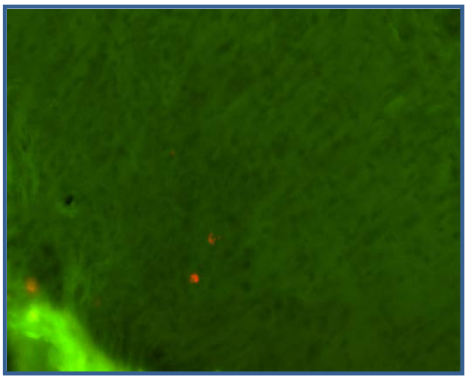
Report:

19th Annual Workshop of the National Reference Laboratories for Fish Diseases

Copenhagen, Denmark
May 27th-28th 2015

Gill necrosis in Koi Carp

SVCV CPE on EPC cell culture



FISH positive staining for Rickettsia like organism in sea bass brain

Organised by the European Union Reference Laboratory for Fish Diseases

National Veterinary Institute, Technical University of Denmark

Contents

INTRODUCTION AND SHORT SUMMARY

The 19th Annual Workshop of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark, 27th-28th May at the Auditorium of DTU Veterinary Institute in Bülowsvej 27, 1870 Frederiksberg C.
A total of 53 participants from 33 countries attended over the two days period, unfortunately 2 participants cancelled right before the meeting. There were five sessions with a total of 25 presentations, 3 of which were given by invited speakers, and a working group session.
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 2014 was provided on the basis of the results obtained from the Survey & Diagnosis questionnaire.
Then the fish disease situation in Norway was presented; a detailed report in Norwegian is available at: http://www.vetinst.no/nor/Publikasjoner/Fiskehelserapporten. An English version will be available at: http://www.vetinst.no/eng/Publications/Fish-Health-Report.
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 talks of the first session were completed by presenting an update on VHS and IHN control program which has been established and maintained in the Trento Region in Italy over the last 30 years, this latter talk included future perspectives and new initiatives.

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 2014.
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 2014. 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 Swedish representative presented an update on infectious disease status describing the recent outbreak of a novel viral disease in wild Eelpout, a wild fish species often monitored for assessing pollution level of sea.
This was followed by a presentation on amoebic gill disease (AGD). The relevance of this disease is increasing and treatment is required with freshwater of hydrogen peroxide. The development of diagnostic tools and interactions of the parasite with the seawater environment (including currents and temperature) were described.

The output of a cooperative project was presented afterwards, starting with the description of the disease situation in Switzerland with focus on a disease outbreak in perch. The description of the aetiological agent and its genetic and serological characterization was presented by the French representative.

This session was closed by a wide and comprehensive description of recently identified salmonid viruses including PRV, PRV-like and PMCV. The talk addressed the question on wether these emerging pathogens should be considered as candidates for future notifications..

The third session, on control and surveillance of relevant pathogens in the EU, started by a presentation on the new aquatic animal health legislation. In May 2013 a new law on Animal Health proposed by the Commission will be adopted by the European Parliament and the Council in the middle of June 2015. This regulation will be the main instrument for implementing the objectives of the Animal Health Strategy (2007-2013) and will constitute a single and robust legal framework for animal health including aquatic animals replacing approximately 40 existing directives and regulations. The new regulation aligns the animal health legislation to the Lisbon treaty and harmonies the rules for aquatic and terrestrial animals where appropriate.

The annexes to the Commission implementing decision, implementing Directive 2006/88/EC as regards requirements for surveillance and diagnostic methods are now finally adopted, after intensive revision and many discussions.

Finally, the third session was concluded with a presentation focusing on the Scottish aquaculture industry, describing how surveillance and disease control measures are applied by Marine Scotland Science. The establishment of models to predict disease outbreak, the code of conduct for best practice, synchronizing treatment and fish movement was described.

In the evening a banquet dinner was held at Restaurant ***"The Italian"*** in Vester Voldgade 25, 1552 København V.

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, a present and future core topic for all the laboratories involved in fish disease diagnosis, vaccination as a strategy to prevent infectious diseases and the establishment of experimental animal model to study infectious diseases. Focus was also given to new research initiative dealing with fish diseases that recently started within the Horizon 2020 framework.

The session started with a presentation showing results of MOLTRAQ, an EMIDA ERA-NET funded project that focus on the molecular tracing of viral pathogens from aquatic animals. This specific talk dealt with genetic characterization of VHSV strains that have been isolated in Denmark during the eradication program.

The session continued with a presentation describing if and how VHSV can evade the immune response that is mounted in rainbow trout after DNA vaccination. Results of the experimental work done so far seems to show that the vaccine can contain the infection at a level that the virus is unlikely to evade the response.

The session continued with an update on ParafishControl, an H2020 project granted project that focus on development of advanced tools and research strategies for parasite control in European farmed fish.

This was followed by a presentation describing the work performed in Finland on genetic analysis of infectious pancreatic necrosis viruses isolated in Finland during 2000-2014.

The next presentation described an innovative technology to be applied in the vaccine industry in aquaculture, which relies on the application of replicon technology. It has recently been demonstrated that it is possible to use salmonid alphavirus as vector for delivering the gene encoding for protective antigens of relevant pathogens for salmon.

Another output of the MOLTRAQ project was presented describing the genetic study on different KHV strains investigating their similarities at whole genome level.

The next presentation focused on Maldi-tof and the output that has been taken in a cooperative project between partners in Denmark, The Netherlands and Sweden. The future that characterizes this innovative diagnostic tool, its capacity of identifying microbial pathogens, benefits and challenges were described.

Findings of long term investigation performed in the Faroe Islands tracking the spread of ISAV HPR0 were described looking into tentative pathways of vertical transmission and horizontal through water transmission.

The session was concluded with 2 joint presentations. The first one defined and described the emergence of skin distress in farmed rainbow trout in UK, the second looking into the transmission studies and investigation performed for the aetiology.

The annual meeting ended with the traditional update from the EURL. The results of the two proficiency tests sent out in 2014, PT1 and PT2, were presented.
The program and application system for the annual training courses that will be provided by the EURL in October 2015 was described. The planned EURL activities in year 2015 were presented and proposals for the EURL work plan for 2016 were discussed.

Minutes from the meeting were taken by Drs. Susie Sommer Mikkelsen and Niccolò Vendramin, and have afterwards been sent to the presenters for correcting in order to avoid misunderstandings. The minutes are included in this report together with abstracts delivered by the presenters. Niccolò Vendramin assembled the report.

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 2016, more details will follow.

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

Copenhagen 18 August 2015
Niels Jørgen Olesen and Niccolò Vendramin

PROGRAM

Wednesday May 27th

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: – Susie Sommer Mikkelsen*
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	Past experience and future plans for the control and management of VHS-IHN in Trento regi in Italy Anna Toffan
10:45 – 11:05	***Coffee break***
11:05 – 12:35	Working groups: Perception of the impact of infectious diseases in Europe.
12:35 – 13:35	***Lunch***

SESSION II:	**Emerging Diseases**
	Chair– Eann Munro and Minutes: – Niccoló Vendramin
13:35 – 13:55	Rhabdovirus disease outbreak in the wild in Eelpout in Sweden Charlotte Axen
13:55 – 14:15	Amoebic gill disease. Current situation in Ireland and diagnostic tools development. Neil Martin Ruane
14:15 - 14:35	Actual fish disease situation in Switzerland Thomas Whali
14:35 – 14:55	First isolation of a rhabdovirus from perch in Switzerland Thierry Morin
14:55– 15:25	Do recently identified viruses in salmonids, like PRV, PRV-like, PMCV cause notifiable emerging diseases? Espen Rimstad
15:25-15:45	Update on the CEV situation in EU Olga Haenen
15:45-16:00	***Coffee break***
SESSION III:	**Control and surveillance of relevant pathogen in the EU**
	Chair–Brit Hjeltnes and Minutes: – Susie Sommer Mikkelsen
16:00-16:20	New aquatic animal health legislation Knut Roenningen
16:20 – 16:35	Diagnostic manuals
16:35 - 16:55	Disease control and sureveillance system in Scotland Eann Munnro
19:00 –	***BANQUET DINNER at Restaurant "The Italian"***

Thursday 28th May

Annual Workshop of the National Reference Laboratories

SESSION IV Scientific research update

Chair Uwe Fischer– and Minutes: – Niccoló Vendramin and Susie Sommer Mikkelsen

9:00 – 9:15	MOLTRAQ – Molecular Tracing of VHSV in Denmark Susie Sommer Mikkelsen
9:15 – 9:35	Ability of viral haemorrhagic septicaemia virus to evade the protective immune response induced in rainbow trout by DNA vaccination Dagoberto Sepulveda
9:35 - 9:50	Parafish - Advanced Tools and Research Strategies for Parasite Control in European farmed fish Niccoló Vendramin
9:50 – 10:10	Genetic analysis of infectious pancreatic necrosis viruses isolated in Finland during 2000-2014 Holopainen Riikka
10:10-10:30	***Coffee break***
10:30 - 10:50	Alphavirus replicon vaccines – a promising vaccine model for Atlantic salmon Espen Rimstad
10:50 – 11:10	Molecular characterization and tracing of KHV Sven Bergmann
11:10 – 11:30	Rapid identification of various pathogenic fish bacteria by MALDI-TOF Olga Haenen
11:30 – 11:50	Tracking ISAV-HPR0 Dissemination Debes Christiansen
11:40 – 11:55	Emerging skin disorders of rainbow trout in the UK: Current situation in the UK Jason Mewett
11:55-12:10	Emerging skin disorders of rainbow trout in the UK: transmission trials establishment Irene Cano
12:10-12:30	General discussion and ideas for future cooperative activities.
12:30 – 13:30	***Lunch***

SESSION V: Update from the EURL

13:30 – 13:50	Results of the proficiency test, PT1 and PT2, 2014 Niccolò Vendramin
13:50 – 14:10	EURL Training courses. Topics and organization for courses 2015 Niccolò Vendramin
14:10 – 14:30	EURL activities in 2014 Niels Jørgen Olesen
14:30 – 14:50	EURL workplan for 2014; Ideas and plans for 2016 Niels Jørgen Olesen
14:50 – 15:00	Next meeting and end of 19th Annual Workshop
15:00-15:30	***Coffee, cake and goodbyes***

Welcome

Niels Jørgen Olesen and Niccolò Vendramin wished everyone welcome to the 19th Annual Workshop. 53 participants consisting of scientists from 33 countries as well as Ph.D. students are attending the meeting.
Unfortunately two delegates had to cancel right before the Workshop; they will be informed through the report. After information on technical and practical issues Niccolò Vendramin describes 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 2014

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

[1]*DTU Vet National Veterinary Institute, Bülowsvej 27 Frederiksberc C, Copenhagen, njol@dtu.vet.dk*

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 evolved and changed a bit over the years it comprise 3 parts:

General data on aquaculture fish production, type, and size, health categorization of fish farms according to Council Directive 2006/88/EC, and information on national surveillance programmes.

Epidemiological data on the disease situation in each Member State with focus on the listed diseases but also including other diseases of interest.

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

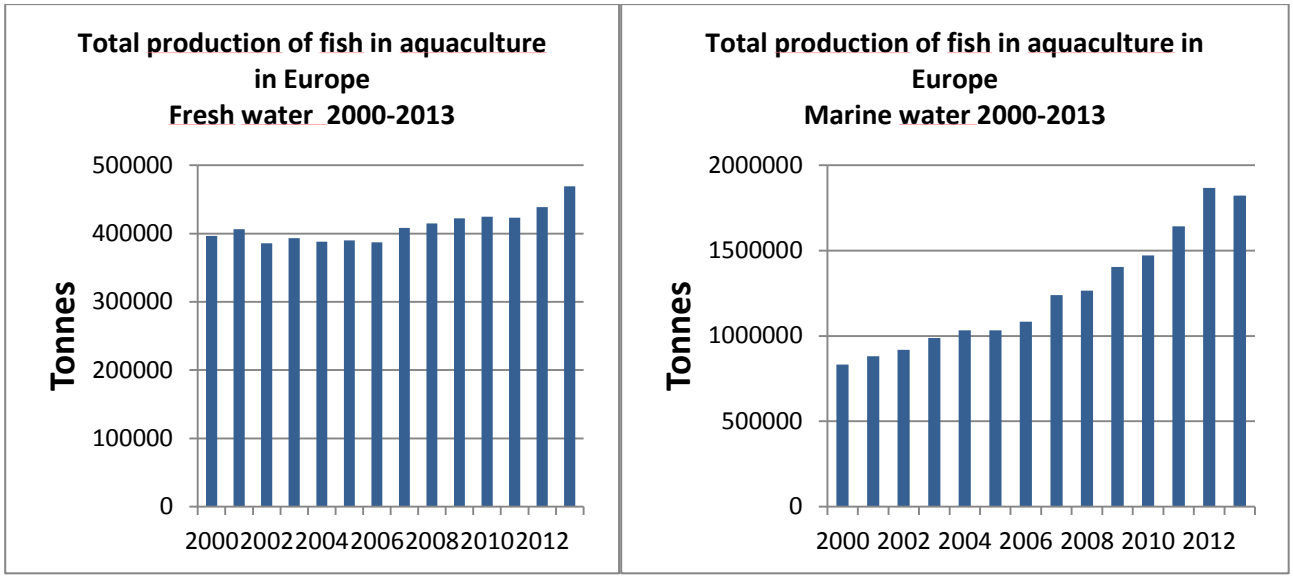
Production data from FIGIS

The data on the European aquaculture production were obtained from the FIGIS database. This database does not include information on the number and size of fish farms, and therefore these data had to be obtained directly in the questionnaire. In FIGIS only data from back to 2013 is available. Surprisingly and for the first time the total fish production in aquaculture in Europe did not increase in 2013. This is primarily reflected by the lower Atlantic salmon production, that might be due to the increase of the Chilean production or to a different reporting system in FIGIS. The Atlantic salmon production, however, still account for 1,43 mill ton against 1.5 mill ton in 2012, and is by far the largest contingency in Europe. The rainbow trout production has now passed the 400 000 t and increased with 12 000 t in 2013. The increase is however, primarily due to the increased production in Turkey! The carp production is still mainly in the Eastern part of Continental Europe and is very stable. Both the production of sea bream and especially sea bass also increased in the Mediterranean countries with a production of 142.000 t and 145.000 t, respectively. Among other fish species of interest are pike-perch (increase to 573t), eel (decrease to 4.017t), sturgeon (increase to 5.584t), turbot (decrease from 12.676t to 9.891t), and halibut (decrease from 1.854t to 1.485t) the cod production have almost collapsed from 22.729t in 2009 to 4.252t in 2013. The production of cleaner fish for lice control e.g. lumpfish 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 increase, but seem to be under way, while the sturgeon production is still on growing and more attention regarding health management might be given to this species.

Health Categorisation of fish farms:

Many countries provided very clear and correct answers but unfortunately a few more countries did not reply to the questionnaire when compared to the previous years. It is therefore still not possible to obtain a complete overview of the status of fish health categorization in Europe. There was however a

significant increase in the reported number of categorized farms (From 8.505 in 2012 to 14.463 in 2013 for VHS and from 7.360 in 2012 to 11.962 in 2013 for KHV!) this was especially due to Germany who successfully included almost all their farms in categorized zones.
All most 3/4 of the authorised farms in Europe are situated in category III zones for VHS and 2/3 for IHN. For both diseases the remaining farms are situated in category I or II. Very few farms are placed in category V infected areas, and it is obvious that the diseases are very underreported. In all countries except Norway almost all salmonid farms are in Category I for ISA. Only very few carp farms are approved KHV free in Category I as almost all are placed in Category III or nor categorized.
There are 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? According to a new proposal for adaptation by the EU Commission Isavirus HPR0 if detected in or in proximity of a farm, the farm can remain its Category I status. 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. However, only fish species listed as susceptible for the given listed disease shall be included, i.e. no sea bass / sea bream / eel farms / pike.perch etc. for IHN categories)
A new Animal Health Law is under preparation and revision and will include aquatic animals; in this connection the categorisation system might be simplified and be made more transparent.
Concerning the epidemiological data, obviously, there is still a severe underreporting of VHS, IHN and KHV in many countries. Only 52 and 54 of 8.896 farms are considered VHS or IHN infected, respectively, while 50 of 11.831 farms are considered KHV infected in the reporting countries. As in 2012 there were no ISA infected farms in Europe 31.12.2013! But there were a few outbreaks in Norway that have been contained.
Many countries have surveillance programmes for SVC (21 of 35 participating countries), BKD (17 of 35 countries), IPN (18 of 35 countries) and Gyrodactylus salaries (9 of 35 countries), for which they are seeking “additional guaranties” according to §42 in CD 2006/88/EC. The number of farms in the programmes varies from very few farms to many farms.
In northern European countries the most common problems are sea lice, pancreas disease, Amoebic gill disease in the salmon production, but in 2012 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, 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 seem to play an increasing role.
There are very large differences between countries on how many samples are tested on cell cultures, ranging from < 100 to several thousands. The total number of samples examined by cell culture decreased with 8% since last year while PCR increased with 16% and is now more used than cell culture, e.g. Norway only tested 226 samples on cell cultures but 5000 samples by PCR, as they also skipped VHS/IHN surveillance by cell cultivation! The large number of PCR-tests conducted in some countries mostly reflects the KHV and ISA testing.



Total production of fish in aquaculture in Europe 2000 to 2013 (http://www.fao.org/figis)

Comments

Concerning ISA, Norway decided to include all farms in cat. 3 (unknown status) while Scotland all in cat 1.

UPDATE ON FISH DISEASE SITUATION IN NORWAY

Hjeltnes B.1

1Norwegian Veterinary Institute, P.O Box 1263 Sentrum, NO-5811 Bergen, Norway,
brit.hjeltnes@vetinst.no

Abstract:

In 2014, Norway produced 1.198900 tons of Atlantic salmon (Salmo salar), 74300 tons of rainbow trout (Oncorhynchus mykiss) 3800 tons of Atlantic cod (Gadus morhua), 1500 tons of Atlantic halibut (Hippoglossus hippoglossus) and 800 tons of other species.
Salmon louse infestation represents one of the most significant challenges to Norwegian aquaculture and increased resistance to anti sea louse 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 louse.
The main viral problem is pancreas disease, infection with salmonid alphavirus (SAV). There has been was an increase in the total number of sites (142) with reported disease outbreaks (SAV3 and SAV2).
Ten cases of infectious salmon anaemia (ISA) was registered in 2014.
IPN was diagnosed in a total of 48 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.
The number of heart and skeletal muscle inflammation (HSMI) was diagnosed in a total of 181 sites, which is the highest number ever reported. HSMI was in 2014 removed from the Norwegian national list of notifiable diseases.
The Norwegian Veterinary Institute diagnosed cardiomyopathy syndrome (CMS), also known as 'heart rupture' on 107 sites. This is an increase over recent years.
Amoebic gill disease, Paramoeba perurans (AGD) has increased in abundance and distribution.
A new disease in rainbow trout was first reported from three hatcheries in 2013 and has also been reported in 2014. A viral etiology is suspected.
Production losses remain a significant problem in Norwegian aquaculture.

Questions

Q: Concerning IPN, did you implement an eradication program?

Brit Hjeltnes: In order to control IPN, the biggest contribution come from the breeding program, selecting eggs resistant to IPN .

UPDATE ON FISH DISEASE SITUATION IN THE MEDITERRANEAN BASIN

Vendramin N.1

1DTU Vet National Veterinary Institute, Bülowsvej 27 Frederiksberc C, Copenhagen, niven@dtu.vet.dk

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 fast in the last 20 years and the production is estimated to be around 1,6 Million Tonns per year with a corresponding value of over 5 billion Dollars (FIGIS 2013).

The aim of this work is to maintain and expand the platform established in 2012, in order to share information and communicate between 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 16 experts were obtained about disease situation in the Mediterranean basin for 2014. 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

Comment

Snjezana Zrncic: In Croatia there has been outbreaks of VER-VNN last summer. Large problems in fry and also other stages, all from batches sold or certified as free from this pathogen. It is important to address this issue also in the legislation.

PAST EXPERIENCE AND FUTURE PLANS FOR THE CONTROL AND MANAGEMENT OF VHS-IHN IN THE TRENTO REGION - ITALY

Anna Toffan1, Valentina Panzarin1, Rosita Quartesan1, Chiara Ceolin1, Luigino Bortolotti2

1Istituto Zooprofilattico Sperimentale delle Venezie, National Reference centre for fish, molluscs and crustacean diseases, Legnaro, Padova, Italy

2Azienda Provinciale per i Servizi Sanitari Provincia Autonoma di Trento

Abstract:

Trento is the most important Italian region for trout/egg production and trade. It was here that in the late '70s the VHS/IHN eradication program was carried out as a voluntary campaign; in 1992 this initiative was put in line with the European legislation (91/67/CE) and obtained a financial support from public institutions. In 2008 the eradication campaigns were further implemented following Council Directive 2006/88/EC. Nowadays, the region counts 68 farms, 45 of which are classified as category I; as well, 12 production sites are classified as category V. This shows that, despite the eradication programs, the several stamping out strategies and fallowing, several infected farms are still to be found throughout the territory.

Council Directive 2006/88/EC requires category V farms to be subjected to minimum control measures and passive surveillance only; on the other hand, thanks to a regional resolution (Reg. delib.n.740 del 19/5/2014) the Trento Region decided that also category III and V farms were to be subjected to annual monitoring processes. At the beginning of 2015, sampling from each infected farm was performed and the results obtained are going to be presented.

In the above mentioned regional resolution, by way of derogation from 2006/88/EC due to economic or practical reason, a sector-based eradication plan may be authorized in order to prompt farmers of category V farm to be upgraded to category I. The sector-based eradication plan must be approved and supervised by the local veterinary authority. Advantages and disadvantages of this approach will be discussed.

Finally, the possibility to use vaccination to help fish farmers to get rid of the disease is under evaluation. To date, no commercial available vaccine against VHS or IHN is available, and from literature, the best vaccine appears to be DNA technology. Since IHNV and VHSV are both present in the majority of infected farms, co-administration of the DNA vaccines could be an option. Unfortunately, DNA vaccination at present is still a sensitive issue, due to the lack of regulatory precedents in Europe and a general aversion to the GMOs.

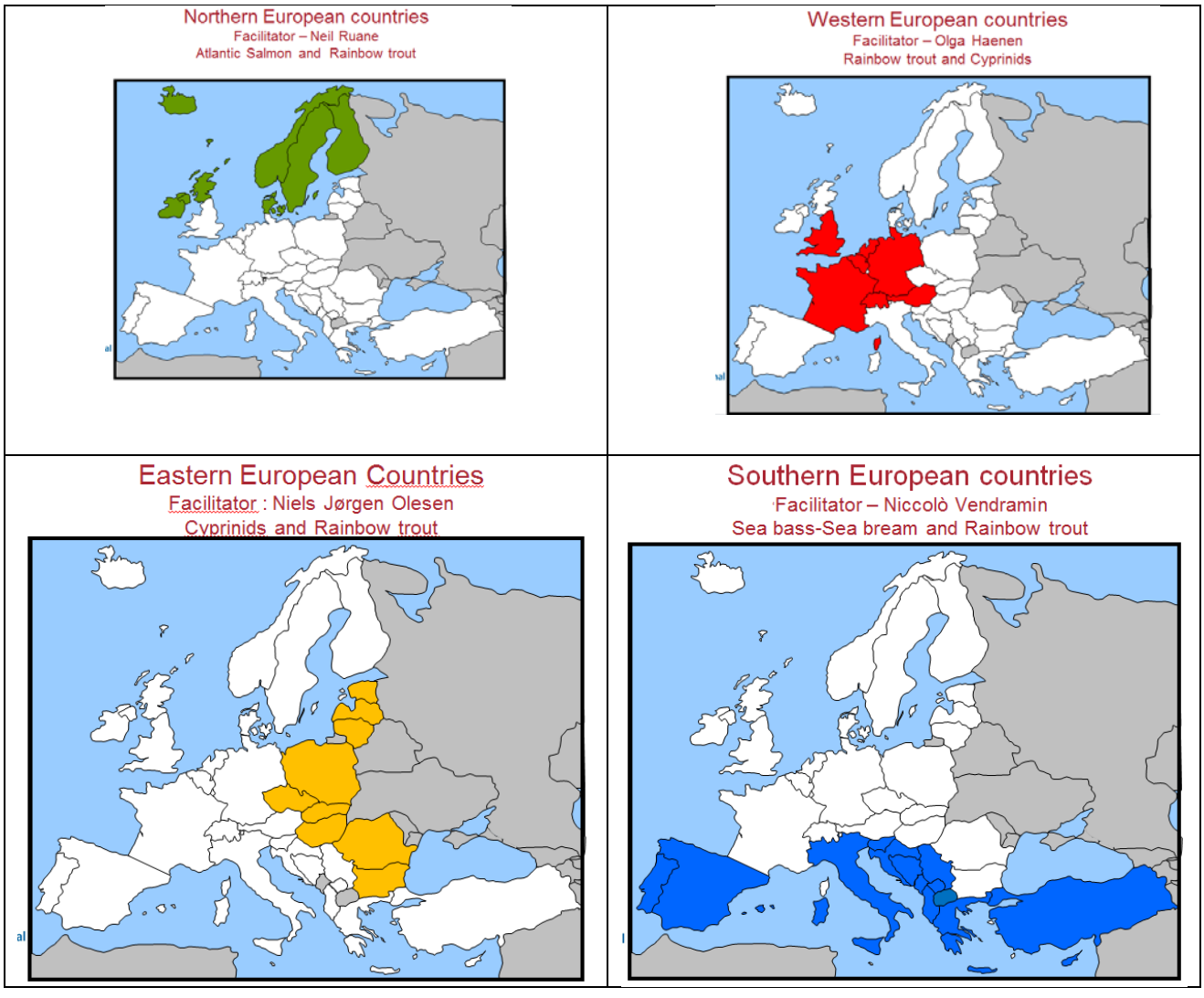
Questions:

Hildre Sinde: Did you test wild fish? It could be important, especially in relation to the eradication plan as they could act as carriers.

Anna Toffan: No, but farms in the same river are quite close related we do not expect to have many wild fish in that trait of river.

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

This year the activity that was introduced for the first time last year was conducted once again.
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 onr 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.

Northern countries

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

Concerning Atlantic salmon the most important diseases were considered to be caused by viral pathogens being PD, caused by SAV-salmonid alphavirus, the most important one. At a lower level of "impact" both CMS and HSMI were considered to have a significant impact.

Concerning bacterial diseases, some of them have still an economical significant impact often linked to the downgrading of the fish at the slaughterhouse; other bacterial pathogens can become relevant as secondary infections. It has to be underlined that the impact is also related to the decision of having the disease at the farm and control it or initiate a strategy that aims to keep out the disease completely from the farm.

Concerning parasitic diseases Sea-Lice and AGD are considered of major importance, AGD is considered a major problem both in Scotland and Ireland.

ISA, BKD and Gyrodactylus have low impact economy wise, whereas they are still relevant for the legislation

Concerning Rainbow trout production the infectious diseases considered to have the highest impact on production are the bacterial diseases, Rainbow Trout Fry Syndrome-RTFS and Enteric Red Mouth – ERM.
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.
Jason Mewett from the English fish health inspectorate had the opportunity to present the output of the discussion.
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.
Looking into bacterial diseases, as for the Eastern European production the two most important diseases in 2014 were RTFS and Furunculosis having a significantt impact both on production and on economy.
Finally among parasitic diseases, *Ichthyophthirius multifiliis* and PKD reach the highest score in production and economy.
For the cyprinid species, as general remark the impact of the diseases is quite different among countries. This fact is linked to different production levels. While in Germany, for example, there is a major production of this fish for food consumption, in UK fish are mostly farmed for recreational purposes. The most important bacterial disease derives from infection with *Aeromonas* spp., while *Ichthyophthirius multifiliis* is the most important parasitic disease. The infection caused by CEV is kept under monitoring.

In **Eastern Europe** the main species addressed were Cyprinids and Rainbow trout.
Dr. Thomas Vesely was in charge of presenting the output of the discussions.
Concerning rainbow trout production the two most important diseases are still considered viral listed diseases VHS and IHN, these cause still relevant and significant losses both in terms of production, economy and legislative consequences. As mentioned for the Nordic countries RTFS is still an important issue, secondarily also Furunculosis caused by *Aeromonas salmonicida* was perceived as an important disease in 2014. Parasitic diseases did not raise major concerns, while as “emerging disease” cases of skin disorders like Strawberry diseases have been reported.
Concerning the cyprinid production KHV is considered to cause the highest impact, while SVC is still considered asa relevant problem. Some countries detected new cases of CEV and reported them as an emerging disease . Infection with *Aeromonas* spp, *Ichthyophthirius multifiliis* and Philometra are considered the most important among bacterial and parasitic diseases, respectively.

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 Zrncic was in charge of describing the discussion output for southern European countries.
Unfortunately the discussion was affected by the absence of representatives from Spain and Greece, however data from Spain were included after the WS to complement this report.
For Seabass and Seabream, VER-VNN is the most important disease. Vibriosis is at the second 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. Concerning parasitic diseases Gill fluke were considered the most important ones.
For Rainbow Trout, the impact of VHS and IHN can be 0 in farms/countries which are free from these diseases whereas score high on criteria 3. Among bacterial diseases RTFS and ERM are considered having the highest impact on production and economy. On a regional basis, Lactococcosis had a significant impact. Concerning infestation with parasites gill diseases are considered to be the most important in 2014.
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 disease with a more holistic view, not focusing only on legislative obligations.

SESSION II: EMERGING DISEASES

Chairman: Dr. Eann Munro

RHABDOVIRUS DISEASE OUTBREAK IN THE WILD IN EELPOUT IN SWEDEN

Charlotte Axén[1], E Blomkvist[1], A Alfjorden[1], M Hakhverdyan[1], T S Boutrup[2], H Ahola[1], F Ljunghager[3], Å Hagström[1], N J Olesen[2], M Juremalm[1], M Leijon[1], J-F Valarcher[1]

[1] National Veterinary Institute, Uppsala, Sweden; [2] Technical University of Denmark, Copenhagen, Denmark; [3] Swedish Agency for Marine and Water Management, Gothenburg, Sweden

Abstract:

In January to October 2014, mass mortalities were reported in eelpout (Zoarces viviparous) along the Swedish south-east coast. Diseased fish were lethargic, sometimes with loss of balance and did not move until touched. Movements were uncoordinated and some fish showed breathing difficulties. Necropsy of seven eelpouts from two separate locations (Outbreak#1-2) did not identify any significant lesions. Liver appearance varied whereas gills and spleens were bright red. Microscopically, the brain was the only organ where specific viral lesions were seen; with cortical histiocytic cell infiltration, single cell neuronal degeneration and perivasculitis. There was stasis and a significant amount of intercellular debris in investigated organs. The gill epithelium was hypertrophic and the liver parenchyma was hyperplastic with apparent loss of structure, fragmented or duplicate nucleoli and double nuclei. Single cell necrosis and oedema was present in the pancreas. Focal oedema was also identified in the kidney interstitium.

A virus was isolated by cell culture, where CPE appeared on BF-2 cells day 5 post inoculation (p.i.), with full CPE day 7 p.i. For Outbreak#1, CPE on FHM cells only occurred after inoculation with BF-2 culture supernatant. For Outbreak#2, CPE on FHM cells appeared on day 7 p.i, with full CPE day 14 p.i. Titration identified a 2.7 x 10^5 $TCID_{50}$/ml for BF-2 cells and 1.9 x 10^4 $TCID_{50}$/ml for FHM cells. EPC and RTG-2 cells did not produce CPE for Outbreak#1. For Outbreak#2 there was full CPE on EPC cells day 7 p.i, and RTG-2 was not done. ELISAs for VHSV, IHNV, IPNV and SVCV were negative. Chloroform inactivation indicated presence of a rhabdovirus. However, further rhabdovirus testing (hirame rhabdovirus, perch rhabdovirus, snakehead rhabdovirus, pike fry rhabdovirus, rhabdovirus anguillae and an un-characterized sculpin rhabdovirus) by IFAT gave negative results. TEM identified a typical bullet-shaped rhabdovirus appr. 140 x 80 nm in size. By deep sequencing of tissue suspension and supernatant from BF-2 culture the whole genome was identified (consensus sequence of 11,139 nucleotides, Outbreak#2). It had a 59.5% overall match to the closest relative, Siniperca Chuatsi rhabdovirus (SRCV). Open reading frames (ORFs) with starting codons exactly aligning with those of SCRV were found for all typical rhabdovirus proteins except for the glycoprotein, that was shifted four amino acids away. Pair-wise comparison of Outbreak#1 and Outbreak#2 sequences revealed an identity of 99.92-99.98 % for sequences within Outbreak#2 and 99.76-99.77 % between Outbreak#1 and Outbreak#2 sequences. A PCR based on the L-gene identified

viral RNA in kidney, spleen and heart as well as in the brain (N=10 fish), although in some fish the signal was lower or lacking in other organs than the brain. PCR of organs from healthy eelpout (N=40) was negative, indicating that the virus is associated with the disease and mortalities.

Conclusions

Deep sequencing shows that this is a previously undescribed rhabdovirus, we name it eelpout rhabdovirus (ERV). Lack of variability between viral RNA from Outbreak#1 and Outbreak#2, both geographically and temporally separated, shows that ERV is an emerging virus in the Baltic Sea. Symptoms and microscopic lesions in the brain indicate that ERV is neurotropic. For ERV isolation, BF-2 is the best of the four routinely used cell cultures. Since at least two cell lines should be used, we recommend a combination with FHM culture if ERV is suspected.

Questions

Olga Haenen: In which species did you find IPN Ab ?

Charlotte Axen : in Rainbow trout

Heike Shuetze : At which temperature did you grow the EPRV

Charlotte Axen: 15° C

Neil Ruane: What RT-PCR did you use to identify the virus?

Charlotte Axen: the primers target a region of the N-gene, I can provide more information if needed

AMOEBIC GILL DISEASE: CURRENT SITUATION IN IRELAND AND DIAGNOSTIC TOOLS DEVELOPMENT

Neil Ruane, Jamie Downes

Fish Health Unit, Marine Institute, Oranmore, Co. Galway, Ireland

Abstract:

Amoebic gill disease (AGD), caused by the amoeba *Neoparamoeba perurans*, has been a significant disease of farmed salmonids in Tasmania since the mid 1980's and until recently, was only reported as causing occasional problems in other salmonid producing countries. It was first reported in Ireland in 1995, resulting in significant mortalities in eight sites. Since that time there have been occasional reports of the disease, mainly confined to a small number of sites. In 2011 the situation changed. In July 2011 the first reports of AGD emerged in a salmon farm in the north of France, followed by reports in Ireland (August) and Scotland (September). Outbreaks continued to occur in both Ireland and Scotland in 2012 and in the autumn the first reports emerged from Norway. In 2013 the number of cases increased in Norway and was followed by the first AGD cases in the Faroe Islands in 2014. On-farm monitoring consists of regular gill scoring complemented by gill smears, histopathology and real-time PCR. The only effective treatments against AGD are freshwater and/or hydrogen peroxide bathing, which may have to carried out a number of times during the production cycle. An overview of the situation in Ireland will be presented along with information on the development of a real-time PCR assay and its use in a longitudinal study on a marine Atlantic salmon farm in the south-west of the country.

Questions:

Eann Munro: You mention that famers normally use freshwater treatments? Is it standard to use freshwater over hydrogen peroxide?

Neil Ruane: It is used, however the summer water temperatures can reach 16 – 18^{o}C which is too high for using peroxide at the recommended dose

Niccoló Vendramin: Do you have any insight on the specificity of RT-PCR? Would be interested to know if it works on what is known to be "Freshwater gill amoeba"

Neil Ruane: Specificity was assessed in silico and on marine amoeba. Not tested on freshwater.

ACTUAL FISH DISEASE SITUATION IN SWITZERLAND

Thomas Wahli

Centre for Fish and Wildlife Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Abstract:

So far in Switzerland no regular active surveillance programs in fish farms have been performed. However farmers, veterinarians and diagnostic laboratories have to report any suspect of a notifiable disease to the competent authorities. This situation will change. Beginning in 2016 a regular active risk based surveillance will be established.

Consequently all cases received to date by the Fish disease laboratory were sent in because of disease problems or voluntary health controls making a reliable overall assessment of the current situation difficult. Nevertheless the data allow generating an overview on the current situation.

In 2014 one case of Viral Haemorrhagic Septicaemia (VHS) was recorded in a farm producing rainbow trout and brook charr. Only rainbow trout were affected. In a farm producing organic trout an outbreak of Infectious Haematopoietic Necrosis was detected associated with high mortalities in affected fish. The farm consisted of two separated parts. As transmission from the upper to the lower part was suspected, fish from all tanks were investigated. Thereby, in the upper part no IHN could be detected but Infectious Pancreatic Necrosis (IPN), a notifiable disease in Switzerland was found. Subsequently, all stocks were destroyed.

In 2013 two new viral diseases had been diagnosed in Switzerland. In a perch producing indoor farm a perch rhabdovirus has caused losses among freshly imported fish. Further, in a part recirculation farm producing rainbow trout, fish showed signs considered as typical for sleeping disease. Indeed a salmon alpha virus could be detected by means of qPCR. The virus was identified as belonging to the subtype 2 clustering together with isolates from UK and continental Europe. In 2014 the virus was detected again in the same farm while so far no fish from any other facility have shown to be infected by this virus.

In a farm producing Siberian sturgeon repeatedly herpesvirus has been detected. This virus led to considerable losses particularly among young fish while older fish seldom succumbed to the infection.

Among bacterial infections Flavobacterioses, be it external infections of the skin or gills or systemic infections, were the most frequent and most devastating diseases.

Several events of mass mortalities of wild fish were ascribed to an infection by *Saprolegnia parasitica*. There is a major debate, whether a new particularly pathogenic strain is actually spreading. Further problems in wild fish are associated with the infection by *Tetracapsuloides bryosalmonae*, the causative agent of Proliferative kidney disease (PKD). In contrast to the situation in wild fish problems with PKD in farmed fish are rather limited.

Questions:

Anna Toffan: Can you give some more details on the PCR used for Sturgeon virus?

Thomas Wahli: Samples were sent to Sven Bergmann.

Sven Bergmann: It is a nested PCR . **See**: Kevin T. Kwak, Ian A. Gardner, Thomas B. Farver, Ronald P. Hedrick (20016) Rapid detection of white sturgeon iridovirus (WSIV) using a polymerase chain reaction (PCR) assay. Aquaculture 254 (2006) 92–101

FIRST ISOLATION OF A RHABDOVIRUS FROM PERCH IN SWITZERLAND

T. Morin[2,*], L. Bellec[2], B. Von Siebenthal[1], J. Cabon[2], H. Schmidt-Posthaus[1], T. Wahli[1].

[1] Centre for Fish and Wildlife Health, Bern, Switzerland
[2] French Agency for Food, Environmental and Occupational Health & Safety, European University of Brittany, Plouzané, France

Abstract:

Rhabdoviruses infect a wide range of hosts, including vertebrates, invertebrates and plants. They are among the most devastating viruses for the aquaculture worldwide and, under certain circumstances, they can cause a significant ecological impact on wild fish populations. *Perca fluviatilis* is a fish species of increasing interest for the Swiss fish farming industry and recirculation systems have been specifically set up in recent years to develop its production. In one of these farms, an aberrant spiraling swimming associated to elevated mortalities occurred repeatedly in imported fish shortly after stocking. No bacterial or parasitic etiology was detected but a Perch rhabdovirus was isolated on BF-2 cells and identified using a specific indirect fluorescent antibody technique (IFAT). Subsequent investigations on other samples suggested a special viral tropism for the central nervous system (CNS). Phylogenetic analysis on the partial N and entire G gene sequences positioned this Swiss isolate in the genogroup C of the Perch rhabdovirus species, with high nucleotide and amino acid (aa) identities with the DK5533 strain isolated in Denmark in 1989. Comparative studies using other isolates allowed to distinguish two serological patterns among Perch rhabdoviruses and to identify an aa position in the glycoprotein potentially involved in the antigenic differentiation. Even if perch recently imported in the farm were tested negative in virology prior to transport, they may have been the origin of this outbreak because CNS was not included in the samples analyzed. Another possibility might be a covert infection in the farm with a virus load in resident fish too low to be detected. This study reports the first case of Perch rhabdovirus disease in a Swiss farm and underlines the difficulty to effectively diagnose infection in asymptomatic fish. The high identity of the Swiss Perch rhabdovirus isolate with a strain described in Denmark in 1989 strongly suggests that movements of fish contribute to the spread of pathogens, particularly for species in development for which animal science and knowledge of infectious diseases are to deepen.

Minutes:

Olga Haenen: The Danish strain of rhabdovirus that was used as reference which species belong to, was it wild or farmed?

Niels Jørgen: It was isolated from farmed pike, it was the first detection of this pathogen.

DO RECENTLY IDENIFIED VIRUSES IN SALMONIDS LIKE PRV AND PMCV CAUSE NOTIFIABLE EMERGING DISEASES?

Espen Rimstad[1], Øystein Wessel[1], Turhan Markussen[1], Hanne Haatveit[1], Stine Braaen[1], Ingvild B. Nyman[1], Maria K. Dahle[2].

1 Faculty of Veterinary Medicine and Biosciences, Norwegian University of Life Sciences, Oslo, Norway

2 Norwegian Veterinary Institute, Oslo, Norway Brittany, Plouzané, France

Abstract:

Infectious diseases of fish do not respect national boundaries and may have detrimental effects on production and on export possibilities. For these and other reasnons some diasese are notifiable.
Properties of *Piscine orthoreovirus* (PRV) and *Piscine myocarditis virus* (PMCV) and the diseases they cause should determine the set of rules made by fish health authorities. Many of these properties are not known in sufficient details and estimations are used. This includes knowledge about reservoirs, susceptibility of infection for the different species of fish, the pattern of shedding of virus and survival of viral infectivity outside the host.The development of effective vaccines offers another way of preventing and controlling risks.
PRV is associated with heart- and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon. HSMI is most often observed a few moths after sea transfer, but an increasing number of cases have been reported from freshwater stage. Taxonomically PRV belongs to the family *Reoviridae*, most closely related to the genus *Orthoreovirus*. A distinct variant of PRV has recently been found in rainbow trout. Reoviruses are non-enveloped viruses with icosahedral capsids surrounding the double stranded RNA (dsRNA) genomes. The PRV genome consists of 10 dsRNA segments distributed in the classical orthoreoviral groups of three large, three medium and four small segments.
Piscine myocarditis virus (PMCV) is associated with cardiomyopathy syndrome (CMS). It is classified as a totivirus, i.e. small, non enveloped, icosahedral virion with a linear dsRNA genome. CMS is a disease observed late in the production cyclus.

Questions:

Torsten Snogdal Boutrup: If the risk of international spread is limited shouldn't it be a notifiable disease so that action is taken to minimize the spreading`?
Espen Rimstad: This decision applies more to the policy and I am not an expert, my understanding of listed disease is that this classification is reserved for more virulent agents.
Comments

Eann Munnro: PRV has been found also in Scotland.
Britt Hjeltnes: The first finding seems to be in Mid-Norway, others not used to look for it.
Espen Rimstad: It seems that from there the strain has been spreading.

UPDATE ON THE CEV SITUATION IN THE EU

O. Haenen[1], K. Way[2], N. Vendramin[3], S. Bergmann[4], H. Schütze[4], L. Bigarré[5], M. Adamek[6], V. Jung-Schroers[6], T. Veselý[7], T. Waltzek[8], M. El Matbouli[9], V. Piačková[10], D. Stone[2], M. Matras[11], M. Gjessing[12], O.B. Dale[12], A. Toffan[13], M. Abbadi[13], V. Panzarin[13], S. Sommer Mikkelsen[3], and N.J. Olesen[3]

[1]CVI of Wageningen UR, Lelystad, The Netherlands; [2]CEFAS, Weymouth, Dorset, U. K.; [3]EURL for Fish Diseases, DTU-Vet, Copenhagen, Denmark; [4]FLI, Germany; [5]ANSES, France; [6]University of Veterinary Medicin, Hannover, Germany; [7]Veterinary Research Institute, Brno, Czech Republic; [8]University of Florida; [9]University of Veterinary Medicine, Vienna, Austria; [10]University of South Bohemia, Czech Republic; [11]PIWET, Poland; [12]NVI, Oslo, Norway; [13]IZSV, Italy

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Abstract:

The pox virus that is the disease agent of koi sleepy disease (KSD) was originally described in Japan in the 1970's as a viral oedema of juvenile koi carp (CEV). The virus was also shown to affect adult koi and cause severe damage to gill lamellae, leading to hypoxia, lethargy (sleepy behaviour) and death from anoxia. Losses from CEV were seen in spring and autumn, over a temperature range of 15 – 25°C, and mortalities reached 80%.

In Europe, outbreaks of KSD and PCR detections of CEV-like virus were reported from 2009. In 2012, a CEV-like virus was detected for the first time at a fishery in England in common carp, displaying KSD-signs, , the disease was later diagnosed at other English fisheries in 2012, 2013 and 2014, CEV was also detected in common carp fisheries in other EU member states during these years. By early 2015, at least Austria, Czech Republic, France, Germany, Italy, The Netherlands and the UK had reported multiple KSD outbreaks in imported koi. CEV-like virus was confirmed, in all cases, using improved PCR assays.

A Carp Edema Virus-CEV Workshop was held at the premises of the EURL for Fish Diseases in Copenhagen, Denmark, 12th- 13th January 2015. Twenty participants from 11 countries (10 European, and USA, including the EURL Fish Diseases, CVI of WageningenUR, FLI, Uni Vet Med Hannover, ANSES, VRI Brno, Uni Vet Med Vienna, Uni S-Bohemia, CEFAS, Piwet Poland, NVI Oslo, IZSVE Italy, and Uni of Florida) attended the meeting, with oral presentations and discussions. The workshop was organized because of the increasing number of diagnostic cases where CEV was detected in diseased cyprinids (both koi and common carp, *Cyprinus carpio*).

The primary aim of the workshop was to share knowledge, diagnostic protocols and material among participants and evaluate different strategies on how to tackle this issue. The first day representatives of all countries participating in the workshop described their experience and the cases where fish poxvirus

was detected. Subsequently diagnostic procedures available for the detection of this pathogen were described and compared. During the second day a common strategy on how to tackle this pathogen with research project was addressed, looking into funding opportunities and cooperative activities.

In this lecture, the main outcome of the workshop will be presented, regarding diagnostic methods (a TaqMan RT-PCR developed at CEFAS (D.Stone & K. Way) is choosen as the European test method), the need of a surveillance method to be able to test if CEV is emerging in Europe and would be a candidate to be listed, types of CEV (CEFAS (D.Stone & K. Way) sequenced and found two lineages, one from Koi and one from Common Carp), water temperatures at outbreaks in carp or koi (carp mostly at 6-9°C, koi mostly at 15-25°C), epidemiology of current and old detections (archive samples tested as well), and papers in preparation (like a joint alert paper on CEV in Europe with this network from Way *et al.*). At the EAFP Conference 2015, a *Novel viruses of cyprinids* workshop lead by Haenen, Waltzek and Way will also discuss this virus.

Financial support is aimed for international networking and research on CEV, to estimate its possible impact.

Comments

Sven Bergmann: There are two qPCRs; one from Hannover and one from CEFAS. It seems that the protocol from CEFAS recognizes better the CEV-like viruses from carp while the protocol from Hannover recognizes better CEV strains from Koi. Conventional and nested PCR protocols are working for both CEV variants.
Olga Haenen: Our SYBR green qPCR sometimes works better than the CEFAS PCR so far.
Sven Bergmann: we detect CEV in Koi Carp for the first time in 2010 in Germany.

SESSION III:

UPDATE ON IMPORTANT FISH DISEASES IN EUROPE AND THEIR CONTROL

Chairman: Dr. Brit Hjeltnes

NEW AQUATIC ANIMAL HEALTH LEGISLATION

Knut Roenningen

European Commission, DG SANTE, 101 Rue Froissart, 1040 Brussels, knut.roenningen@ec.europa.eu

Abstract:

A new Regulation on Animal Health proposed by the Commission in May 2013 will be adopted by the European Parliament and the Council in the middle of June 2015. This regulation will be the main instrument for implementing the objectives of the Animal Health Strategy (2007-2013) and will constitute a single and robust legal framework for animal health including aquatic animals replacing approximately 40 existing directives and regulations. The new regulation aligns the animal health legislation to the Lisbon treaty and harmonies the rules for aquatic and terrestrial animals where appropriate.

With regard to the specific requirements for aquatic animal health this new regulation in most parts maintains the principles set out in Directive 2006/88/EC. There are, however, important differences to be mentioned:

a) The regulation with its delegated and implementing acts will set out specific requirements and responsibilities for operators, transporters, veterinarians, fish health inspectors etc. which will be directly binding all over the Union and eventually also the EEA–area.

b) The scope of this regulation is significantly broader than Directive 2006/88/EC with regard to wild aquatic animals and provides a legal framework for setting out detailed rules also for movement of all wild aquatic animal species relevant to listed diseases.

c) The regulation also provides more possibilities for setting out animal health rules for use of aquatic animals for specific purposes other than aquaculture such as zoos, garden ponds etc.

c) There are established new criteria for the listing of diseases and the listed diseases will in accordance with the new regulation be divided into five categories (a – e).

d) The list of diseases will be set out in an annex of the regulation and amendment of this annex is foreseen to be done by a delegated act, which means that deleting or adding diseases to that list has to be adopted both by the Council and the European Parliament.

The new regulation is foreseen to enter into force 1 July 2020 following a transposing period of 5 years. Within the first three years of this period the Commission is obliged to review the list of diseases and present the appropriate delegated and implementing acts for adoption.

DIAGNOSTIC MANUALS FOR LISTED NON-EXOTIC FISH DISEASES: STATUS AND IMPLEMENTATION

N. J. Olesen[1], Susie S. Mikkelsen, and Niccolò Vendramin [1]

[1]*DTU Vet National Veterinary Institute, Bülowsvej 27 Frederiksberc C, Copenhagen,njol@dtu.vet.dk*

Abstract:

The annexes to the Commission implementing decision, implementing Directive 2006/88/EC as regards requirements for surveillance and diagnostic methods are now finally adopted, after intensive revision and many discussions.

The annexes 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 Annexes will replace the Sampling plans and the diagnostic methods for the detection and confirmation of VHS and IHN are laid down in the Commission Decision 2001/183/EC and for ISA in Commission Decision 2003/466/EC. No Commission Decisions on KHVD have been in place before this decision.

Compared to the previous Decisions on VHS, IHN and ISA the major changes are risk based surveillance, the possibility for conducting surveillance for the 3 diseases by RT-qPCR alone (before cell cultivation was mandatory for VHS and IHN), Surveillance and notification only for ISA-deleted is now made possible. New updated technics are included and others are removed. While the 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 should be made aware of them. The updated manuals for each disease will be presented and can be found at www.eurl-fish.eu .

THE SCOTTISH AQUACULTURE INDUSTRY - Surveillance and disease control measures applied by Marine Scotland Science

Eann S Munro & Neil L Purvis

Marine Scotland Science, Marine Laboratory, 375 Victoria Road, AB11 9DB, Aberdeen, Scotland, UK.

Abstract:

Scottish finfish aquaculture production has increased significantly over the past 10 years. In 2013, 163 234 tonnes of Atlantic salmon (*Salmo salar* L.) was produced with a farmgate value of £677 million, an increase of 26 per cent compared to the previous year.

An important strategy adopted within Scotland to maintain a high health status is the use of disease control management areas. Marine finfish aquaculture production is divided into defined management areas around active fish farms based on overlapping tidal excursion zones (3.6 km radius in Shetland, 7.2 km elsewhere). Therefore, sites with overlapping separation distances will be grouped within the same disease management area. The strategy has been successfully applied to the eradication of infectious salmon anaemia virus in 1998 and 2009. Scotland also has a policy of eradication for most serious listed diseases. This is an important control measure in terms of disease status.

A risk based surveillance strategy has been implemented by MSS and is designed to identify those aquaculture sites which are of the greatest risk of introducing and transmitting diseases and their agents. The frequency of site inspections are dependent upon the result of the risk assessment applied to each individual fish farm and the health status of the management area that the farm is situated within. This approach is combined with passive and intelligence led surveillance initiatives, both of which can trigger further investigations.

Fish farm risk classification	Minimum number of site visits
High	1 per year
Medium	1 every 2 years
Low	1 every 3 years

A voluntary agreement exists between aquaculture companies and the competent authority regarding the reporting of increased mortality levels over defined threshold limits* on marine salmonid sites. These reports will trigger further investigation with the potential for a site inspection and subsequently a diagnostic investigation, if appropriate.

Recently, an approach to provide an alert system for detecting potential emerging diseases has been considered by MSS. A Generalized Linear Model (GLM) identifying trends and anomalous observations from diagnostic case reports has been tested by investigating retrospective outcomes focusing on amoebic gill disease (AGD). This infectious disorder of marine farmed salmonids was first reported in Scotland in 2006 and is caused by the protozoan parasite *Neoparamoeba perurans*.

It is essential that the Scottish aquaculture industry continues to grow in a sustainable manner and applying appropriate disease control measures along with adherence to the ‘industry code of good practice’ are an important aspect of this process.

*threshold limits = for fish <750 g, mortality over 1.5% per week, or 6% over a five week rolling period; for fish >750 g, mortality over 1% per week, or 4% over a 5 week rolling period should be reported.

Minutes

Knut Roenningen: I would like to know about the compliance to best practice manual, how this has been accepted by the farmers

Eann Munro: The industry code of good practices is a voluntarily agreement, it is not compulsory , and it can vary quite much depending on the site, some are extremely strict in its application, others are more flexible.

SESSION IV: Scientific research update

Chairman: Dr. Uwe Fischer

MOLECULAR TRACING OF VHS IN DENMARK

S. S. Mikkelsen*[1], H. Schuetze[2], H. Korsholm[3], B. B. Jensen [4], M. S. Bruun[1], N.J. Olesen[1]
[1]*National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark*
[2]*Friedrich-Loeffler Institut, Insel Riems, Germany*
[3]*Danish Veterinary and Food Administration, Vejle, Denmark*
[4]*Norwegian Veterinary Institute, Oslo, Norway*

Abstract:

MOLTRAQ is a pan-European project that aims to increase knowledge on a wide array of economically important viral diseases in fish and molluscs on both the epidemiological and the genetic level. It centers on the use of spatio-temporal and phylogenetic information to create phylogeographic and scenario-simulation models to identify important factors for the spread of disease and to develop and evaluate new control strategies.

Viral haemorrhagic septicaemia Virus (VHSV) is one of the most important viral fish diseases and is widely spread all over Europe and creates significant losses every year for European fish farmers. VHSV has been endemic in Denmark since the 1950's but after an effective control and eradication programme that spanned more than 45 years the virus was finally eradicated from Denmark in 2009.

As part of MOLTRAQ more than 200 Danish isolates, including isolates from both marine and freshwater outbreaks, spanning from 1978-2003 were selected for analysis. The full-length G-gene was sequenced for all isolates and together with epidemiological information these data are being used to create phylogenetic and phylogeographic models to help infer the relationship between VHS outbreaks in Denmark and to look into the spread of the disease over a historical period as well as the effectiveness of containment and eradication programmes.

Molecular tracing shows that the numerous VHS outbreaks in marine fish farms were due to stocking these with VHS infected rainbow trout in the incubation phase and not to infection with VHSV from the marine environment. From evaluating more than 400 VHSV isolates from Denmark it appears that evolution of low virulent VHSV from marine fish species is a very rare event and is most likely related to feeding with fresh fish which is now prohibited in rainbow trout farming.

MOLTRAQ is funded under the EMIDA-ERA Net under the EU 7th Framework program.

Partners into the project are: Norwegian Veterinary Institute (NO, Coordinator), Technical University of Denmark-National Veterinary Institute (DK), Agence Nationale de Sécurité Sanitaire (FR), Friedrich-Loeffler Institut (DE), Institut Francais de Recherche pour l'Exploitation de la Mer (FR), Institut de Recherche pour le Développement (FR) and Norwegian Computing Center (NO).

Question
Uwe Fischer: DK is surrounded by water and many VHSV strains have been described in sea. Focusing on the isolates from marine farms, how is the interaction between the genotypes?
Niels Jørgen Ia is dedicated to rainbow trout in freshwater, this genotype has never been found in free living fish in the marine environment only in sea water farmed fish, whereas other genotypes like Ib-II-III are all described in the sea only.

Torsten Boutrup: From sea cages a number of fish can escape every year, it is important thereby in the strategy to control the disease to stock only sterile female so that there will not be a possibility to establish a population reservoir for the virus in the wild environment surrounding the cages

Snjezana Zrncic: Which is probability for turbot to become infected?
Niels Jørgen: Turbot is susceptible species to mainly to genotype III, furthermore isolates belonging to genotype Ib and Ie have shown to be pathogenic for turbot.
Snjezana Zrncic: Should turbot be checked for VHS?
Niels Jørgen yes should be checked for VHS especially if wild fish are introduced in the farm for breeding

ABILITY OF VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS TO EVADE THE PROTECTIVE IMMUNE RESPONSE INDUCED IN RAINBOW TROUT BY DNA VACCINATION

D. Sepúlveda*, N. Lorenzen.
Department of Animal Science, Aarhus University, Aarhus, Denmark

Abstract:

Viral haemorrhagic septicaemia virus (VHSV) is a negative strand RNA virus, which belongs to the genus *Novirhabdovirus* within the family *Rhabdoviridae*. This virus is the causative agent of VHS, a serious disease in rainbow trout and other economically important fish species. The DNA vaccine encoding the viral glycoprotein has been successful as an experimental prophylactic treatment against this disease, inducing a strong innate and adaptive immune response. However, since RNA viruses are known to possess high variability and adaptation capacity, this work aims to evaluate whether VHSV is able to evade the protective immune response induced by the DNA vaccination.

The evasion capacity of VHSV was evaluated through two approaches. First, *in vitro* approach focus on isolate VHSV variants in cell culture able to escape the neutralizing antibodies of serum from fish immunized with the DNA vaccine. And second, the *in vivo* approach to evaluate the possibility to isolate a VHSV variant able to evade the protection of the fish vaccinated with the DNA vaccine. The experiments comprise repeated serial passages of the highly pathogenic VHSV isolate DK3592b (parental virus) in EPC cells in presence of neutralizing fish serum for the *in vitro* approach, and in rainbow trout injected with the DNA vaccine for the *in vivo* approach.

For the *in vitro* approach, the virus isolated after 11 passages in EPC cell was as sensitive as the parental virus to the treatment with neutralizing antibodies from serum. For the *in vivo* approach, after successive passages of infection, the comparison between the passaged viruses and the parental virus showed that all of them caused low mortality rates in vaccinated fish. Further analysis of the survivor vaccinated fish revealed that all viruses were able to persist in only few vaccinated fish. However, this was enough to spread the infection to cohabitant naïve fish.

The DNA vaccine triggers a broad range of protective mechanisms including both the innate immune response and the humoral and cellular arms of the adaptive immune response. This might explain why it was not possible for the virus to evade the vaccine-induced protection against disease. Since the vaccine does not protect against infection and asymptomatic vaccinated fish can be infected and spread the virus to naïve cohabitants, it is important not to transfer vaccinated fish from geographical regions with VHSV into VHSV free zones.

Questions.
Anna Toffan: Which is the homology between G gene sequence of the vaccine strain and challenge virus
Dagoberto Sepulveda: it is the same virus

Brit Hjeltnes: you showed in your experiment that this virus was not able to escape, but what about a different challenge virus
Dagoberto Sepulveda: sure it would be important to test another strain. But if the homology of the vaccine strain is close to the circulating strains the chance of an escape mutant should be very low.

Sven Bergmann: which is challenge dose?
Dagoberto Sepulveda: the challenge is 10 ^5TCID 50/ml

Brit Hjeltnes: some of vaccinated fish are shedding virus, do you know how long they will shed it?
Dagoberto Sepulveda: we tried for 4 weeks, there is a clearance of the virus in the vaccinated fish.

Uwe Fischer: do you know where the virus persists?
Dagoberto Sepulveda: no we haven´t check yet which organ or tissue is mainly involved.

Sven Bergmann: I would suggest to look into the endothelial cells or the endocard for the virus.

PARAFISHCONTROL:

ADVANCED TOOLS AND RESEARCH STRATEGIES FOR PARASITE CONTROL IN EUROPEAN FARMED FISH

Ariadna Sitjà-Bobadilla (IATS), Javier Diéguez RJB, Santiago Pascual (IIM), Oswaldo Palenzuela (IATS), Ángel González, Niels Lorenzen, Helle Frank Skall , Astrid Holzer, Steve Feist, Richard Paley ,Birgit Oidtmann, Niels Jørgen Olesen, Niccoló Vendramin, George Rigos, Pantelis Katharios, CostasTsigenopolous, Elena Sarropolou, Ivona Mladineo, Carolina Tafalla, Kurt Buchmann, Csaba Székely, Gábor Cech, Réka Borzák, Kálmán Molnár, Jesús Lamas, José Leiro, Marco Galeotti, Paola Beraldo, Donatella Volpatti, Marialetizia Fioravanti,Monica Caffara, Roberta Galuppi, Andrea Gustinelli, Frank Nilsen, Ivar Hordvik, Chris Secombes, Pieter van West, James Bron, Alexandra Adams, Kim Thompson, Herve Migaud, Michael Bekaert, Geert Wiegertjes, Miguel Angel Pardo, Begoña Pérez-Villarreal, Charles Mc Gurk, Bao Diep, Enric Belles-Boix, Bénédicte Ferreira, Panos Christophlogiannis, Joanna Tavla, Ayham Alnabulsi, Javier Villa

Abstract:

European aquaculture production provides direct employment to 80,000 people and a 3-billion € annual turnover. Parasites cause severe disease outbreaks and high economic losses in finfish aquaculture. The overarching goal of ParaFishControl is to increase the sustainability and competitiveness of European Aquaculture by improving understanding of fish-parasite interactions and by developing innovative solutions and tools for the prevention, control and mitigation of the major parasites affecting Atlantic salmon, rainbow trout, common carp, European sea bass, gilthead sea bream and turbot. To achieve these objectives, ParaFishControl brings together a multidisciplinary consortium comprising 30 partners possessing world-leading, complementary, cross-cutting expertise and drawn from public and private research organisations, and the aquaculture industry. The consortium has access to excellent research facilities, diverse biological resources including host-parasite models, and state-of-the-art vaccinology, genomic, proteomic and transcriptomic technologies. The project will: 1) generate new scientific knowledge on key fish parasites, including genomics, life-cycle, invasion strategy and host-parasite interaction data, with special emphasis on host immunity, pathogen virulence and immunomodulation, providing a scientific basis for improved prophylaxis; 2) determine the transfer of parasites between farmed and wild host populations; 3) develop a wide range of novel prophylactic measures, including vaccines and functional feeds; 4) provide a range of advanced or alternative treatments for parasitic diseases; 5) develop cost-effective, specific and sensitive diagnostic tools for key parasitic diseases; 6) assess the risk factors involved in the emergence, transmission and pathogenesis of parasitic diseases; 7) map the zoonotic risks due to fish helminths and; 8) provide a catalogue of good husbandry practices to obtain safe and high-quality fish products.

Questions

Brit Hjeltnes: You are setting up an infectivity model and want to find out a water treatment model?

Niccoló Vendramin: Yes the plan is to set up an infectivity model, and manipulate different environmental factor looking into the impact of these to farmed fish. Our main target will be the bryozoans. And as this is parasitic disease the idea is to see if it is possible to mitigate.

Thomas Wahli: it's geared towards farmed fish? We have the problem in wild fish.

Niccoló Vendramin: it is addressed to farmed fish, but sure the interaction with wild fish will be considered

Torsten Snogdal Boutrup: the idea of the project is that you can manipulate the factors to find out what you can change to get rid of the infection. For example is it relevant how many pathogens you put into the fish or not?

GENETIC ANALYSIS OF INFECTIOUS PANCREATIC NECROSIS VIRUSES ISOLATED IN FINLAND DURING 2000-2014

Holopainen R, Eriksson-Kallio AM, Gadd T

Finnish Food Safety Authority Evira, Helsinki, Finland

Abstract:

Infectious pancreatic necrosis virus (IPNV) is the type species of genus *Aquabirnavirus* in the family *Birnaviridae*. It causes a highly contagious viral disease of salmonids that has a serious economic impact on aquaculture worldwide. IPNV is a non-enveloped double-stranded RNA virus with a bi-segmented genome that encodes five viral proteins. IPNV and other aquabirnaviruses can be categorised into seven genogroups based on their genetic properties.

IPNV infections occur regularly in the coastal fish farms in Finland. The inland farms, however, were IPNV-free until 2012, when the virus was detected in rainbow trout in six inland farms from three different freshwater systems. In 2013 and 2014, IPNV was isolated again in several inland farms. The aim of this study was to investigate the genetic relationships between the 2012-2014 inland isolates and other Finnish IPNV isolates collected in the past 15 years. Altogether more than 120 IPNV isolates were analysed based on partial viral capsid protein (VP2) gene sequences. Additionally, complete viral genome analysis was performed to selected isolates. The results indicate that the newly discovered inland IPNV isolates belong to genogroup 2, whereas the isolates from the coastal fish farms belong to genogroups 2, 5 and 6.

This work was partly supported by a grant from the Finnish Foundation of Veterinary Research.

Questions:

Olga Haenen: Did you look into also Eel, as that also might have IPNV Ab Genotype and migrate.

Rikka Holopainen: No we didn’t.

Uwe Fischer: Finland share waters with Russia. Do you also include isolates with Russia?

Rikka Holopainen: No

Uwe Fischer: Any risk of introducing something from Russia:

Rikka Holopainen: Always a risk but don’t know.

Niels Jørgen: Do you have some insights about isolation of IPNV in relation to clinics? Did you isolated fish from clinical fish?

Rikka Holopainen: No, most of them are detection and isolation from non-clinically affected fish

ALPHAVIRUS-REPLICON VACCINES – A PROMISING VACCINE MODEL FOR ATLANTIC SALMON

Astrid Wolf[1], Christel M. Olsen[1], Stine Braaen[1], Petter Frost[2], Kjartan Hodneland[2], Stephane Villoing[2], Espen Rimstad[1]

1 Faculty of Veterinary Medicine and Biosciences, Norwegian University of Life Sciences, Oslo, Norway

2 MSD Animal Health Innovation AS, Thormøhlensgate 55, N-5008 Bergen, Norway

Abstract:

Alphavirus vectors, based upon alphaviruses from terrestrial animals, have been used for vaccine development in various animal models for protection against lethal doses of various pathogens. The exploitation of the replication machinery of *Salmonid alphavirus* (SAV) as a vaccine platform has just begun, and initial experiments from our laboratories are promising. We have found that the SAV-replicon is functional for expression in cells from various taxa, (fish, mammals, insects, shrimps) in a rather large temperature range (4-37°C), and alphavirus dsRNA is present for several days after transfection of fish cell lines. The combination of prolonged dsRNA production, low toxicity, and wide temperature range for expression may potentially be advantageous for the use of the SAV replicon to induce immune responses in fish. The pSAV replicon is versatile and can be used for induction of immune responses against several fish pathogens.

A SAV replicon encoding the infectious salmon anemia virus (ISAV) hemagglutinin-esterase (HE), pSAV/HE, is an efficacious vaccine against infectious salmon anemia (ISA). Delivered intramuscularly (i.m.), the replicon vaccine provides high protection against subsequent ISAV challenge in Atlantic salmon, and induces a strong innate response locally at the injection site. Protection was also achieved against SAV when the envelope protein cassette was expressed. Intraperitoneal (i.p.) administration of the pSAV/HE replicon vaccine did not induce protection. When pSAV/HE was co-injected with the replicon encoding the VHSV G protein, previously reported to induce cross protection against heterologous virus challenge in fish, reduced protection to ISA was observed, indicating antagonistic effect.

Furthermore, studies to develop the SAV replicon to generate virus-replicon particles (VRPs) have been initiated. VRP are propagation-defective particles with a single replication cycle and are not able to spread.

Questions.

Heike Schuetze: You mention the CMV promotor, which other construct can be used?

Espen Rimstad: In the first prototype DNA, you need CMV promotor. If you consider the Replicon defective particles there is no DNA in those.

MOLECULAR CHARACTERIZATION AND TRACING OF KHV

S. M. Bergmann[1], M. Cieslak[1], H. Schütze[1], S. Hammoumi[2] and J.-C. Avarre[2]

[1]*FLI Insel Riems, IMED, Germany;* [2]*University of Montpellier, IRD, France*

Within der order *herpesvirales*, a family *alloherpesviridae* was created. Beside other genera the genus *cyprinivirus* is taxonomic grouped into this family. Recently there are four species which are taxonomically grouped in this genus: cyprinid herpesvirus 1 (CyHV-1, carp pox virus), CyHV-2 (goldfish herpesvirus), CyHV-3 (koi herpesvirus) and the *angullid herpesvirus* 1 (Ang-HV-1, *herpesvirus anguillae*, HVA). Different KHV isolates obtained from cell culture and KHV DNA detection in samples obtained from gill tissues were sequenced with probes after using new methods for enrichment of specific virus DNA in the frame of the EU project "Moltraq" (Molecular tracing of pathogens in aquaculture, EMIDA-ERA-Net). For differential diagnostics also DNA from CyHV- 1and CyHV-2 were prepared.

To fill up gaps mainly due to insufficient DNA date, 19 isolates and specimens were completely sequenced and from 9 specimens a partial sequence was established. At least three different KHV groups were distinguished: an European linage, an Asian linage and a third linage which was called "atypical" KHV. The letter group was established because those viruses were not detectable by recommended routine PCRs (Gilad et al. 2004, Bercovier et al. 2005) but by other PCRs (Bergmann et al. 2010, Engelsma et al. 2013) with and without sequence analysis of the amplicons. Over the complete genome (approx. 295 kbp) all KHV were very similar (99,98 % similarity). So far, only in one case of an atypical KHV the complete genome departed from the published sequence of about 3%. Using KHV antibody detection assays like serum neutralization assay and ELISA, no differentiation between antibodies derived from typical and atypical reacting KHV were possible. In both cases the humoral immune response was similar: antibodies against KHV are present.

It was also found that even from DNA of a cloned and plaque purified virus 4 – 6 different virus variants were present in the cell culture (e.g. KHV-T) which was confirmed using gill tissue samples obtained from naturally infected carp / koi. This was also valid for samples from carp where different variants of CyHV1 und CyHV-2 were verified from samples of infected fish (CEFAS, Dr. David Stone).

Questions:

Niccoló Vendramin: The differences in the nucleotide sequence targeted by Gilad qPCR region, could they affect sensitivity of the diagnostic method?

Sven Bergmann: For some cases yes, for some others no.

RAPID IDENTIFICATION OF VARIOUS PATHOGENIC FISH BACTERIA BY MALDI-TOF

Haenen, O.*[1], Jansson, E.,[2], Roozenburg, I.[1], Helstrøm , A.[2], Nonnemann, B.[3] Dalsgaard, I.[3]
[1] *CVI of WUR, NRL for Fish, Crustacean and Shellfish Diseases, Lelystad, The Netherlands,*
[2] *SVA, Fish Diseases Lab, Uppsala, Sweden*
[3] *National Veterinary Institute, Technical University of Denmark , Frederiksberg C, Denmark*

Abstract:

Aquaculture in brackish and marine water is growing worldwide. New cultured species are introduced, and types of aquaculture vary from outdoor to indoor and from flow through to recirculated water, at various temperatures. Various bacterial species play an important role as causative agents of fish, crustacean and shellfish diseases, and sometimes of man.

At the 2013 EAFP, a workshop on Vibriosis was held in Tampere, Finland. There, a relative new method ***Matrix-assisted laser desorption/ionization – Time of Flight*** for identification of bacteria was presented by Anders Helstrøm. MALDI-TOF is a soft ionization technique used in mass spectrometry. This technique is thought to be a three-step process. First, the sample is applied to a metal plate and covered with a suitable matrix. Second, a pulsed laser irradiates the sample, triggering ablation and desorption of the sample and matrix material. Finally, the analyte molecules are ionized by being protonated or deprotonated in the hot plume of ablated gases, and can then be accelerated into whichever mass spectrometer is used to analyze them.

Additionally, I will show the use of MALDI-TOF in daily practice. You take a colony from an agar plate, and a micro 96 well plate. The sample can be prepared in 3 ways. 1) Direct application (add 1 µl matrix fluid), or 2) the Overlay direct method, or 3) The formic acid extraction method. For details please see Bruker´s website.
Within 15 minutes the plate can be analysed and, in theory, provide identification of 96 different bacterial isolates in approximately one hour.

For Club 5 (CoVetLab), a MALDI-TOF project on (shell)fish bacteria was running from 2013-2014. MSPs (= Main Spectra Projection) were produced for: *Aeromonas salmonicida* (11 MSPs), *Flavobacterium columnare* (8 MSPs), *F. psychrophilum* (16 MSPs), *Yersinia ruckeri* (3 MSPs), *Renibacterium salmoninarum* (11 MSPs), *Vibrio anguillarum* (8 MSPs), and one of each of *Vibrio ichthyoenteri*, *V. splendidus, V. vulnificus, V. aestuarianus* and *Nocardia crassostreae*.

Bacterial isolates from type cultures, routine bacterial diagnostics and isolates from the laboratories own collections have been tested by MALDI-TOF and compared with standard techniques for identification, as biochemical assays and 16S rRNA sequencing/PCR by use of the standard database and the new MSPs. *Flavobacterium psychrophilum, F. columnare, Renibacterium salmoninarum, Vibrio anguillarum* and *Yersinia ruckeri* were all successfully identified to species level.

So far, differentiation to serotype or biotype for *V. anguillarum, V. vulnificus* or *Y. ruckeri* was not possible with MALDI-TOF and needs further development. MSPs were put into Bruker's so called *Projects*, to run in parallel to the Bruker database. In 2014 and 2015, Club 5 organized a Ring trial. Furthermore, a MALDI-TOF workshop was held June 2014 at SVA Uppsala (SE), and another April 2015 at CVI Lelystad (NL). One of the problems we faced was, that *Vibrio scophthalmi/ichthyoenteri* were often not differentiated to species with 16S rRNA sequencing, nor with MALDI-TOF.

As a conclusion, MALDI-TOF is a useful, fast, specific method for identification of bacteria, but there are still challenges in species identification for some (shell)fish bacteria. Also subtyping needs further attention.

Questions
Uwe Fischer: Do you think that in future the method can replace completely classical bacteriology for fish diseases?
Olga Haenen: The company "Bruker" was part of meeting in spring, we get improved the database every year. There is a very broad potential in using MALDI-TOF.
Uwe Fischer: Can you explain why some strains are not correctly identified?
Olga Haenen: lack in the database and that is why we have it improved by adding spectra. The more isolates Bruker gets from users the more they can improve and extend the Bruker database.
Espen Rimstad: can you compare with PCR performances?
Olga Haenen: Yes compare to 16 s RNA typing. There are papers available for human strains.
Triin Tedersoo: if the database is ready, will you share it?
Olga Haenen: we want to, we need to agree with Bruker and you would need the Bruker MALDI-TOF software to be able to read the added MSPs. Contact us for further information as new partners are welcome in the network.
Debes Christiansen: do you have to pay license to Bruker?
Olga Haenen: yes to use the Bruker software, and only then you can update your database with extra MSPs.

TRACKING ISAV-HPR0 TRANSMISSION PATHWAYS IN FAROESE ATLANTIC SALMON AQUACULTURE

Debes H. Christiansen

Faroese Food & Veterinary Authority

Abstract:

Infectious salmon anaemia (ISA), caused by the ISA virus (ISAV), is an economically important infectious disease in Atlantic salmon (*Salmo salar*, L) and has caused major epidemic outbreaks in most salmon farming countries. Phylogenetic and epidemiological evidence suggests that most ISA outbreaks are caused by horizontal transmission of virulent ISAV between neighboring salmon sea sites. Recently, a non-virulent ISAV was shown to cause a transient infection in most marine Atlantic salmon farming sites and circumstantial evidence suggests that this non-virulent ISAV is the ancestral virus to all virulent ISAV associated with ISA outbreaks. The transition into virulence of the non-virulent ISAV is likely caused by various deletions in the highly polymorphic region (HPR) of the hemagglutinin-esterase (HE) gene and point mutations or insertions in the fusion (F) gene. Hence, the non-virulent and virulent ISAV have been designated ISAV-HPR0 and ISAV-HPR deleted, respectively.

Although ISAV-HPRO has been identified in all three compartments of Atlantic salmon production i.e. in Brood fish, in fresh water pre-smolt farms and in marine production sites knowledge on the transmission pathways of ISAV-HPR0 between the compartments and what triggers the mutation of ISAV-HPR0 to virulent ISAV is very limited. This knowledge is fundamental for optimal management of ISAV and ISA outbreaks. Whereas the major transmission pathway of ISAV-HPR deleted has been shown to be horizontal the major transmission pathway of ISA-HPR0 has been suggested to be vertical.

The aim of the present study was to investigate possible transmission pathways of ISAV-HPR0 between the three compartments in Faroese Atlantic salmon aquaculture. Preliminary results of this study will be presented.

Questions.

Niels Jørgen: Are there infection with different ISA isolates in the same site, how to deal with those?
Debes Christiansen: In the same site we have different isolates in the same populations, some in the same fish. The approach taken is to compare sequences of a single isolate; the sequences presented are from 4-20 single fish.
Espen Rimstad: HPR0 infected, how do they perform compare to naïve ?
Debes Christiansen: I haven´t tracked that yet. We don´t see any difference in performance.
Sven Bergmann. Vaccination, do you find vaccine virus variants into vaccinated fish?
Debes Christiansen; I had 2 cases. It vanished after couple of days. Ct values were very high (over 30).

EMERGING SKIN DISORDERS OF RAINBOW TROUT IN THE UK: CURRENT SITUATION IN THE UK

Jason Mewett

Cefas, Weymouth Laboratory, Weymouth, UK

Abstract:

There has been an increase in skin disorders of Rainbow Trout in the UK during the last ten years. Conditions such as Red Mark Syndrome (RMS), Strawberry Disease (SD) and Puffy Skin Disease (PS) have become common and cause significant financial losses to the industry due to downgrading at harvest and / or rejection from processors. A condition known as Cherry Fin has also appeared but has remained isolated to the two sites where it initially occured.

Causative organisms have not yet been identified but RMS and PS both appear to be transmissible suggesting an infectious aetiology. The current situation is discussed including photographic examples of each condition and it's prevalence across the UK trout industry.

EMERGING SKIN DISORDERS OF RAINBOW TROUT IN THE UK: TRANSMISSION TRIALS ESTABLISHMENT

Cano I, Verner-Jeffreys D, van Aerle R, Rimmer G, Paley R, Feist SW

Cefas, Weymouth Laboratory, Weymouth, UK

Abstract:

The transmission of puffy skin (PS) condition to rainbow trout *Oncorhynchus mykiss* (Walbaum) was tested by co-habitation challenge with PS-affected fish collected from the field. Two separate challenges, using PS affected fish (Trojans) sourced from two different sites, were conducted. Both challenges lasted for 49 days.

Diploid and triploid naïve fish were used in the infection trials. Development of PS symptoms was observed in both group of naïve fish, in overall 66 % of the fish sampled during the challenge showed PS symptoms of different severity. PS clinically presented first as white oval patches in one or both flanks in the skin 15-21 days post challenge (dpc). The extent of the patches ranged between 10 to 90% of the body surface, depending of the severity of the lesion. Severity and number of the fish affected increased during the challenge. Macroscopically, dermal hyperplasia and multifocal petechial haemorrhaging were observed at the end of the trials. Abnormal fish behaviour consisting of "flashing" and excessive mucus production was noted from 15 dpc to the end of the challenge. Fish with severe PS lesions also displayed inappetence and associated emaciation.

Unidentified cells containing rod-like cytoplasmic inclusions were observed in 41% of the fresh skin scrapes analysed from the second trial. *Ichthyobodo necator* was also identified at low levels in 10% of the skin scrapes analysed. Histologically epidermal oedema was observed in 31% of the naive fish showing gross pathology, with additional 12% displaying epidermal hyperplasia, mostly observed at the end of the challenge. Other concomitant features of the PS lesion developed in the naive fish were epithelial erosion and sloughing, and occasionally mild or focal inflammation.

The parasites *Ichthyophthirius multifiliis* and *I. necator* were observed in a small proportion in the skin of naïve challenged fish and in Trojans but not in control fish. No consistent pathology of internal organs was observed. The results of analysis of metagenome sequence data, obtained by Miseq sequencing of DNA, RNA and rRNA depleted RNA extracted from PS samples, were inconclusive. In summary we have showed that PS is a transmissible condition. However, the aetiology remains elusive.

Questions

Olga Haenen: Did you check for fungus or molds?

Irene Cano: Yes we could not detect it

Torsten Boutrup: 2 comments. Change in feed and feed ratio, might have change the pathology of something we have been seeing for years. For example *Ichtyobodo necator* has been observed more and more in big fish. It can be that the same etiological agent causes a different pathology.
In relation to cherry fin, I observed something similar in rainbow trout in an experimental facility. That case was linked to wrong management procedure during the hatching. Fish got an initial lesion constantly mechanically irritated and the lesion could not recover.
Jason Mewett: According to my knowledge there was no change of management procedure in the farm where the Cherry fin appeared so far is 1 case in 1 site. Puffy is becoming more problematic than RMS
Uwe Fischer: Do you think that you can treat these syndromes?
Jason Mewett: I think some treatment with formalin was tried, but with no response.
Irene Cano:: After treatment lesions seems to help but reappeared.
Olga Haenen:. Could be genetic?
Jason Mewett: this is a theory, maybe linked to the limited gene pool of rainbow trout use in a single farm.

SESSION V: Update from the EURL

RESULTS OF THE PROFICIENCY TEST, PT1 AND PT2, 2014

Niccoló Vendramin[1], Anemone Ojala, Susie Sommer Mikkelsen and Niels Jørgen Olesen

[1] EU Reference Laboratory for Fish Diseases,
DTU Vet National Veterinary Institute, Bülowsvej 27 Frederiksberc C, Copenhagen, niven@dtu.vet.dk

Abstract:

A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).
PT1 was designed to primarily assess the identification of the fish viruses: 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). 41 laboratories participated in PT1 while 40 participated in PT2.
The tests were sent from the EURL in the beginning of October 2014.
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.

PT1 consisted of five coded ampoules (I-V). These ampoules contained IPNV, EHNV, SVCV, IHNV and VHSV, respectively, see table 1. The proficiency test was designed to primarily assess the ability of participating laboratories to identify the listed fish viruses VHSV, IHNV and ENHV (Council Directive 2006/88/EC) and the non-listed viruses SVCV and IPNV if present in the ampoules, bearing in mind that the test ampoules also could contain other viruses (e.g. other fish rhabdoviruses, ranaviruses, or birnaviruses). In addition the participants were asked to quantify the viruses in PT1 by titration in order to assess the susceptibility of their fish cell lines for virus infection. Participants were encouraged to use their normal standard laboratory procedures. However, the identification should be performed according to the procedures laid down in Commission Decision 2001/183/EC using fish cell cultures followed by e.g. ELISA, PCR, immunofluorescence (IFAT) or neutralisation test.
If ranavirus was present in any of the ampoules, it was mandatory to perform sequence analysis or a restriction endonuclease analysis (REA) of the isolate in order to determine if the isolate was EHNV or another ranavirus and it was recommended to follow the procedures described in Chapter 2.3.1 in the OIE Manual of Diagnostic Tests for Aquatic Animals 2014. Laboratories were encouraged to identify VHSV and IHNV isolates as far as possible by means of genotyping in addition to the standard methods. It was recommended to use the genotype notification described in Einer-Jensen et al. (2004)

for VHSV and in Kurath et al. (2003) for IHNV. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.

PT1 Conclusion

The inter-laboratory proficiency test 2014 was conducted without major constraints. 91% of parcels were delivered by the shipping companies within 8 days after submission. It was, however, unfortunate that one parcel was 27 days on the way and one parcel was 57 days on the way before delivered to the laboratory primarily due to border controls.

EHNV was included in the proficiency test for the first time in 2009. This year 40 participants were able to correctly identify the virus. Of the laboratories performing PCR based methods, 38 laboratories performed sequencing. Of these laboratories all correctly identified the content.

This year it has to be remarked that a problem with the batch of ampoules containing IPNV Ab has appeared, this has been taken into consideration in the process of giving score to participants. This year variation between virus titres obtained in the various laboratories was more pronounced than usually with up to 6 log differences between highest and lowest titres. It might reflect variation in the stability of the virus in the respective batches. Special precautions will therefore be taken in the following PT´s to ensure uniformity of the amount of viable viruses in the ampoules.

PT2 consisted of four coded ampoules (VI-IX). Two ampoules contained KHV, one ampoule contained ISAV and one Sterile Medium, see table 9. The test was designed to primarily assess the ability of participating laboratories to identify the notifiable fish pathogens ISAV and KHV (listed in Council Directive 2006/88/EC) if present in the ampoules, bearing in mind that the test ampoules could also contain other pathogens. Participants were expected to use their normal PCR or real-time PCR methods for detection of the pathogens. It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses were not inactivated and, thus, it might had been possible to replicate them in cell cultures.

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.

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. Collected accreditation data will not be presented in this report but will be presented at the 19th Annual Workshop of the NRLs for Fish Diseases May 2015 in Copenhagen. Participants were

asked to reply latest November 21st 2014. Laboratory (EURL) for Fish Diseases. 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.
The tests were sent from the EURL in the beginning of September 2013.
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.

PT2 conclusion

Considering that this was the fifth time that the EURL provided a proficiency test on ISAV and KHV identification, we consider that most participants obtained very good results. All 39 laboratories testing for KHV identified the virus in ampoule VI and VIII! Out of 40 laboratories 39 laboratories identified Not KHV or ISAV in ampoule VII. With only one false positive this is much less than observed in the PTs from previous years. All 40 laboratories testing for ISAV identified the virus in ampoule IX. Thereby very significant improvement in the proficiency of identifying and typing these pathogens has been observed during these past 5 years, especially in relation to the sensitivity, as this year the viral content in the ampoule was low. After the European Commission in autumn 2012 de-listed Epizootic Ulcerative Syndrome cause by *Aphanomyces invadans* it has been agreed not anymore to include this pathogen in the PT for fish diseases.
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.
Of the 24 laboratories sequencing the ISAV virus all found that the isolate was with deletion in segment 6 and thus not belong to HPR0. Some of the participant also noticed that this year the HPR13 isolate from the Faroes was used instead of the Gleasvaer isolate that we have included in all the former PT´s.
The results of the proficiency tests will be further discussed at this presentation.

EURL ACTIVITIES IN 2014

N. J. Olesen, A. Ojala, and N. Vendramin
National Veterinary Institute, Technical University of Denmark

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 18th Annual Meeting of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark, 3-4 June 2014 at the premises of the Veterinary Institute. A total of 53 participants from 32 countries attended over the two days period. There were five sessions with a total of 29 presentations, 2 of which were given by invited speakers, and a working group session. A report was submitted in August 2014.

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 + SVCV and IPNV. PT2 was developed in order to test the proficiency of participating laboratories to identify ISA and KHV. The proficiency test is covering all 5 listed exotic and non-exotic diseases. 41 National Reference Laboratories (NRLs) participated in the proficiency test. A report was submitted primo March 2015. 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.
In order to update the sampling and diagnostic procedures for KHV the EURL invited 3 experts for a 2 day scientific meeting and passed the outcome to the Commission in order to recommend and finally adopt a Commission Decision on KHV along with the other non-exotic aquatic animal diseases. The Commission Decision on sampling and diagnostic procedures for the listed non-exotic diseases are expected to be adopted by the Commission primo 2015 and will be finally in force as soon as accepted.

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 virusY, the putative causative agent of a new disease observed in Rainbow trout in Norway.

During 2014, 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 produce anti-sera; to update the EURL webpage (www.eurl-fish.eu); and finally to attend international meetings and conferences.

In 2014 the fish diseases activities of DTU Veterinary were established in Copenhagen after the transfer from Aarhus in 2013. The number of colleagues within the group is now 5 academics and 4 technicians, but the EURL still collaborate with the fish diseases research group conducted by prof. Niels Lorenzen who, due to the transfer, jumped from DTU to Aarhus University.

The new placement also resulted in a close localization together with scientists conducting the function as NRL for mollusc diseases and who are internationally recognised researchers in fish bacteriology, as well as close distance to excellent scientists and research facilities in the Copenhagen area.

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EURL TRAINING COURSE FOR 2015

Niccoló Vendramin

DTU Vet National Veterinary Institute, Bülowsvej 27 Frederiksberc C, Copenhagen, niven@dtu.vet.dk

Abstract:

Also for 2015 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 5th to Friday the 9th of October

- **Introduction to Histopathology in fish diseases.**
  The course will be held in week 42 from 12th -15th October 2015

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 WORKPLAN FOR 2015

Niels Jørgen Olesen, Teena Klinge, Susie Sommer Mikkelsen, and Niccolò Vendramin

[1] *EU Reference Laboratory for Fish Diseases,*
DTU Vet National Veterinary Institute, Bülowsvej 27 Frederiksberc C, Copenhagen, niven@dtu.vet.dk

1. Coordination and training			
1-1	Annual workshop	Organize and prepare for the 19th Annual Workshop for the National Reference Laboratories for Fish Diseases (NRLs) in 2015	To be held 27-28 May 2015
1-2	Annual workshop report	Produce a technical and financial report from the Annual Workshop 2015.	To be finalized and submitted August 2015
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 2015 and data collated for the Annual Workshop 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 at 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 (temptatively November) 2015; two courses of 3-4-day 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 emerging diseases problems management and control.	One meeting gathering 5 to 7 international experts will be held at our premises or on spot according to disease case. Scientific report and recommendations will be delivered afterwards to relevant stakeholders.
2. Proficiency test			
2-1	Proficiency tests	Prepare the Annual Inter-laboratory Proficiency Tests year 2015 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 etc)	To be shipped fall 2015 (tentatively primo September)
2-2	PT report	Collate and analyze information gained from the Inter-laboratory Proficiency Tests	Report for the proficiency test 2014 will be submitted February 2015, while results of the 2015 test will be finally collated December 2015,

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 2015
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

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 and EUS on the EURL web page.	The diagnostic manuals are finally adopted by the Commission in 2014. A number of comments are expected from the Member States. The EURL will revise these and update the manuals accordingly
4-3	Fishreflabnet	Maintain and further develop the interactive network with the NRLs, Fishreflabnet, in order to promote a more proactive data sharing and communication with and between reference laboratories in 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 characterise selected isolates of listed viruses (pathogenicity testing in-vivo and in-vitro, serological and genetic characterisation).	The EURL receive every year strains and samples for corroboration of diagnostic results in EU Member states. Regularly these strains must be characterised 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.

4-6	Molecular epidemiology	Perform molecular epidemiology analysis to improve knowledge on diseases spreading mechanisms of viral 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 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	It is that more focus should 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 continued in 2015 (e.g. CEV – Koi sleepy disease; virusY in Rainbow trout, RLO-Rickettsia like organism in Sea bass)
4-9	Producing virtual teaching material (e-learning)	Preparing virtual guidelines for conducting proficiency tests (receiving and opening ampules inoculation etc.)	Set up tools for producing e-tutorials in-house. One tutorial on opening ampoules for PT produced.
5. Missions			
5-1	Missions	Organizing missions to relevant laboratories. 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, the laboratories to visit will be appointed in order to strengthen collaboration in the NRL network. (e.g. Spain, Croatia, Iceland 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.

DRAFT EURL WORKPLAN FOR 2016

Niels Jørgen Olesen, Susie Sommer Mikkelsen, Teena Klinge, Tine Iburg and Niccolò Vendramin

EURL FOR FISH DISEASES, 2016

OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2016

1. Coordination and training

1-1 Organise and prepare for the 20th Annual Workshop for the National Reference Laboratories for Fish Diseases (NRLs) in 2016. Cph primo June 2016?

1-2 Produce a report from the Annual Workshop 2016.

1-3 Collect and report data on the fish disease situation in EU, including all the listed non-exotic fish diseases. With more focus on emerging diseases.

1-4 Facilitate and provide training in laboratory diagnosis. The training courses in methods used for diagnosis of fish diseases is offered annually at the premises of the EURL-Fish. The courses will primarily be for training of staff from NRLs and the content will depend on request from participants. The courses will be conducted autumn 2016

2. Proficiency test

2-1 Prepare the Annual Inter-laboratory Proficiency Tests year 2016 for the NRLs. The test will include testing for VHSV, IHNV, EHNV, ISAV, KHV and in addition upon request SVC and IPN. If accepted by the NRLs is will also include SAV.

2-2 Collate and analyse information gained from the Inter-laboratory Proficiency Tests

3. Reagents and products

3-1 Supply reference reagents to the NRLs in Member States.

3-2 Produce a panel of well characterized VHSV and IHNV isolates to be distributed to interested NRLs for e.g test validation and implementation.

3-3 Update and maintain a library of isolates of ISAV, VHSV, IHN, KHV, EHNV and pathogens causing disease that might be listed in future, e.g. SAV, nodaviruses.

3-4 Maintain a library of tissue material from fish infected with listed pathogens

4. Scientific advice and activities

4-1 Update and maintain the webpage for the EURL, www.eurl-fish.eu

4-2 Update the diagnostic manuals for VHS, IHN, ISA, KHV disease, and EHN on the EURL web page.

4-3 Collect information on strain variation occurring within pathogens causing the listed diseases VHS, ISA, EHN and KHV disease and provide recommendations on how to discriminate between various strains.

4-4 Identify and characterise selected isolates of listed viruses (genetic characterisation).

4-5 Update and expand www.fishpathogens.eu with more pathogens.

4-6 Assessment and standardisation of real-time PCR tests for the diagnosis, identification and typing of the listed non-exotic fish diseases.

4-7 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.

5. Missions

5-1 Organizing missions to relevant laboratories. Missions will focus on NRLs where on-site communication would be beneficial.

5-2 Attending missions, international meetings and conferences in order to be updated on emerging and listed exotic and non-exotic fish diseases.

Apart from all the mandatory plans for next year suggestions for other topics to work on for the EURL would be most appreciated

PROPOSAL AND DISCUSSION FOR DEVELOPMENT OF FURTHER ACTIVITIES

1. **Inclusion of SAV in PT2**
   After the delivery of the PT report for 2014, a questionnaire was send to all participants asking if and what could be improved for the following one. Among the questions included in the questionnaire it was asked how participants would perceive the inclusion of SAV in PT2. As some participants gladly welcome the initiative while others did not accept the proposition, this point was discussed at this general assembly. Different issues were raised about accreditation of the methods in the laboratory, effort needed to perform an extra analysis, the disease being listed at OIE level but not at EU level etc. It was agreed that the EURL would have come up with a final proposal included in the report of the AW.
   The EURL for fish diseases has decided that SAV will be include in PT2 2015. Each participant will have to declare when submitting results whether it has tested or not for SAV and then the EURL will scrutinize results and assign a score on the background of what each participant has declared when submitting the results.

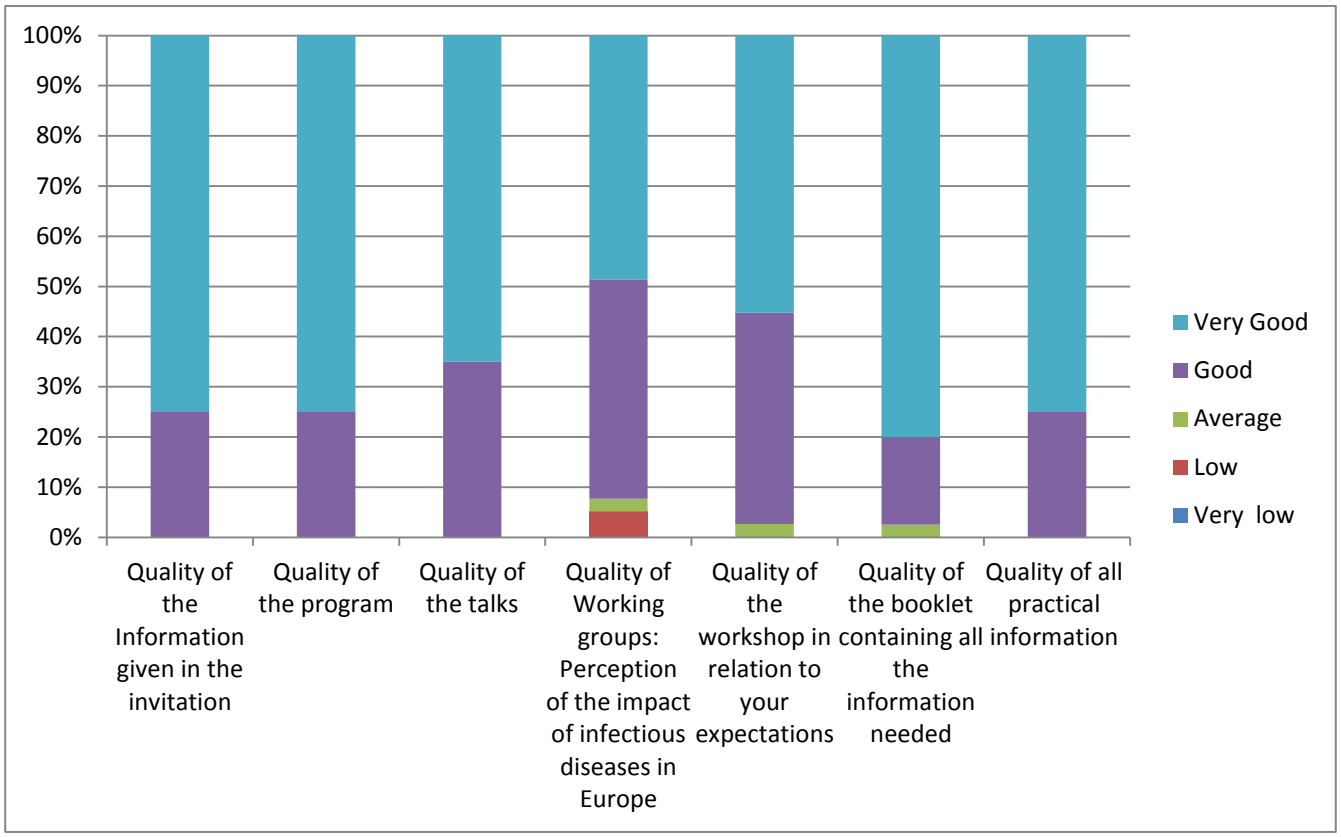
2. **One activity to be developed is the use of non-lethal samples for surveillance of listed fish diseases.**
   A project based on this will target whether it is possible to maintain satisfactory level of sensitivity and specificity using for example swabs instead of euthanizing the fish for organ sampling

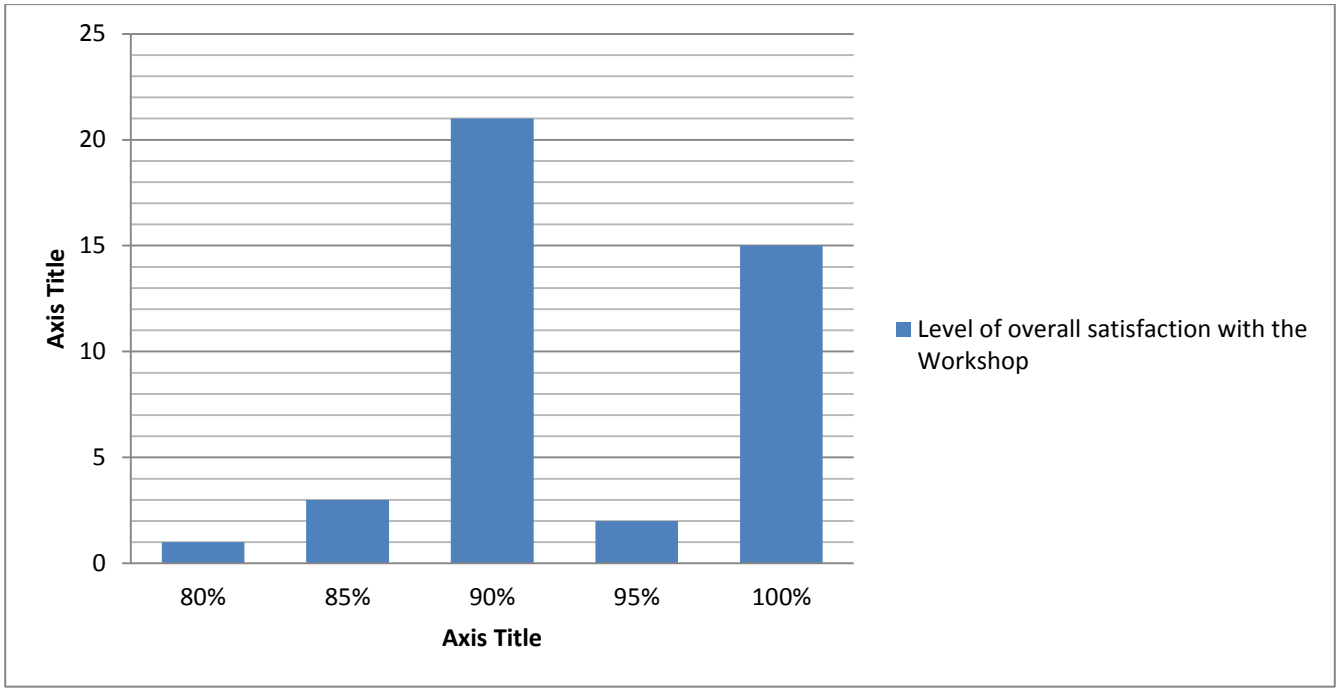
3. **Another point raised was about the publication of accredited SOP in English on EURL website**

4. **CEV- Carp Edema Virus.**
   After the WS organized by the EURL and Dr. Olga Haenen on CEV, different laboratories are networking on this topic. The role of this pathogen in European aquaculture is still not clear and further efforts are needed to decide whether it is emerging or not. The Network Coordinator, Dr. Olga Haenen, suggests that further action are taken and sustained with a proper project. A meeting for the network to apply for a specific project would be appreciated.

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.




25
20
15
10
5
0
Axis Title
80%
85%
90%
95%
100%
Axis Title
Level of overall satisfaction with the Workshop

GREETINGS AND CONCLUSION OF THE MEETING

The next meeting will be held at the end of May 2016. It will be organized in our facilities here in Copenhagen, 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