously into consideration by the EURL and managed both with the participants and DANAK the accreditation body that audit the QA system at DTU.

PT2 conclusion

Concluding remarks PT2

This was the first time that the EURL provided a proficiency test on SAV identification. Considering that 33 laboratories participated (of which 32 correctly identified SAV in ampoule VII) this was regarded as a proper initiative that strengthen the diagnostic capacities of the NRLs in detecting emerging pathogens, and it will be included in the coming years as well.

All 43 laboratories testing for KHV identified the virus in ampoule VI.

Out of the 33 laboratories that tested for SAV 32 laboratories identified SAV in ampoule VII.

Out of 44 laboratories 42 laboratories identified Not KHV or ISAV in ampoule VIII and there were “only” two false positive.

All 43 laboratories testing for ISAV identified the virus in ampoule IX, though one laboratory also wrongly identified KHV in ampoule IX.

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).

Of the 27 laboratories sequencing the ISAV virus all found that the isolate was with deletion in segment 6 and thus not belong to HPR0.

EURL TRAINING COURSE FOR 2016

Tine Iburg

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Abstract:
Also for 2016 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 10th to Friday the 15th of October

- **Introduction to Histopathology in fish diseases.**
The course will be held in week 42 from 17th -20th October 2016

The content of the training courses and the procedure to register will be described.
More information will be soon made available on the EURL website
http://www.eurl-fish.eu

EURL ACTIVITIES IN 2015

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Abstract:
The duties of the EURL are described in Council Directive 2006/88/EC of 24 October 2006 (Annex VI). The duties mainly concern fish diseases listed as exotic diseases: epizootic haematopoietic necrosis (EHN); and fish diseases listed as non-exotic diseases: infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN) and koi herpes virus disease (KHVD).

The 19th Annual Meeting of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark, 27th-28th May at the premises of the Veterinary Institute. A total of 53 participants from 33 countries attended over the two days period. There were five sessions with a total of 25 presentations, 3 of which were given by invited speakers, and a working group session.

Again this year an inter-laboratory proficiency test was distributed to the NRLs mainly within the EU but there were also participants from countries outside EU. For the fifth year, the proficiency test consisted of two tests, PT1 and PT2. PT1 was designed to primarily assess the ability of participating laboratories to identify VHSV, IHNV and EHNV. PT2 was developed in order to test the proficiency of participating laboratories to identify ISA and KHV; in 2015 the identification of SAV was included as well on a voluntary base. The proficiency test is covering all 5 listed exotic and non-exotic diseases. 45 National Reference Laboratories (NRLs) participated in the proficiency test. A report was submitted primo March 2016. Most 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 web page.
The final version of the Commission Decision 2015-1554 on sampling and diagnostic procedures for all non-exotic diseases listed in Council Directive 2006/88/EC have finally been adopted and the procedures for the fish diseases are available on the EURL webpage http://www.eurl-fish.eu/Diagnostic_Manuals.

Another important focus area was the development, implementation and validation of diagnostic tools for identification of the listed and emerging diseases and their accreditation. One outcome of these efforts was the implementation of a real time RT-PCR for detection of piscine orthoreovirus (PRV) and testing of more than 200 samples from wild salmon, and the first phases of optimization and validation of a real time RT-PCR for surveillance and diagnosis of IHNV.

During 2015, 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 webpage (www.eurl-fish.eu); and finally to attend international meetings and conferences.

EURL WORKPLAN FOR 2016 AND 2017; IDEAS AND PLANS

Niels Jørgen Olesen

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Abstract:

	Description	Objectives	Expected outputs
1. Coordination and training			
1-1	**Annual workshop**	Organize and prepare for the 20th and 21st Annual Workshops for the National Reference Laboratories for Fish Diseases (NRLs) in 2016 and 2017, respectively	To be held during the final week of May in 2016 and 2017
1-2	**Annual workshop report**	Produce a technical and financial report from the Annual Workshops in 2016 and 2017, respectively.	To be finalized and submitted August 2016 and August 2017
1-3	**Survey& diagnosis**	Collect and report data on the fish disease situation in EU, including all the listed non-exotic fish diseases given in Council Directive 2006/88/EC Annex IV Part 2.	A questionnaire will be submitted in January 2016 and January 2017 and data collated for the Annual Workshops in May.
1-4	**Training**	Facilitate and provide training in laboratory diagnosis. The yearly training courses in methods used for diagnosis of fish diseases will be offered to the EURL laboratory facilities. The courses will primarily be for training of staff from NRLs and the content will depend on request from participants.	Training courses are provided fall October-November 2016 and 2017; two courses each year of 3-5 days each with expected 15 participants are foreseen.
1-5	**Scientific working group**	Organize specific scientific meeting collating international experts to assess and provide recommendations on management and control of emerging diseases problems in EU.	One meeting gathering 5 to 7 international experts will be held at our premises or on spot according to disease case in 2016 and 2017 respectively. Scientific reports and recommendations will be delivered afterwards to relevant stakeholders.

2. Proficiency test			
2-1	**Proficiency tests**	Prepare the Annual Inter-laboratory Proficiency Tests year 2016 and 2017 for the NRLs. The tests will include VHSV, IHNV, EHNV, ISAV and KHV and will also address other common viral pathogens in fish farming (IPNV, SVCV, SAV, Ranaviruses etc)	To be shipped fall 2016 and 2017, respectively (tentatively mid-September)
2-2	**PT reports**	Collate and analyse information gained from the Inter-laboratory Proficiency Tests	Report for the proficiency test 2015 will be submitted February 2016, while results of the 2016 tests will be finally collated December 2016 and reported in 2017. The 2017 PT will be reported early 2018.
3. Reagents and products			
3-1	**Supply of Reagents**	Supply reference reagents to the NRLs in Member States.	Reagents as monoclonal antibodies, rabbit antisera, pathogen isolates or cell cultures are expected to be send to approx. 15 laboratories in 2016 and 2017, respectively.
3-2	**Production of reagents**	Production of diagnostic reagents against selected pathogens when necessary	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
3-3	**Pathogen library**	Update and maintain a library of isolates of infectious salmon anaemia virus (ISAV), Viral Haemorrhagic Septicaemia virus (VHSV) and Infectious Hematopoietic Necrosis virus (IHNV), Koi Herpes virus (KHV) and Enzootic Hematopoietic Necrosis virus (EHNV) and other relevant putative emerging fish pathogens.	The library will be updated with 10 to 20 pathogen isolates both year

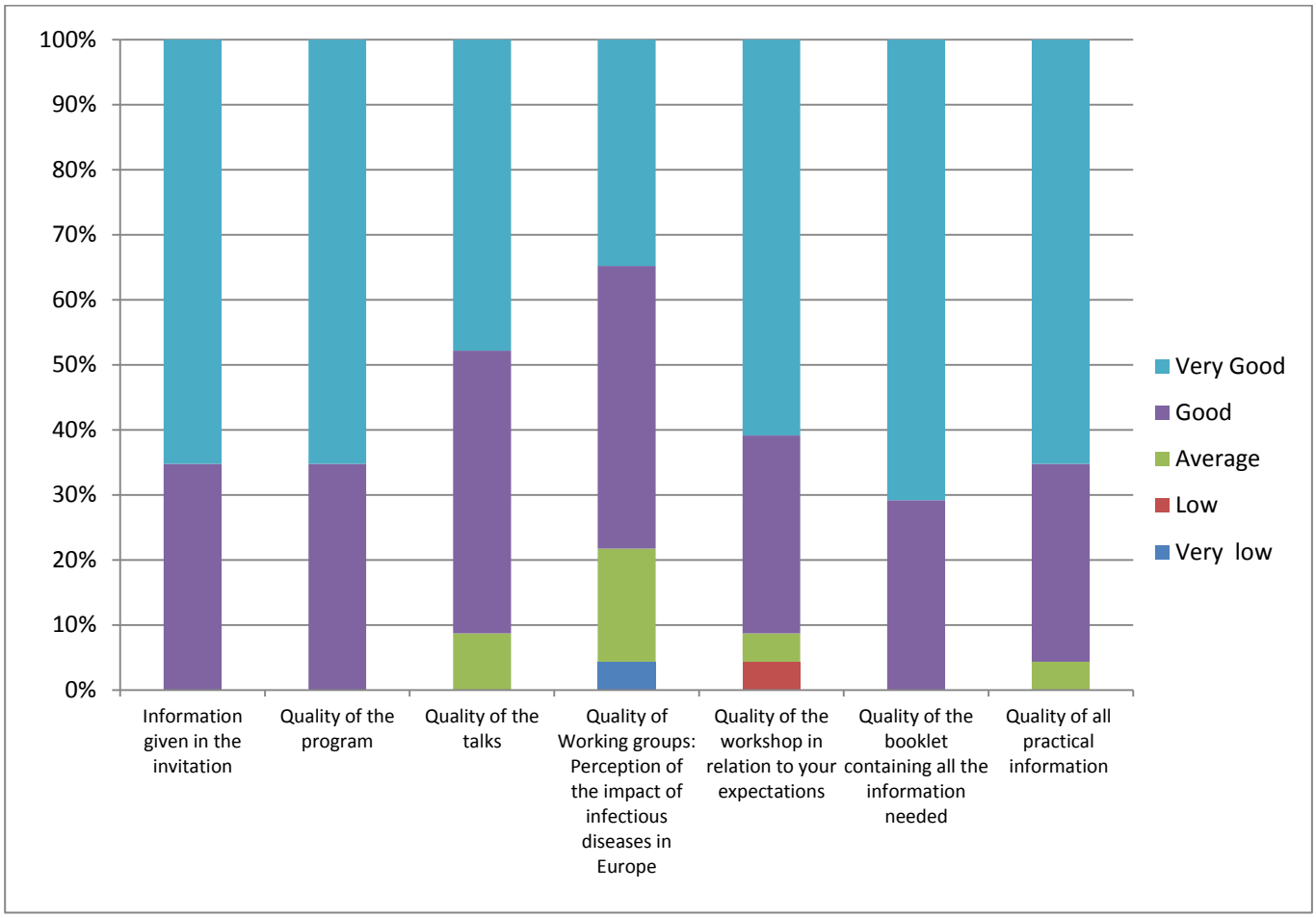
4. Scientific advice and activities			
4-1	**Webpage**	Update the webpage for the EURL, www.eurl-fish.eu	Keep the webpage constantly updated, uploading relevant material (e.g. AW report, AW presentations, Training course report etc.,)
4-2	**Diagnostic manuals**	Update the diagnostic manuals for VHS, IHN, ISA, KHV disease, EHN on the EURL web page.	The diagnostic manual for sampling and detection of listed non-exotic diseases was finally adopted in 2015. But as the diagnostic procedures for identification and surveillance of the listed diseases is rapidly evolving new procedures will be assessed and validated for inclusion in the first revision of the diagnostic manual.
4-3	**FishRefLabNet**	Maintain and further develop the interactive network with the NRLs, FishRefLabNet, in order to promote a proactive data sharing and communication with and between reference laboratories in the Member States.	The webpage and mailing list based platform for communication and data sharing will be continued with periodical updates sent to all members that subscribed.
4-4	**Pathogen characterization**	Identify and characterize selected isolates of listed viruses (pathogenicity testing in-vivo and in-vitro, serological and genetic characterization).	The EURL receive every year strains and samples for corroboration of diagnostic results in EU Member states. Regularly these strains must be characterized properly as an emergency response to avoid unwanted spreading of new pathogens in EU
4-5	**www.fishpathogens.eu**	Update and expand www.fishpathogens.eu with more pathogens.	The database is a valuable tool for virus characterisation and molecular epidemiology. The more isolates included the stronger the tool. New databases on other listed and emerging pathogens are in the pipeline such as a database on SAV (pancreas disease and sleeping disease viruses). At least 50 new isolates are envisaged to be included and 1 new database opened in 2016 and 2017.
4-6	**Molecular epidemiology**	Perform molecular epidemiological analysis to improve knowledge on diseases spreading mechanisms of the listed viral fish pathogens.	A study involving isolates from several Continental European countries is envisaged.

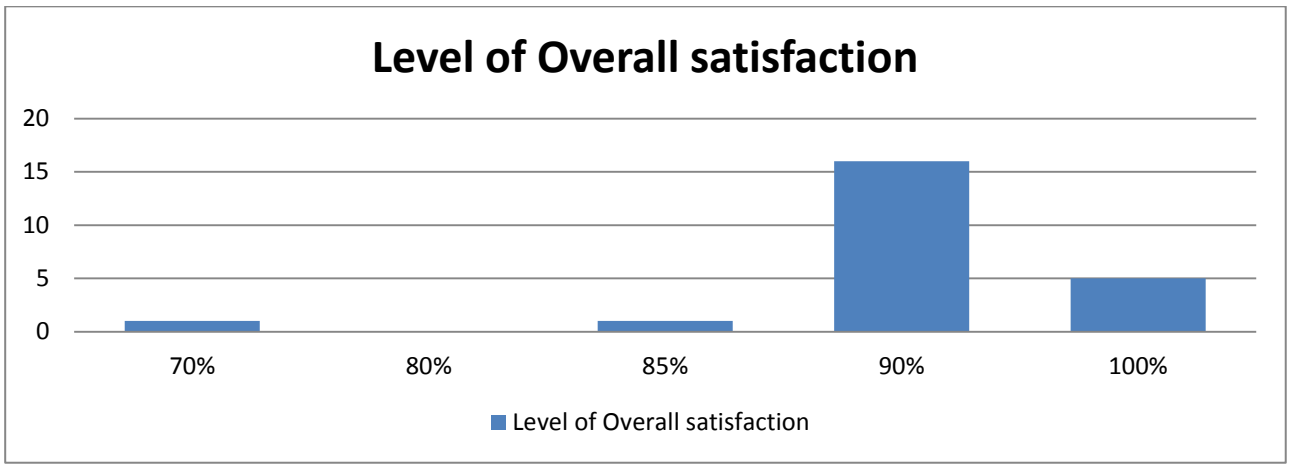
4-7	**Real-time PCR**	Assessment and standardisation of real-time PCR tests for the diagnosis, identification and typing of emerging and the listed non-exotic and exotic fish diseases.	Real-time PCR is a highly sensitive and specific tool for diagnosis and surveillance of a number of listed pathogens. Published and non-published methods will be assessed in our premises in order to offer validated protocols for the NRL´s
4-8	**Emerging diseases**	In collaboration with specialised experts WW to review selected emerging fish diseases in Europe and assess their potential listing as exotic or non-exotic diseases	Due to increased international trade of fish focus will be given to emerging diseases and rapid response. An assessment of risk for contracting and spreading emerging and re-emerging diseases in EU will be enforced in 2016 and 2017 (e.g. CEV – Koi sleepy disease; Piscine orthoreovirus infections in Rainbow trout and salmon, RLO-Rickettsia like organism in Sea bass, new
4-9	**Producing virtual teaching material (e-learning)**	Preparing virtual guidelines for conducting proficiency tests. For sampling and shipment of material for laboratory examination; and for receipt and processing fish tissue material for virology (inoculation on cell cultures and for PCR analysis) and histopathology	Set up tools for producing e-tutorials in-house. One tutorial on Dissection of fish for sampling for histopathology will be produced.
4-10	**Molecular characterization of fish cell lines**	Perform molecular analysis to "bar-code" and certify cell lines routinely used for viral diagnostics.	Misclassification of cell culture has been an issue constantly affecting cell culture work in terrestrial animals (including humans). In order to guarantee uniform and certified cell lines, genetic characterization and certification of relevant fish cell lines (i.e. EPC, BF-2) will be implemented

5. Missions and international meetings			
5-1	**Missions**	Organizing missions to relevant laboratories in EU and in third countries. Missions will focus on NRLs where on-site communication would be beneficial. As collaboration with NRLs in 3rd countries from where EU is importing large amount of fish products is increasing, missions to these, e.g. China and Korea is foreseen	1-2 missions will be conducted each year. The laboratories to visit will be appointed in order to strengthen collaboration in the NRL network. (e.g. Spain, France, Korea, Iran etc…)
5-2	**International meetings**	Attending missions, international meetings and conferences in order to be updated on emerging and listed exotic and non-exotic fish diseases.	The EURL expect to participate in 2 to 3 international conferences each year.

WORKSHOP EVALUATION

A questionnaire was delivered to the participant asking to evaluate various aspect of the workshop. 40 questionnaires were retrieved. Data compiled are shown hereunder. Specific comments will be considered for the next annual workshop organization.




Level of Overall satisfaction
20
15
10
5
0
70%
80%
85%
90%
100%
Level of Overall satisfaction

GREETINGS AND CONCLUSION OF THE MEETING

The next meeting will be held at the end of May 2017. It will, most likely, be organized in our facilities here in Copenhagen, either at Frederiksberg or in Lyngby we could also consider other facilities in Europe but we have to provide proper justification to the Commission to do so.

Thanks a lot to the people arranging the meeting as well as those of you who helped running the meeting by being chair persons, presenting stuff and being here