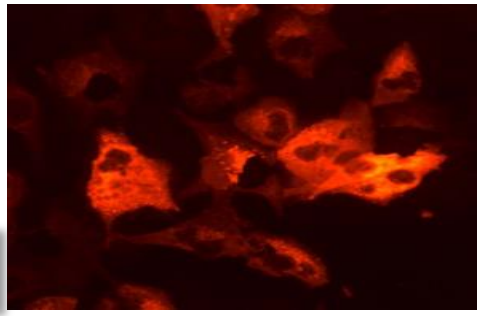
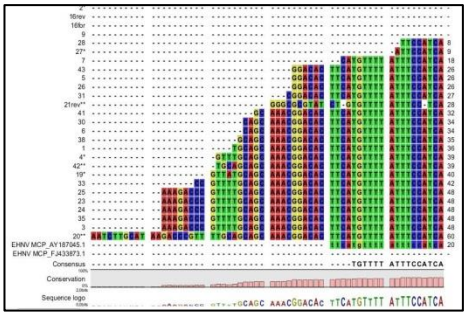
# Report:
# 18th Annual Workshop of the National Reference Laboratories for Fish Diseases

## Copenhagen, Denmark, June 3-4 2014


Myeloma in pike-perch


Positive IFAT staining on KHV infected CCB cells


Alignment of EHNV sequences

Organised by the European Union Reference Laboratory for Fish Diseases

National Veterinary Institute, Technical University of Denmark

# Contents

## INTRODUCTION AND SHORT SUMMARY

The 18th Annual Workshop of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark, 3-4 June 2014 at the Auditorium of DTU Veterinary Institute in Bülowsvej 27, 1870 Frederiksberg C.
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.
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 2013 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.
This was followed by an update on VHS and IHN recent outbreaks occurred in Croatia shortly after its entry in the EU.
The talks of the first session were completed by presenting data on surveillance of Atlantic salmon and Arctic Charr in Iceland.
The second half of the morning was allocated to a new activity, introduced for the first time during the Workshop. Each participant was asked to consider the relevant infectious diseases for the 2 most important fish species farmed in his country. 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 was dedicated to Emerging diseases.
Firstly, the Swedish representative presented an update on infectious disease status describing unsolved cases and the appearance of haemorrhagic smolt syndrome - HSS.
The second talk gave an interesting and detailed overview of CMS and HSMI, two important viral diseases that affect Atlantic Salmon.
In connection to this topic, the results of a screening for Piscine Reovirus (PRV) causing HSMI and Piscine Myo-Cardiopathy virus (PMCV) causing CMS conducted on wild salmon used for restocking purposes in Denmark was presented.
A talk describing how new farming practices and technologies affect the appearance and severity of infectious diseases was given focusing on Danish aquaculture production using recirculating facilities.
Finally the new virusY, a virus causing a not previously described disease in Rainbow trout in Norway was addressed.

The third session, on control and surveillance of relevant pathogens in the EU, started by a presentation on surveillance and diagnosis of koi herpesvirus disease (KHVD).
Then the output from the work of an expert group established by the EURL on surveillance and diagnostic methods for KHV in order to finalize a draft of the new Commission decision Part 2 on sampling and diagnostic procedures for KHV was presented.
This was followed by an introduction to the new Aquatic Animal Health Law. In May 2013 the European Commission launched a proposal for a new EU Animal Health Regulation, and the work is currently in the process of being adopted by the legislative bodies of the Union. The Regulation aligns with the Lisbon Treaty and lay down horizontal principles and rules for transmissible animal diseases in kept and wild animals and their products
Finally the health categorization of fish farms in Europe in 2013 based on answers from the questionnaire on "survey and diagnosis" that is delivered every year to all national reference laboratories was presented.
In the evening a banquet dinner was held at Restaurant "***Restaurant Maven, Nicolai Kirke".***
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, and vaccination as a strategy to prevent infectious diseases.
The session started with a presentation on Maldi-tof a new diagnostic tool able to identify microbial pathogens, benefits and challenges with this new technology were described.
Then a new tool for evaluating antimicrobial resistance in fish pathogens was described.

The session continued with an update on TARGETFISH, an FP7 granted project that focus on development of improved and targeted vaccination strategy for important farmed fish species in European Aquaculture.
This was followed by a presentation showing results of MOLTRAQ, an EMIDA ERA-NET funded project that focus on the molecular tracing of viral pathogens from aquatic animals.
The next presentation described findings of PKD infection in wild trout in Denmark, focusing on the relation between wild and farmed species in the epidemiology of this important disease.
The selection of new candidate fish species in aquaculture has to consider health management, this topic was addressed by interesting talk evaluating sensitivity of Redfin Perch and Marble Trout to VHS and IHN under experimental conditions.
An update on CEV, a virus appearing in carp in Europe was provided, stressing the need for the development of reliable diagnostic techniques.
As reliable validated techniques are beneficial for the whole Europe, the validation testing of serum-neutralisation allowing the detection of specific antibodies against Koi Herpes virus was given.
As follow up on presentation from last year, describing VHS outbreak in Wrasse in the Shetland, the output of experimental trials and pathology of this disease in this species was provided.
Finally this session was concluded by presenting the finding of suspicious of VHS in Iran.

The annual meeting ended with the traditional update from the EURL. The results of the two proficiency tests sent out in 2013, PT1 and PT2, were presented.
The program and application system for the annual training courses provided by the EURL in September 2014 was described. The planned EURL activities in year 2014 were presented and proposals for the EURL work plan for 2015 were discussed.

Minutes from the meeting were taken by Drs. Morten Sichlau Bruun, Susie Sommer Mikkelsen, Torsten Snogdal Boutrup 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 Anemone Ojala, 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 2015, more details will follow.

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

Copenhagen 09 August 2014
Niels Jørgen Olesen and Niccolò Vendramin

## PROGRAM

## *Tuesday June 3rd*

| | |
|---|---|
| 8:45 – 9:15 | **Registration and welcome address** |
| 9:15 – 09:30 | *Welcome Address and announcements*<br>*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<br>Niels Jørgen Olesen |
| 9:50 – 10:10 | Update on the disease situation in Norway<br>Brit Hjeltnes |
| 10:10 – 10:25 | Update on the disease situation in aquatic organisms in the Mediterranean<br>NiccolòVendramin |
| 10:25 – 10:40 | VHS and IHN outbreaks in Croatia- Diagnosis and management of new outbreaks.<br>Snježana Zrnčić |
| 10:40- 10:55 | Surveillance on Atlantic Salmon and Arctic Charr in Iceland.<br>Sigríður Guðmundsdóttir |
| 10:55 – 11:15 | ***Coffee break*** |
| 11:15 – 12:35 | Working groups: Perception of the impact of infectious diseases in Europe. |
| 12:35 – 13:35 | ***Lunch*** |

## SESSION II: Emerging Diseases

| | |
|---|---|
| | *Chair Uwe Fischer– and Minutes: – Niccoló Vendramin* |
| 13:35 – 13:55 | Update on the emerging fish disease in Sweden.<br>Charlotte Axén |
| 13:55 – 14:25 | HSMI and CMS epidemiology and pathogenesis<br>Trygve Poppe |
| 14:25 - 14:45 | PMCV and PRV occurrence in wild and farmed fish in Denmark<br>Susie Sommer Mikkelsen |
| 14:45 – 15:05 | Old diseases and new trends in Danish rainbow trout farming<br>Torsten Snogdal Boutrup |
| 15:05 – 15:25 | A new infectious rainbow trout disease in Norway and detection of potentially associated virus<br>Monika Hjortaas |
| 15:25-15:45 | ***Coffee Break*** |

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

| | |
|---|---|
| | *Chair Brit Hjeltnes – and Minutes: – Torsten Snogdal Boutrup* |
| 15:45 - 16:15 | KHVD surveillance and diagnosis<br>Keith Way |
| 16:15 – 16:35 | A new Animal Health Law in Europe – consequences for aquatic animals.<br>Knut Roenningen |
| 16:35 - 17:55 | Health Categorization of zones and compartments in Europe.<br>Niels Jørgen Olesen |
| 19:00 – | ***BANQUET DINNER at Restaurant Maven, Nicolai Kirke*** |

## *Wednesday 4th June*

### SESSION IV Scientific research update

| | |
|---|---|
| | *Chair Richard Paley– and Minutes: – Morten Sichlau Brunn* |
| 9:00 – 9:20 | Maldi-Tof: new diagnostic tool for identification of fish pathogens<br>Inger Dalsgaard |
| 9:20 – 9:40 | VetMic Aquatic VetMic panel adapted for fish bacteria<br>Charlotte Axen |
| 9:40 - 10:00 | Targetfish FP7 - Developing a targeted vaccination strategy, to prevent important fish diseases in European aquaculture industry.<br>Niels Lorenzen |
| 10:00 – 10:20 | Molecular Tracing of aquatic viruses Moltraq<br>Susie Sommer Mikkelsen |
| 10:20 - 10:45 | ***Coffee break*** |
| 10:45 – 11:05 | PKD infection in wild trout in Denmark with focus on wild farmed fish interactions.<br>Kurt Buchman |
| 11:05 – 11:25 | Susceptibility to VHSV and IHNV of redfin perch and Marble trout<br>Anna Toffan |
| 11:25 – 11:45 | First case of Koi Sleepy Disease by CEV in the Netherlands.<br>Olga Haenen |
| 11:45 – 12:05 | The description of the validation of a seroneutralisation test allowing the detection of antibodies specific To Koï Herpesvirus<br>Thierry Morin |
| 12:05-12:25 | VHSV in wrasse: experimental challenge and pathology.<br>Iveta Matejusova |
| 12:25-12:40 | Isolation and identification of viral hemorrhagic septicemia virus in farmed rainbow trout in Iran<br>Mohaddes Ghasemi |
| 12:40 – 13:40 | ***Lunch*** |

## SESSION V: Update from the EURL

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|---|---|
| 13:40 – 13:55 | EURL activities in 2013<br>Niels Jørgen Olesen |
| 13:55 – 14:15 | EURL workplan for 2014; Ideas and plans for 2015<br>Niels Jørgen Olesen |
| 14:05 – 14:25 | EURL Training courses. Topics and organization for courses 2014<br>Niccolò Vendramin Susie Sommer Mikkelsen |
| 14:25 – 14:45 | Results of the proficiency test, PT1 and PT2, 2013<br>Niccolò Vendramin |
| 14:45 – 15:00 | Comparative analysis of sequences from PT 2013<br>Susie Sommer Mikkelsen |
| 15:00 – 15:15 | Next meeting and end of 18$^{th}$ Annual Workshop |
| 15:15-15:30 | ***Coffee, cake and goodbyes*** |

## Welcome

Niels Jørgen Olesen and Niccolò Vendramin wished everyone welcome to the 18th Annual Workshop. 53 participants consisting of scientists from 32 countries as well as Ph.D. students are attending the meeting. 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 2013

**N. J. Olesen[1] and Anemone Ojala[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, for 2013 it comprise 3 parts:

1. 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.
2. Epidemiological data on the disease situation in each Member State with focus on the listed diseases but also including other diseases of interest.
3. Laboratory data from the NRLs and other laboratories, including the numbers of samples examined, and diagnoses of fish diseases made.

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 2012 is available. The production has increased quite significantly from 2011 to 2012. The increase primarily account for the Atlantic salmon production, especially in Norway. With a raise from 0.68 mill t A. salmon in 2001 to 1,36 mill t in 2011 and 1.5 mill ton in 2012, Europe is following the global development towards increased aquaculture production (Figure 1). The rainbow trout production has stabilised in Europe in 2012- however with some increases in the last years coming back to the level in 2009. The carp production is still mainly in the Eastern part of Continental Europe and also increased a litte compared to the years before. Both the production of sea bream and especially sea bass also increased in the Mediterranean countries. Among other fish species of interest are pike-perch (548t), eel (4.701t), sturgeon (5.249t), cod (10.926 t), turbot (12.676t), and halibut (1.854t). Pike-perch have not yet obtained the expected increase, but seem to be under way, while the sturgeon production is the same as

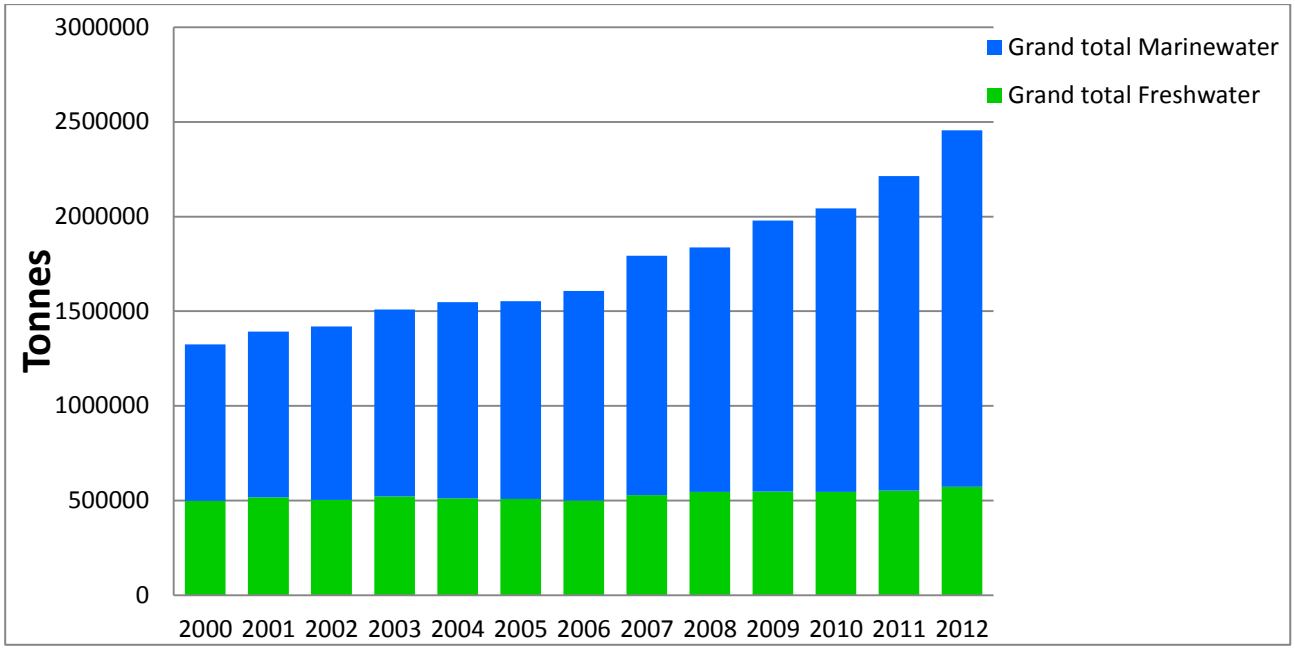
in 2011 after a significant increase. The cod production still decreases dramatically. Data on the health categorisation of fish farms will be given in a later presentation.

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 2012 (http://www.fao.org/figis)

**Minutes:**

For the 16th year in a row the Survey & Diagnosis investigation was conducted. Concerning data of production we refer to FIGIS data, unfortunately data are updated only to 2012.
Considering the most important species for European Aquaculture including Atlantic Salmon, Common carp, Rainbow trout, European Sea bass and Gilthead Sea bream the production in 2012 has increased compared to 2011.

Considering other important species for aquaculture in Europe:

- For eel there has been a decrease in production over the years due to the problems of getting elvers
- The pike-perch production is still very small but seems to be rising
- Turbot production is also increasing
- Atlantic cod and Halibut are hardly decreasing

Among the countries there are big differences in the size distribution of farms. Also the number of national program varies quite a lot:

- SVC: 21 countries have national surveillance program
- BKD: 17 countries with BKD programs, it is mainly Atlantic salmon farms targeted for this disease
- IPN: 18 countries, it is mostly rainbow trout producing countries.
- Gyrodactylus salaris: 9 countries

Concerning the diseases situation, in 2012 56 farms are declared VHS infected, while in 2013 52 farms out of 27688 were declared infected. 9 countries reported VHS infected farms.
In 2013 the first outbreak of IHN was described in Hungary, it is possible to observe a decrease in the number of infected farms concerning VHS, IHN, KHV in Austria. Also in Switzerland and in Germany there is a decrease in the number of VHS infected farms.
As follow up from 2012 no VHS has been detected in Estonia.
In the Netherlands there is a decrease VHS and IHN.
In England and Wales there is a small increase of KHV.
After the VHS outbreak in Cleaner fish in Scotland, no other outbreaks appeared.
For Norway a detailed update will be provided with a specific presentation.

For this year the data will be uploaded on the EURL-Fish website

**Questions:**

**Sigríður Guðmundsdóttir:** we would like to ask to correct data in the graph related to the number of samples tested in Iceland.

**Niels Jørgen Olesen:** we will update the data.

# UPDATE ON FISH DISEASE SITUATION IN NORWAY

**Hjeltnes B.**[1]

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

**Abstract:**

In 2013, Norway produced 1143700 tons of Atlantic salmon (*Salmo salar*), 73900 tons of rainbow trout (*Oncorhynchus mykiss*) 6700 tons of Atlantic cod (*Gadus morhua*), 2000 tons of Atlantic halibut (*Hippoglossus hippoglossus*) and 700 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 was a decrease in the total number of sites (99) with reported disease outbreaks (SAV3 and SAV2), but the no of reported cases of SAV2 increased. Ten cases of infectious salmon anaemia (ISA) was registered in 2013. For several of these cases, the source of infection has not been identified.

IPN was diagnosed in a total of 56 sites and the decline in the number of outbreaks seen in the recent years, continues. Selective breeding and virus eradication programs carried out by the industry; appear to contribute to this significant reduction of disease outbreaks.

Heart and skeletal muscle inflammation (HSMI) is an infectious disease in farmed salmon which has in recent years become extremely widespread. The disease was diagnosed in a total of 134 sites. The no of cases are approximately at the same level as previous years. The Norwegian Food Safety Authority has suggested HSMI to be removed from the national list.

The Norwegian Veterinary Institute diagnosed cardiomyopathy syndrome (CMS), also known as ’heart rupture’ on 100 sites. This is an increase over recent years.

Cold water vibriosis, infection with *Vibrio salmonicida*, was diagnosed on 13 marine sites in Northern Norway. This is a decrease from precious year when 21 cases were reported.

Amoebic gill disease, P*aramoeba perurans* (AGD) was diagnosed in 56 sites in Western Norway. Screening revealed that several sites were infected. The amoeba has previously been reported in Norway in 2006.

A new disease in rainbow trout was reported from three hatcheries in 2013. A viral etiology is suspected.

Production losses remain a significant problem in Norwegian aquaculture.

**Minutes:**

The most important farmed fish species in Norway is Atlantic salmon and Norway is the main producer in Europe, therefore most of the presentation will be on salmon although other fish species are currently produced in Norway.
Concerning viral diseases:

- For ISA 10 outbreaks in 2013, so far 3 2014
- SAV is an important viral disease in Salmon aquaculture and in Norway will be appointed an OIE refrence lab for SAV. In 2013 99 cases has been detected. Outbreak where SAV 3 is detected are decreasing (mainly Western Norway) while outbreaks where SAV 2 is detected are increasing (Midnorway). In the first part of 2014 there has been a higher number of outbreaks than in the same period of 2013, this could be connected to the higher water temperature.
- PRV: more or less steady state.
- PMCV: Slight increase and mainly affects large market fish.
- IPN: Mainly in Atlantic Salmon, both marine and fresh. Large, decrease in outbreaks. Succesful breeding program with genetic markers for IPN. Eradication programs run by industry.

Concerning bacterial diseases:

- Vibrio salmonicida: 2012 outbreaks in 21 sites. After implementing vaccination program, in 2013 the outbreaks were reduced to 13 sites
- Yersinia: 2013: 20 sites, 2012 16 sites

Concerning parasitic diseases:

- Salmon lice have been treated with chemical compounds since 1978 with new compounds in 1990 and 2000 with increased resistance from 2007. Today it is a big problem. It is also a major problem for wild fish. This issue is mitigated with Cleaner fish that carry along their own diseases (mainly atypical forucolosis and Pasteurella infections).

- *Neoparamoeba perurans* has now been detected in Norway causing amoebic gill disease. There are infected sites in the southern part of Norway. AGD: 1$^{st}$ detec in 2006, 2$^{nd}$ in 2012. 2013 58 sites. 90% in oct.-nov. Several treatments.

**Questions:**

**Niels Jørgen Olesen:** it is impressive the results obtained through the breeding program for IPN. Have you seen increase in other disease in IPN-resistant fish?

**Britt Hjeltnes:** No reports yet on negative effects.

**Niccoló Vendramin:** Do you still vaccinate fish against IPN?

**Britt Hjeltnes:** yes

# UPDATE ON FISH DISEASE SITUATION IN THE MEDITERRANEAN BASIN

**Vendramin N.**[1]

[1]*DTU 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 1,5 Million Tonns per year (FIGIS 2012) with a corresponding value of 4,9 Million Dollars.

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 24 experts were obtained about disease situation in the Mediterranean basin for 2013. 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

**Minutes:**

Aquaculture production in the Mediterranean basin is a relevant activity. This particular area is the point of connection of 3 different continents, where different legislation, different control methods are applied. The three most important settings in this area consist in raceways for rainbow trout, ponds for carp and cage for sea bream and sea bass.

Specifically referring to bass and bream, these species are out of any legislation as they are not susceptible to listed disease, however their production is bigger than the trout production, and this pose a serious gap in the health system in Europe.

Again this year a questionnaire was delivered to a panel of expert in the area asking to list the 3 most important diseases for freshwater and saltwater environment.

In Saltwater environment to be mentioned as most important disease are “gill fluke”, Vibriosis and Viral Encephalopahty and Retinopathy (VER). At the second place the 2 most important are considered VER and Enteromyxosis in bream. At the third place the situation is more composite, including Marine Flexibacteriosis and the complex of gill flukes and Enteromyxosis. Among emerging problems Rickettsia like organisms giving Neurological disease in Sea bass, and a new enteromyxosis in sea bream caused by *Enterospora Nucleophila*.

Concerning Freshwater environment, Rainbowtrout Fry Syndrome -RTFS and Lactococcossis are the 2 prominent diseases considered the most important problems, at the second place Lactococcosis and the 2 rhabdovirus (VHS-IHN) are considered the two diseases characterized by higher impact, finally at the third place Enteric Red Mouth ERM and RTFS.

For the future it would be interesting to characterise more the impact of diseases generating diagrams describing their importance.

**Questions:**

**Olga Haenen:** What about different Vibrio species in Italy?

**Niccolò Vendramin:** Typical vibriosis caused by *V. anguillarum* is a large problem in aquaculture, recently also atypical vibriosis are becoming important.

# VHS and IHN outbreaks in Croatia - Diagnosis and management of new outbreaks

**Zrnčić S.[1], Oraić D.[1,] Brnić D.[1], Sučec I.[2]**

[1] *Croatian Veterinary Institute, Savska 143, Zagreb, Croatia, zrncic@irb.hr*
[2] *Ministry of Agriculture, Directorate for Veterinary and Food Safety*

**Abstract:**

Croatia is implementing surveillance programme with regard to viral listed diseases according to the "Decree on the measures of animal health protection against infectious and parasite disease" issued yearly by Ministry of Agriculture. Control of aquatic animal diseases is based on the Council Directive 2006/88/EC implemented in national legislation in 2008. and the control of VHS/IHN on salmonid farms is mandatory.

Concerning viral diseases, until 2013. IPNV was present on several salmonid farms, but in 2013. there were two single outbreaks of both listed viral diseases on two different Croatian farms.

In July 2013. an owner of small rainbow trout farm (*Oncorhynchus mykiss*) with annual production of about 20 tonns per year noticed high mortalities in two raceways with fry weighing about 60 grams. An official vet submitted samples to NRL for diagnosis. Affected fish showed exophtalmia, pale gills, distended abdominal wall with haemorrhages on the skin and hemorrhages in the eye. Dissection revealed haemorrhages on the liver, piloric caeca, swimbladder, intestine, in dorsal musculature and enlarged spleen. VHS virus was isolated on cell cultures and identified by ELISA. The diagnosis was confirmed by RT-PCR and partial sequencing of nucleocapsid and glycoprotein genes was performed. The obtained nucleotide sequences were deposited in Gene bank as HVI-CRO 72/2013 and typed as genotype Ia. The diagnosis and sequencing was confirmed and typed by EURL (genotype Ia, subtype 2, grouped into subclade Pol II that includes isolates from Poland and Slovenia) and IZSVE (Ia2 showing 99% similarity to Polish strain). Epidemiological investigation was performed in cooperation with CA on the farms from which fry was introduced. At the same time measures were lifted on all farms including sampling, movement control, inspection on fish transporter – determining routes of movement, disinfection records, sampling of susceptible species. All collected samples were negative.

In December 2013 samples from newly established rainbow trout were submitted to NRL as a part of routine monitoring programme. Two samples of rainbow trout weighing from 160 to 200 grams showed exophthalmia, pale gills, and just two of them showed few hemorrhages on the skin. Necropsy revealed abundant hemorrhages on the fat tissue, piloric caeca, peritoneum and intestines and in single

specimen on the liver and in dorsal musculature. Examination of organ pools on the cell culture resulted by virus isolation and identification was performed by ELISA. Obtained results were confirmed by RT-PCR and extended mid-G region (615 instead of 303 nucleotids) was sequenced and according to given results we concluded that it is IHNV isolate belonging to M group. Detailed typing is ongoing. Epidemiological investigation included three farms which were source of fish for populating raceways. The same measures as in the case of VHS outbreak were lifted on all farms involved.

In both cases we could not find out the source of infection and finally we set up the suspicion on illegal movement of fish.

**Minutes:**

In Croatia before entering in the EU, a surveillance programs has been carried out for 20 years according to the national legislation.

At the beginning of 2013 VHS and IHN were not present in our country.

In July 2013, in a small family-run farm, an increase of mortality was noticed, sample collected and processed according to standard procedure. After CPE developed, VHSV was identified with ELISA, the isolate was genetically characterized.

In December 2013 samples for surveillance in a newly established farm tested positive for IHN. Further inspection were made at farm level, epidemiological investigation performed and control measures applied.

Finally in May 2014 samples for routine surveillance were collected from a repro-center for brook trout repopulation for anglers purposes. 3 out of 15 pools showed CPE on cell culture and co-infection with VHSV and IHNV demonstrated. Again eradication measures applied.

Concerning epidemiology Fish transported by same vehicle and the same driver!

**Questions:**

**Olga Haenen:** Double infection: did you see any different clinical signs compared to single infection?

**Snježana Zrnčić**: No pronounced sings appeared apart from haemorrhages.

# SURVEILLANCE OF ATLANTIC SALMON AND ARCRIC CHARR IN ICELAND

**Gudmundsdóttir S.**[1]

[1] Department of Fish Diseases, Institute for Experimental Pathology, University of Iceland, Keldur, Keldnavegi 3, 112 Reykjavík, Iceland. siggag@hi.is

**Production**

Present: Atlantic salmon and Arctic charr are the main species is Icelandic salmonid aquaculture. The production of Atlantic salmon suffered a serious reduction starting in 2007. A wide spread epidemic of bacterial kidney disease, caused by *Renibacterium salmoninarum* (Rs), played a major role in this episode. In 2013 the production had reached 50% of the amount produced in 2006. The impact on Arctic charr culture was on a lesser scale.

Future: Production of both species is planned to increase in the nearest future and there are plans for growth in the rainbow trout culture as well.

**Surveillance**

Screening: According to regulations, a proportion of cultured female brood fish is screened for Rs each year as well as every wild female brood fish stripped for restocking purposes. Progeny of positive individuals is destroyed.

History of the last epidemic: Starting in 2003, on a farm that kept wild brood fish on the premises, the epidemic lasted 6 years. Stamping out, vigorous sanitary measures, extensive restrictions of movements and increased surveillance were the tools applied to bring the epidemic to an end.

The wild fish situation: In 2006, the prevalence of Rs positive samples in wild brood fish rose markedly and reached 27.5% in 2008, in contrast to the 0-3% experienced in previous years. This turned out to be due to worsening husbandry practices in the keeping of brood fish between catching and stripping.

Current status: In 2013 there were no positive Rs tests in cultured fish and the prevalence for wild brood fish was 3.5%.

Methods: The screening method used is an ELISA test using polyclonal antibodies (1). When needed, a sample from the homogenate of the kidney sample used for ELISA (before the solvent is added) is put onto FTA paper that can be used to run nested- or semi-nested PCR (2).

(1) Gudmundsdóttir et al. Journal of Fish Diseases, 16 (1993), 185-195.

(2) Arnason et al. Icelandic Agricultural Sciences, 26 (2013), 49-57.

**Minutes:**

BKD was detected for the first time in 1968, in the 80's this disease was epidemic in 4 big farms, since then the number of farms infected has increased. There is a specific BKD surveillance program that includes a proportion of female to be tested from the aquaculture facilities and all the salmon females from the wild for restocking.

BKD had a huge impact on salmon production with an incredible drop between 2007 and 2008, now it is increasing again.

In 2013 no RS has been detected in aquaculture, while there is a prevalence of 3.5% in wild fish, coming from different rivers.

A research project was started studying the efficacy of diagnostic methods with diagnostic samples from infection trials including Atlantic salmon and Arctic charr. Methods applied were ELISA both with polyclonal and monoclonal antibodies, furthermore PCR and qPCR protocols were used to test samples. DNA was extracted from of FTA cards and with commercial kits.

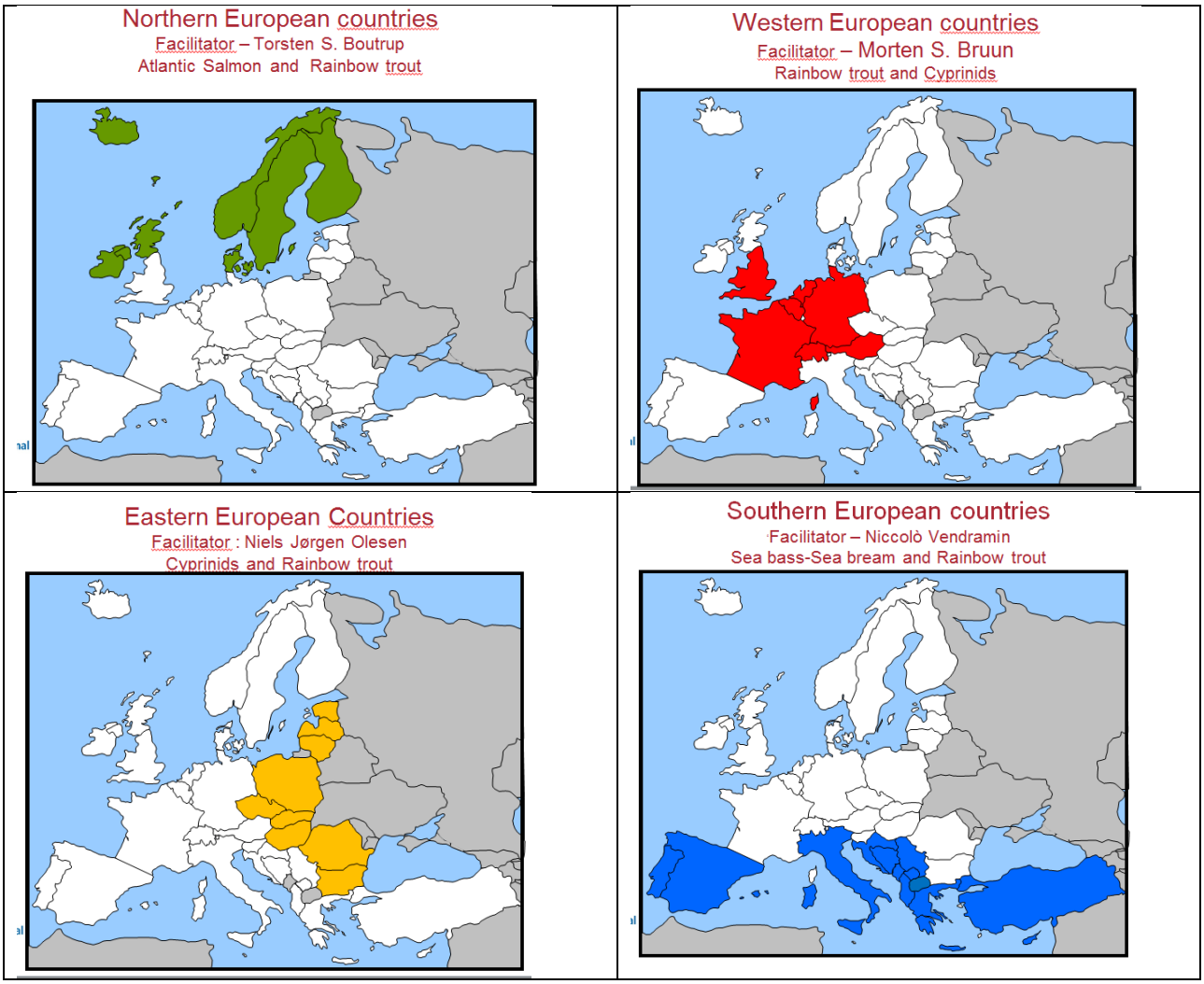
A comparison of results obtained in different trials has been shown. No single detection method is always "the best one". Various factors may affect the "pattern of results" obtained.

**Questions: No.**

## Working group:

## Perception of the impact of infectious diseases in Europe

This year a new activity has been introduced in the Annual Workshop program.
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 for 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..

Then 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.

Each group were asked to select a rapporteur to present the outcome of the work for the full audience.

The output of this work was as follows.

**Northern countries** focused on salmon production. They underlined the importance of Sea lice. Despite this pathogen is not causing mortality as such, it has a number of implications as treatment cost and on the wild salmon. Secondly, amoebic gill disease got very high score, especially in other salmon producing countries than Norway. Looking into viral diseases Pancreas disease is the one scoring the highest, while ISA has not an high direct impact on production, not high economy whereas score the maximum in legislation because it is a listed disease. Among bacterial diseases the one getting the highest score is Columnaris disease.

In **Western Europe** the main species were Rainbow trout and Cyprinids.
For Rainbow trout, the impact at farm level for the two rhabdoviruses IHNV and VHSV can vary quite much, while the legislation impact is the maximum being listed disease.
In some countries IPN has also a high impact under legislative legislations because of national control plans for this disease, while the impact on production and economy is not considered high.
Looking into bacterial diseases, the most important is Rainbow Trout Fry Syndrome- RTFS having a relevant impact both on production and on economy. Finally among parasitic diseases, *Ichthyophthirius multifiliis* 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 productions level. 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; Among viral diseases KHV and SVC are the most important cyprinid viral diseases both on production, economy and legislative wise. There is a raising interest for Koi Sleepy Disease (KSD) caused by Carp Edema Virus (CEV). The most important bacterial disease derives from infection with *Aeromonas* spp., while *Ichthyophthirius multifiliis* is the most important parasitic disease.

In **Eastern Europe** the main species addressed were Cyprinids and Rainbow trout.
For Cyprinids KHV and SVC get the highest score among viral diseases, and infection with *Aeromonas* spp. and *Ichthyophthirius multifiliis* are considered the most important among bacterial and parasitic disease respectively.

For Rainbow trout, VHS is the most important disease, despite the situation among countries is different and the impact can vary. The second and the third most important diseases are bacterial ones, first Enteric Red Mouth (caused by *Yersinia ruckeri*) and RTFS (caused by *Flavobacterium psychrophilum*) have the highest impact in terms of production and economy.
There is actually a gap into the impact of SAV as it is not part of surveillance plan for many countries.

In **Southern Europe** the farmed species targeted by this surveillance were the two important marine species European seabass and Gilthead seabream, and Rainbow trout for freshwater production.
For Seabass and Seabream, VER-VNN is the most important disease. Vibriosis is at the second place because of its high impact on economy. At the third place there is parasitic infestation with gill flukes. As general remark, parasitic diseases are having a high impact because there is a lack of authorized treatment
For Rainbow Trout, VHS and IHN score the highest impact on all the categories. Among bacterial diseases RTFS and ERM are considered having high impact on production and economy. under specific conditions (RT farmed in seawater) Vibriosis has a high impact . Concerning infestation with *Ichthyophthirius multifiliis* and most of the parasitic diseases, they are often overlooked as managed directly by the farmer or the practitioner without including the laboratory in their diagnosis.

As final remark, the knowledge exchange between diagnostic laboratories and other stakeholders as farmers, private consultants and veterinarians is encouraged in order to retrieve more and more data on the impact of disease in the aquaculture production in Europe. This data collection aims to depict the impact of infectious fish disease with a holistic view, not focusing only on legislative obligations.

# SESSION II: EMERGING DISEASES

*Chairman: Dr. Uwe Fischer*

# EMERGING DISEASES IN SWEDEN DURING 2013 to SPRING 2014

**Axén C.**[1]

[1]*National Veterinary Institute, SVA, SE-751 89* Uppsala, Sweden, *charlotte.axen@sva.se*

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**Abstract:**

The swedish aquaculture industry is not challenged by serious disease outbreaks. A few cases with diseaese not generally diagnosed occurred during 2013 – 3 cases of ASS (2 in the inland zone), 1 ERM (inland), 1 BKD (inland) and 1IPNab (coast). The inland cases of ASS and BKD are problematic – we do not now if they occured just by chance (last cases were in 2008 and 2011, respectively) or if it indicates a decline in biosecurity measures. Infection with Sarcocystidae parasites, first shown in 2011, was suspected in a few cases with massive columnaris or cold water disease, but IHC could ot be done to confirm the findnings and we still lack other diagnostic methods such as PCR.

In January 2014, low mortality (3-5%/month) was reported in one farm in Rainbow trout from 2013 The only external lesion observed was corneal opacity. Authopsy revealed hypertrophy of the gills (direct microscopy), multiple small indentations of the lenses and residues of vaccine in the abdominal cavity.The farmer then reported that he had vaccinated the fish in December. Viral and bacterial cultures were negative except for *Lactobacillus* in one fish and an isolate that gave negative results in Maldi-Tof. Mortality continued despite treatments with saline, formalin, hydrogen peroxide and tetracycline, equally in vaccinated and non-vaccinated fish, and six more fish were autopsied. These showed pale, saggy mid-sections of the kidney, one had an enlarged spleen and all had vaccine in the abdominal cavity. Histology sections have not been thoroughly examined yet, but there was debris within the tubuli lumen, fibrosis surrounding kidney tubuli and degeneration of glomeruli.In the liver, nuclei wer distinct, some with two nucleoli. This is the same farm where the first Sarcocystidae case occurred, and some possible parasitic structures have been identified. However, whether this is an infectious disease or due to water conditions has not been clarified. The effect of vaccinating at a water temperature ~0°C, when the fish cannot absorb the vaccine and the immune system is at a low activity has been discussed, but the mortality rates are equally high in unvaccinated fish. At this date, mortalities are stil occuring but at a lower rate.

In the beginning of March 2014, a farmer called for the veterinary services because he had noticed a slight mortality (appr. 1‰ per day) in 1-year old salmon smolt that he was about to export. The farm also had Rainbow trout and Brown trout but these were healthy. The veterinarian noted anemia, abdominal swelling and petechial bleedings in the abdomial fat of investigated fish and sent fish to SVA for autopsy. SVA also noted these symptoms, as well as petechial muscle bleedings and a viral culture was started. Because the symptoms agreed with the clinical presentation of VHS and Atlantic salmon is listed as a susceptible species, the farm was put under restrictions and further sampling was done to rule out VHS – 30 1-year old salmon, 20 Rainbow trout (~500 g) and 10 Brown trout were sampled. Tissues for histology were also secured. Viral cultures (VHS, IHN, IPN, SVC) were negative, as well as qPCR for ISAV and SAV. Histology sections showed a pathology agreeing with Haemorrhagic Smolt Syndrome (HSS), hitherto only reported from Scotland and Norway. Then it turned out 2-year old salmons had the same symptoms, and these were also samled for histology, which showed HSS-like lesions. Histology slides were sent to the Veterinary Institute in Bergen, Norway for confirmation of diagnosis. The farmer reported that he and colleagues believe these low mortalities in presmolt and smolt are common in early spring, and do not reflect that the fish is diseased. Thus, HSS could be something that has been present in Sweden for several years but has gone by unnoticed by veterinarians.

**MINUTES**

In 2014 in Sweden we had observed disease outbreaks that will be presented.

The first outbreak occurred in January in Rainbow trout, it was characterized by mortality rate of about 3-5 %. Water temperature was around 0 degree Celsius. The main finding was opacities in eyes; both vaccinated and unvaccinated suffered the mortality thereby excluding a side effect from vaccinations. Virological and bacteriological examinations tested negative. Histology was performed as well. The gills displayed branchitis status with severe modifications of gills structure and debris at secondary lamellae. Various treatments were tried by the farmer including both disinfectant and antibiotics without success. Since the mortality did not stop, new samples were delivered for further investigation. Necropsy performed one month after the first sampling showed changes in coloration of fish, gill anemia, residues of vaccines in abdomen some adherences kidney pale.

Once again histopathological examination revealed severe changes in the gills with bronchitis and the presence of inclusion body next to nuclei of red blood cells.

The second outbreak occurred in Atlantic salmon, at a smoltification phase. Mortality was 1‰.

Necropsy of affected fish revealed anemia, abdominal swelling, petechial hemorrhages in the abdominal fat. Laboratory investigations were performed: virus cultivation tested negative, PCR investigations ruled out the presence of ISAV and SAV, finally no bacterial growth was observed. Histological examination showed massive bleedings in the gill's lamellae and in the cardiac muscle. In the kidney it was observed the presence of debris in tubule, and haemorrages affecting the tubuli and the interstitial space. Massive hemorrhages were observed also in the intestinal wall. Samples were sent to Norway with suspicion of Hemorrhagic Smolt Syndrome - HSS. The suspicion was confirmed by NVI Norwegian Veterinary institute.

The third Outbreak occurred in wild fish, affecting Eelpout population on the east coast of Öland in May. Necropsy of moribund fish displayed yellow livers, filled icteric gall bladder, Gills were affected as well. Laboratory investigations allowed isolation of Vibrio Anguillarum; BF-2 cell culture displayed CPE. The isolate was not identified; it is suspected the presence of a birnavirus or perch rhabdovirus.

**Questions:**

**Kurt Buchmann:** I would like to ask a question about the Sarcocystis case in Swedish trout presented last year. How did you confirm the diagnosis? Did you see spores of coccidians? I am a little puzzled because there exist only one Sarcocystis record from fish (from Salvelinus in Canada in 1943). And this diagnosis has been strongly doubted.

**Charlotte Axen**: We observed inclusion body in blood cells; so far Sarcocystis infection has been diagnosed and confirmed in mammals. We observed some positive staining with in house produced polyclonal antibody against Sarcocystis.

**Uwe Fisher**: I would like to ask if you have seen a connection with the increase in seal population and the parasite infestation in Cod wild stocks.
**Charlotte Axen**: yes.

# CMS AND HSMI IN ATLANTIC SALMON (*Salmo salar* L.), PATHOGENESIS AND EPIDEMIOLOGY

**Prof. Poppe T.T.**[1]

[1]*NMBU, Norwegian University of Life Sciences, Campus Adamstuen/*

*Dep. Of Pathology, National Veterinary Institute, P.b. 750 Sentrum, 0106 Oslo*

*trygve.poppe@nmbu.no*

**Abstract:**

**Cardiomyopathy Syndrome (CMS)** is a chronic heart disease primarily affecting marine farmed atlantic salmon. The first cases were reported from Norway in 1985, but subsequently described from other salmon-farming countries including Scotland, The Faroe Islands and Canada. CMS is a chronic progressive disease that develops over several months, with mortality typically occuring in large fish 12-18 months after transfer to sea water and in fish close to slaughter. The economic losses are not due to high mortality during production, but to the effect of sudden death on large and valuable market sized fish.

Acute death is the result of cardiac tamponade and blood loss, and haemopericardium and/or blood clots in the pericardial cavity are typical findings at necropsy. Histologically, lesions initially occur in the atrium with pleomorphic nuclei and sub-endocardial infiltration of inflammatory cells. This progress to the spongy part of the ventricular endocardium, and from focal to multifocal or diffuse degeneration and increased number of mononuclear inflammatory cells, lymphocytes and plasma cells. The compact ventricular myocardium is usually unaffected, but a highly cellular epicarditits is common. Important differential diagnosis are heart and skeletal muscle inflammation (HSMI) and salmonid pancreas disease (PD).

Piscine myocarditis virus (PMCV) has recently been recognised as the aetiology of CMS. PMCV belongs to the Totiviridae family and the virus appears to be widespread in farmed fish. All identified isolates in Norway seems to belong in the same geno group. The recent identification of the causative virus will help in developing control strategies.

**Heart and skeletal muscle inflammation (HSMI)** is a systemic viral disease of seawater farmed Atlantic salmon.The first cases were identified in Norway in 1999, and the disease is currently widespread in Norwegian aquaculture, where it causes substantial losses. The condition is also described from farmed salmon in Scotland, and PCR-screening of marine fish caught along the

Norwegian coast has revealed PRV in great silver smelt, capelin, Atlantic herring and horse mackerel. Clinically outbreaks typically occur 5-9 months after transfer to sea water. Morbidity may be very high in affected cages, while mortality may reach 20%.

Clinical signs include anorexia and abnormal swimming behaviour and internally, pale heart, yellow-orange liver, ascites, splenomegaly and visceral petechiae. Red skeletal muscle is usaually heavilly affected with myocyte degeneration and infiltration of inflammatory cells. In the heart, early lesions in the ventricular compactum typically include coronary vessel perivasculitis, endocarditis and focal myocarditis. A highly cellular epicarditis can also be observed. Cardiac lesions subsequentially spread to the entire myocardium developing an extensive panmyocarditis, multifocal necrosis and inflammation in both spongy and compact myocardium. Atrial lesions are similar to those seen in the spongy myocardium, but often milder. Lesions in other organs are few, but general congestion and multifocal liver necrosis may be seen. In addition, haemorrhage and accumulation of erythroytes can be recorded in gills, kidney and spleen. PD and CMS are important differential diagnosis.

Piscine reovirus (PRV) has recently been suggested as the aetiological agent of HSMI. PRV belongs to the reovirus group and appears to be widespread in farmed salmon. The route of infection is precently unknown, but there seems to be a complex relationship between disease, carriers and virus reservoirs.

For both diseases, diagnosis is based on the characteristic histopathological lesions in heart and, if present, other organs (like red skeletal muscle in HSMI), viral detection in situ by immunohistochemistry and the presence of virus by PCR. There is no treatment or vaccines available yet. Reduction of stress is an essential prophylactic measure.

**MINUTES**

We are talking of two diseases characterized by Emerging impact on the Norwegian aquaculture.

They are two different diseases affecting heart:

- HSMI- PRV first diagnosed in 1999
- CMS- PMCV first diagnosed 1985

In order to comprehend these diseases it is important to focus on Heart anatomy- normal histology. The Ventriculum consists in 2 different muscular parts: an outer compact layer part that is supplied by arterial blood and an inner spongy part which is supplied by venous blood. The most internal layer of the hearts is the endocardium while outside we have the Epicardium.

HSMI occurred for the first time in 1999 diagnosed also Scotland. This disease is widespread and tipically occurs in sea water, however recently has been detected also in smolt farm (no contact with sea water). The virus considered the causative agent has been detected in more wild species.

This disease does not show gross external pathology, affect moderate size fish that have spent 5-8 months in sea water. Histopathology reveals several lesion in the in the ventricular compactum layer, these typically include coronary vessel perivasculitis, endocarditis and focal myocarditis. The pathology might evolve to pancarditis at a later stage. The Red skeletal muscle is usually heavily affected with myocyte degeneration and infiltration of inflammatory cells.

CMS found also in Scotland and Faroe Islands, it is considered a major problem for Norwegian salmon aquaculture. Virus has been demonstrated 9 months before outbreak in healthy fish.

Few clinical signs – sudden death disease develop over very long time, it is in fact a chronic progressive disease. Fish mainly affected are 15-18 months old salmon in Saltwater. Affected fish display a general congested status, dilated atrium, fibrinous coat on liver. It is often possible to observe a blood clot of big dimensions in the heart, it is hemopericardium that develop after the atrium wall is damaged.

It has to be remembered that PD, CMS and HSMI can occur at same time in same fish posing diagnostic question.

**Questions:**

**Niels Jørgen Olesen**: With IHC is it possible to show the viral antigen in the tissues?
**Trygve T. Poppe**: Yes, you can demonstrate it, staining the muscle in degenerative changes. We have strong association with demonstration of the virus, and lesions. The main unanswered question is that we can demonstrate virus in healty fish.
**Uwe Fisher**: Did you try cell culture from heart?
**Irene Ørpetveit:** It is in process.
**Niccoló Vendramin:** Can you comment on the distribution of the lesions in relation to the virus?
**Trygve T. Poppe**: They are certainly characterized by different tissue-trophism. HSMI spreads from blood while CMS tropism for the endocardium
**Uwe Fisher** Have you tried explants from red muscle?
**Trygve T. Poppe:** Not yet.
**Niels Jørgen Olesen:** have you considered regulation for this diseases. Could they be included in the legislation?
**Brit Hjeltnes**: HSMI is listed at a national level while CMS it is not listed disease. Only recently it was possible to associate the causative agent with the disease.

# PMCV and PRV occurrence in wild and farmed fish in Denmark

**Mikkelsen S. S.[1], Arnö J.[1], Bruun M.S.[1]**

[1]*DTU Vet National Veterinary Institute, Bülowsvej 27 1870 Frederiksberg C, susmi@dtu.vet.dk*

**Abstract:**

Every year salmon are restocked in the 7 rivers Storå, Skjern Å, Varde Å, Sneum Å, Kongeå, Ribe Å and Gudenåen.

Six-month-old and 1-year-old salmon for about 2 mio. Kr (€268000) are restocked every year. The salmon are restocked in both the main rivers as well as the larger inlets. 6-month-old salmon are restocked in smaller rivers where the spawning and growth is ideal. They are released from a boat drifting downstream between September and October.

1-year-old smolt are restocked in a few places in the main rivers in April. They are restocked in large numbers to provide better protection against birds and other predators.

In 2007 more than 200000 6-month-old and 1-year-old salmon were restocked in the west-facing rivers. At first Irish, Scottish and Swedish wild salmon were used for restocking, but in 2001 it was discovered that there were still original populations in the rivers and since then all the broodstock have been genetically tested and now only broodstock from the original populations in western Denmark are being used.

The broodstock are caught by electrofishing in a collaboration between local sports fisheries organizations and the Danish Center for Wild Salmon, DCV and used for breeding at the premises of DCV close to the towns of Skjern and Randers, respectively. Before being selected for breeding the broodstock are tested for an array of pathogens, including ISA, VHSV, IHNV, IPNV and BKD.

Piscine Reovirus (PRV) is a double-stranded non-enveloped RNA-virus in the family of Reoviridae, while Piscine myocarditis virus (PMCV) is a double-stranded RNA virus of the Totiviridae family. Wild and farmed salmon and trout have not been tested for PRV or PMCV in Denmark before, but both viruses are found in Norway, where they are suspected of causing Heart and Skeletal Muscle Inflammation (HSMI) and Cardiomyopathy Syndrome (CMS), respectively.

In 2013, broodstock from four different rivers in Denmark were received for surveillance. These rivers are Ribe, Varde, Skjern and Store Å, which are all west-facing rivers. Of these fish 8 were Sea Trout and the rest Salmon. 184 fish were tested by real-time RT-PCR for PRV and 30 fish from each river were tested by real-time RT-PCR for PMCV. This is the first time wild and farmed fish have been tested for either virus in Denmark.

Furthermore, 120 farmed fish (Rainbow Trout) were tested for both PMCV and PRV. These fish were all from fish farms at Vejle Å, which is east-facing. Four historical isolates from Grasscarp from 1990 and 6 pools of 10 fish of Halibut from 1999 that had been found positive for reovirus were also tested.

All tested fish were negative for PMCV, but 11 of 184 tested fish proved positive for PRV. Two isolates were from Store Å, two from Skjern Å, three from Ribe Å and four from Varde å. All Sea trout were negative for PRV and so were the historical samples.

**Minutes**

During last year we looked at causative agent of HSMI and CMS in farmed and wild fish in the Danish aquaculture system. One main effort was performed on the Salmon stocked in the 2 reproduction centers we have in Denmark. Fish are caught when they are going upstream in the river, they are made spawning in captivity and the progeny maintained up to 1 year, when smolts are seeded in the river system again. No PMCV was detected while 11 samples out of 184 tested positive for PRV. After these findings 2 batches of eggs from the reproduction centre were introduced in the stable, one batch was disinfected according to standard procedure while the other untreated. The testing of the fish hatched from these eggs is in process. We are also sequencing the PRV detected.

**Questions:**
**Kurt Buchmann**: Which tissues have been tested?
**Susie Sommer Mikkelsen**: Heart, kidney and gills.
**Henrik Korsholm:** Fish tested so far were only females.
**Olga Haenen**: Have you tried virus isolation on cell culture
**Susie Sommer Mikkelsen** Not tried yet
**Uwe Fischer**: Can you comment about the reintroduction of the progeny from this fish? Is there a risk that these fish will go and match with other infected fish?
**Susie Sommer Mikkelsen**: This is the first time we perform such a survey, we don't know if incidence in the wild is increasing or decreasing
**Torsten Snogdal Boutrup**: We have tested 90 fish and found a relevant prevalence, how to deal with it? It seems that biosecurity measures applied at the farm reduce virus load.
**Brit Hjeltnes**: Thanks for the very interesting presentation, in Norway work with restocking programs for wild salmon. The parents are caught in the wild, one hypothesis is that the virus can spill over from the infected farms and wild fish can harbor it.
**Susie Sommer Mikkelsen**: We need to sequence our viral strain and compare also with Norwegian sequences.
T**orsten Snogdal Boutrup**. Based on the experience in tagging danish salmon, they will travel along west coast of Norway so there is the possibility that they will meet Norwegian stocks and exchange pathogens.
**Trygve T. Poppe**: Have you considered also salmon that are swimming up in the wrong river and how to deal that the virus is spread among different river system.
**Torsten Snogdal Boutrup**: we can estimate that about 5% of the salmon will take the wrong river.
**Henrik Korsholm:** each parent is tested and if there are form wrong river they are excluded but we cannot exclude they spawning in the wild.

# OLD DISEASES AND NEW TRENDS IN DANISH RAINBOW TROUT FARMING

**Boutrup T. S.** [1]

[1]*DTU Vet National Veterinary Institute, Bülowsvej 27 1870 Frederiksberg C, tosb@dtu.vet.dk*

**Abstract:**

During recent years Danish rainbow trout aquaculture has experienced several structural changes. In turn this has led to the disappearing of some diseases, the change of the clinics of others and also some new and not yet fully elucidated syndromes have emerged.

During the last decades changes in the production of rainbow trout in Denmark has happened several ways. This includes at first implementation of recirculation technology as a reaction to environmental demands, and later as a mean of intensifying production as a major milestone. But more stepwise changes in feeding and farming intensity, vaccination, cost of manpower and treatment regimes, to mention some, can change situation less dramatically but over time be the reason for development of new hygiene practices and incresed levels of stress. Also the development of consultancy services with specific knowledge in production management, disease diagnostics and treatment has changed over time.

This talk will present the major disease problems at present in Danish rainbow trout aquaculture, and some of the emergences that has beeen seen of new disease complexes and changes in relevance of "old" well established pathogen driven diseases.

Many changes seen in Danish rainbow trout aquaculture is seen and reported by private field practitioners. These monitor the farms in Denmark very closely, making clinical on site diagnosis and initiate treatment. This, of course, give valuable information on what happens in the field, on the other hand some disease problems might be under reported since they are just taken care of in the field, at a practical level. This point will also be discussed.

**Minutes**

This presentation will try to update on the main fish disease problem in Danish aquaculture system. It can be challenging to deal with Koch´s postulates when new trends in fish farming makes the diagnosis more difficult.
In Denmark we have three main actors that are managing the fish health: fish inspectors that mainly sample for surveillance purposes, the private practitioners and the laboratory. It has to be said that the private vet service are very autonomous and thereby there is a limited spill over to diagnostic laboratories that work when there are difficult cases. In this sense little information are going into the system.
The trend is to increase feeding level, and this might probably lead to a situation where low pathogenic microorganisms will appear once the stress level exceeds a certain limit. Instead of old ponds system recirculating system are increasing. Considering oxygen supply for example in some farm there is the need to maintain hyper saturation of oxygen (200-300% oxygen) to sustain the fish, this is a factor to consider on the fish and on how they perform.
Recirculation has huge impact on infection with *Ichtyopthirius multifilis* .
Because of economic crisis there is reduction in manpower and this is affecting the hygiene level at the farm. There is a constant compromise between the known optimal procedures and the perception of what need to be done.

- Concerning viral diseases, Denmark is declared free from VHS.

IPNV has still an important impact. This disease is endemic at a country level, however there are farms that are declared free and maintain disease free status, for example some of the major producers have screened and selected the broodstock so they can produce IPN free fish. One of the key aspect is that when the virus come from the eggs there is a very little impact on production, but the survivors after the IPN outbreak are performing very poorly, especially considering the immune system, it is possible to relate this with vaccine failure and increased susceptibility of ERM.

- Concerning bacterial diseases:

RTFS is the most severe disease in DK, highest impact on production, now become to affect larger fish.
ERM thanks to the vaccination has reduced its impact, in some cases seen in very cold water, it pose a diagnostic problem as severe cases can be misdiagnosed at a preliminary level with VHS.
Foruncolosis is a problem in sea farmed rainbow trout.
Vibrio anguillarum has been observed also in Fresh water.
BKD this disease is a major problem with RAS farm.
Bacterial gill diseases are mainly a relevant problem in fry and RAS.
Start feeding enteritis, this syndrome is observed 1 week after beginning feeding and it is connected to an important increase of various bacteria in the fish. In the single outbreak is 1 bacteria, but within cases vary the population that is connected to the enteritis.
RTGE few cases link to feeding and hygiene

- Among parasites

ICH- *Icthyophtirius multifiliis* is an important problem in RAS
*Icthyobodo necator* major problem in fry involved in larger fish up to 200 gr.
For PKD the situation is composite, there are farms where is has a huge impact and others where there is no impact at all. With RAS reduced.

Gill amoebas. These pathogens pose a serious diagnostic question, they are suspected to be related with gill disease outbreaks but it is hard to diagnose them at a farm level, thereby there is a gap in the knowledge.
Other opportunistic parasites have very little importance, they are on the other hand indicators of wrong treatment of water quality
Skin lesions potentially associated with RED MARK SYNDROME have been observed, this disease will have to be considered in future.

**Questions**

**Snježana Zrnčić:** Do you have an explanation for the outbreak of *V. anguillarum* in freshwater?
**Torsten Snogdal Boutrup**: we don't know how it entered.
**Brit Hjeltnes**: We have observed the presence of *V anguillarum* in hatchery
**Torsten Snogdal Boutrup**, I think that there is a direct epidemiological link that we are missing. For example it could be related to un-proper disinfection of vehicles.

# A NEW INFECTIOUS RAINBOW TROUT DISEASE IN NORWAY AND DETECTION OF A POTENTIALLY ASSOCIATED VIRUS

**Hjortaas M[1], Johansen R[1], Dahle M[1], Lyngstad TM[1], Tengs T[1], Hauge H[1], Taksdal T[1], Tavornpanich S[1], Olsen AB[1]**

[1] *Norwegian Veterinary Institute, Norway, monika.hjortaas@vetinst.no*

**Abstract:**

During autumn 2013, The Norwegian Veterinary Institute (NVI) received material from three cases of diseased rainbow trout from hatcheries in the western part of Norway (Vestlandet).
The fish, 30-100g, showed signs of a novel disease characterized by circulation failure, pale viscera, anaemia and liquid (ascites) in the abdominal cavity. Hematocrit analysis confirmed severe anaemia. The histopathological examination revealed inflammation of the heart and red muscle and liver necrosis. Moderate to high mortalities have been observed. Diseased fish had been exposed to fresh water only or water with low salinity (< 1‰). The farms had received eggs or fry from two interconnected broodfish farms.
Extended microbiological examination has been performed and did not reveal any known pathogenic viruses, apart from small amounts of infectious pancreas necrosis virus (IPNV) in one of the hatcheries. No pathogenic bacteria were detected in the diseased fish and antibiotic treatment performed at one occasion showed no effect.
A short sequence from an RNA virus has been detected in blood and tissue from the affected fish at all 3 farms. High-throughput pyrosequencing is ongoing to characterize the total genome of the virus; meanwhile the virus is termed “virus Y”. A real-time RT-PCR assay for specific detection of virus Y has been established and we are currently working on the validation of the test. This assay has been used to map the distribution of virus Y in affected and in contact farms. Large amounts of virus Y are found in both diseased and healthy fish in the affected farms. No disease has been observed in contact farms so far, but small amounts of virus Y are found at a rather high prevalence
at some of these farms.

Our investigations so far do not prove whether virus Y is the cause of the new disease alone or in concert with other factors. An ongoing small scale challenge trial on both rainbow trout and Atlantic salmon has been executed, aiming to answer acute questions like

- Does virus Y cause the new disease in rainbow trout?
- Does virus Y replicate in rainbow trout and salmon after intraperitoneal injection?
- Is virus Y transferred to cohabitation fish and if so, does it cause disease?
- What are the target cells and organs for virus Y

**Minutes**

Mortality outbreak occurred in rainbow trout in 4 hatcheries in Norway. Diagnostic examinations ruled out all the known pathogen, only IPNV detected to low level. Disease pattern symptoms and lesions were not consistent with IPN infection alone. Further investigation detected the sequence of a reovirus in diseased fish a new virus defined as Y virus. This new disease appeared in 2013 in Norway. The pathogen is not yet confirmed to be the causative agent of the disease and it has been detected also in healthy fish.
A pilot study was designed and conducted to furhter investigate this case. SPF rainbow trout and Atlantic salmon were intraperitoneally injected with blood pellet from diseased fish collected during the outbreak, this donor fish were further cohabitated with SPF fish. Samples were taken at various time points. Blood was collected for hematocrit analysis and PCR, organs collected for histology and for PCR.
In rainbow trout it was possible to detect the virus RNA both in injected and cohabitated fish and 2 fish (1 injected and 1 cohabitated) displayed inflammation in the heart.
Also in Salmon it was possible to detect the virus in the IP injected fish,and 8 week post cohabitation in one of the cohabitated fish. No histopathological lesions were detected in Salmon.

**Questions**
**Anna Toffan:** Do you suspect this to be a reovirus? Have you tried Electron Mycroscopy?
**Monika Hjortaas:** We cannot be sure on the classification now, we have a limited nucleotide sequence analysis. We are working also with Electron Mycroscopy.
**Torsten Snogdal Boutrup** Do you have any indication if it is close related virus to Reovirus of Atlantic Salmon?
**Monika Hjortaas:** Hard to say, we have introduction of relatively high number of point mutations in the investigated part of the genome. We are not able to detect it with diagnostic PCR for piscine reovirus (PRV).
**Niels Jørgen Olesen:** How are you trying to contain disease?
**Brit Hjeltnes**: There is a close contact with farmers to check if and where they will stock the fish. Fish disease service are alerted so they have strict control of the farm, we have to consider also that is very recent the identification of the causative agent, we have now some evidence that could be infectious. We are constantly evaluating of development. We notified this to the OIE.

# SESSION III:

# UPDATE ON IMPORTANT FISH DISEASES IN EUROPE AND THEIR CONTROL

*Chairman: Dr. Brit Hjeltnes*

# KOI HERPESVIRUS DISEASE (KHVD) SURVEILLANCE AND DIAGNOSIS

**Way K.[1], Bergmann S.[2], Engelsma M. [3], Mikkelsen S. S.[4], Vendramin N.[4], Olesen N. J. [4]**

[1]CEFAS: Aquatic Health and Hygiene Division, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, England.
[2]FLI: Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute for Infectiology, Suedufer 10,17493 Greifeswald - Insel Riems, Germany
[3]CVI: Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands
[4]DTU VET National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, DK-1870, Frederiksberg C, Denmark

**Abstract:**
According to the European Council Directive 2006/88/EC, additional legislation should be implemented describing sampling and diagnostic procedures for the diseases listed in Annex IV Part 2 of the Directive. The sampling plans and the diagnostic methods for the detection and confirmation of VHS and IHN diseases and for ISA disease are described in commission decisions from 2001 and 2003, respectively. However, KHV was only included as a non-exotic disease at the implementation of the Council Directive and no descriptions of procedures were available for this disease. A preliminary version, describing sampling and diagnostic procedures, was later provided on the EURL Fish web page. This version was based on recommendations from the report of a KHV expert working group under the EPIZONE network "KHV PCR diagnosis and surveillance" convened at the Central Veterinary Institute, Lelystad, The Netherlands, in 2009. However, significant new knowledge based on new research on KHV has appeared in recent years. So, the EURL asked the Commission for permission to organize an expert meeting in order to discuss and agree common new recommendations for sampling and diagnosis of KHV for implementation in a new Commission Decision.
The two day meeting was held at the premises of the EURL at Frederiksberg, Denmark and three of the top experts in the field of KHV from Germany, Netherlands and UK, respectively, were invited to participate. The meeting was very successful and produced final drafts of two documents:

1) The Commission decision Part 2 on surveillance and diagnostic methods for KHV

2) Diagnostic procedures for the surveillance and confirmation of KHV disease.

Significant changes from the former versions were accepted and recommended for inclusion in the commission decision. Among the changes are:

- The splitting of sampling and diagnostic tests for diagnostic and surveillance purposes respectively.

- Inclusion of real-time PCR as the method of choice for surveillance.

- Specification on how to define a CyHV-3 strain.

The participants agreed that the meeting had been fruitful and brought together skills and experience on this fish disease from different parts of Europe. In the report of the meeting sent to the commission important issues concerning serology and cyprinid herpesvirus variants were raised. We hope that our

recommendations to resolve these issues will be considered by the Standing Committee On the Food Chain and Animal Health (SCOFCAH).
This presentation will provide more details of these issues as well as providing detail from the final documents described above.

**Minutes:**
A draft for the EU diagnostic manual on KHV has been made in the spring 2014, following a meeting at the EURL, including experts on KHV from Germany, Netherlands and UK. It was agreed that Koi Herpes Virus, belonging to the Alloherpesviridae (KHV), is the aetiological agent of koi herpes virus disease (KHVD). This virus is scientifically defined as cyprinid herpesvirus 3 (CyHV-3) having a 100 % homology to sequences from Genbank submitted by Aoki et al. 2007.
Key points included targeted recommendations for diagnostic procedures when handling diseased fish and procedures for surveillance in clinically healthy fish. For surveillance, sampling should be performed when water temperature is above 17 $^{o}$C, furthermore sampling should not be carried out earlier than two weeks after water temperature reaching this level. If possible fish should be kept at 2-3 weeks at temperatures between 20 and 26$^{o}$C to enhance the establishment of viremia. Also stressing the fish 24 hours prior to sampling e.g. by transport or netting can be a way of inducing viremia, and can be used to increase the chances of finding virus in latent carrier fish. In certain cases (e.g. very valuable fish) non-lethal sampling can be carried out by gill swaps, gill biopsy or blood sampling. For surveillance purposes tissues from no more than 2 fish may be pooled, whereas for fish with clinical disease 5 fish pool is allowed. Assay of choice is the Gilad real-time PCR, alternatively the conventional Bercovier assay can be used, or real-time/ conventional methods with proven equal sensitivity and specificity. If KHV is detected in a new area, confirmation should be done by sequencing. Hopefully this diagnostic manual will help implementing more accurate screening procedures.

**Questions:**

**Olga Haenen:** So the viral strains genetically closely related to KHV, found in the Netherlands are not to be considered KHV.
**Keith Way:** No.

**Uwe Fischer:** I think 100 % similarity is too strict. There could be possible single nucleotid errors or substitutions, where it is still needed to be seen as KHV.
**Keith Way**: Yes, it might be an area to look into for reconsideration.

# A NEW ANIMAL HEALTH REGULATION IN THE EU – CONSEQUENCES FOR AQUATIC ANIMALS

**Roenningen K.** [1]

[1] Legislative veterinary officer – SNE European Commission

**Abstract:**

In May 2013 the European Commission launched a proposal for a new EU Animal Health Regulation, which currently is in the process of being adopted by the legislative bodies of the Union – the Council and the European Parliament. This Regulation is the main instrument for implementing the Animal Health Strategy (2007 – 2013) "Prevention is better than cure" and is a single and robust legal framework for the animal health of all animals including the aquatic. The Regulation aligns with the Lisbon Treaty and lay down horizontal principles and rules for transmissible animal diseases in kept and wild animals and their products.

New elements in this Regulation compared to current legislation are more preventions in terms of biosecurity at farms, in transport, assembly and at borders; enhanced surveillance, diseases notification and reporting; clearer policy for the use of vaccines; and more tools to control emerging diseases. In addition the Regulation is intended to provide easier and safer trade by enhanced convergence with international standards on animal health (OIE), compartmentalisation and requirements for export.

With regard to aquatic animal health the intentions of this new Regulation are the following:

- To keep the principles of Directive 2006/88/EC
- To align to the Lisbon Treaty
- To harmonise with the terrestrial animals where appropriate
- To simplify and clarify when appropriate.

**Minutes:**
The new legislation will make a common ground for regulation of legislation towards transmissible diseases in both terrestrial and aquatic animals. At present, the new regulation has been voted for in the European Parliament, and around 300 amendments have been made. A first common position can not be expected before autumn 2014, and hereafter negotiation between the European Parliament and the European Council will take place before final adoption. The new regulation will be more flexible compared to previous legislation and harmonization between terrestrial and aquatic animals are obtained whenever appropriate. The focus is on transmissible diseases and as such not on a range of other important veterinary issues e.g. welfare, medication and education. The new regulations will give better possibilities to act on disease situations in the wild fish, furthermore categorization is reduced from 5 to 3 categories and the categorization is done on a more risk based system. Finally responsibilities is put on operators and the responsibilities for different levels of professionals is made more clear.

**Questions:**
**Uwe Fischer:** How is one health implemented?

**Knut Roenningen**: It is integrated in the regulation, it is more significant in the parts for terrestrial animals with focus on interaction between animal- and human proffesionals.

**Niccolò Vendramin**: How can the flexibility be seen in disease management ?

**Knut Roenningen**: It is for instance in a less rigid use of vaccines e.g. use of vaccines in strategies toward exotic diseases.

**Niccolò Vendramin**: Will it be easier to use vaccines across countries and e.g. use DNA vaccines ?

**Knut Roenningen**: This is in details another legislation. Some principles are taken care of in the animal health legislation.

**Neil Ruane**: How is requirements regarding NRL accreditation and accreditation of methods ?

**Knut Roenningen:** It is too specific at present time, it is something to be dealt with following implementation.

**Britt Hjeltnes**: Will this increase the competences needed to apply with the regulations?

**Knut Roenningen:** Competences are dealt at different levels, e.g. veterinarians and farmers are qualified facility operators and they need to be qualified according to responsibilities in the legislation.

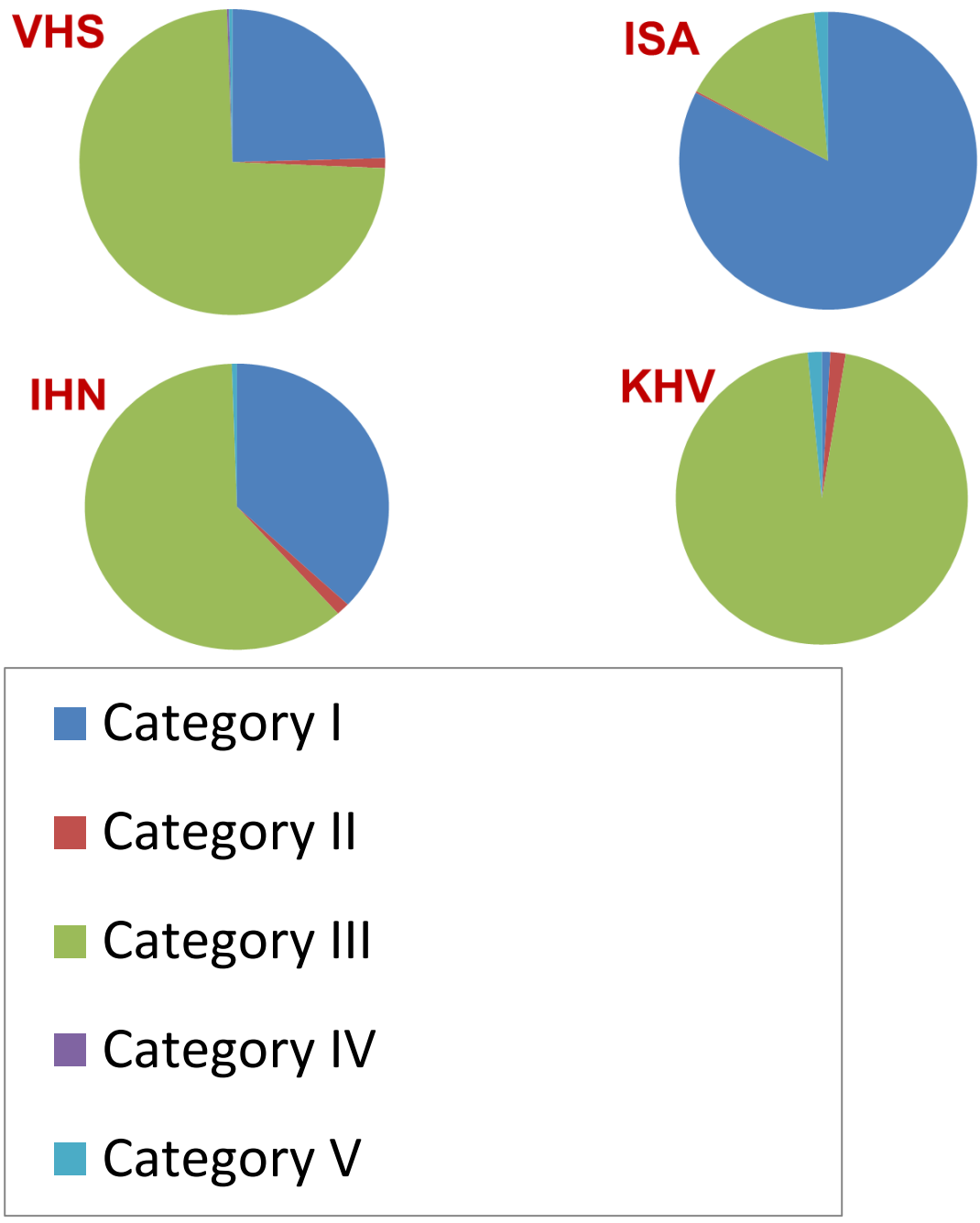
**Niels Jørgen Olesen:** How is disease and spread dealt with in stamping out procedures and emergency slaugthering?

**Knut Roenningen**: There is a specific set of rules for slaughterhouses to deal with diseased fish.

# HEALTH CATEGORISATION OF FISH FARMS IN EUROPE IN 2013

**N. J. Olesen** and Anemone Ojala
*National Veterinary Institute, Technical University of Denmark* njol@vet.dtu.dk

**Abstract:**
The Questionnaire on Surveillance and Diagnosis (S&D) included questions on how fish farms are health categorised according to Council Directive 2006/88/EC in the respective countries. 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.


VHS
ISA
IHN
KHV
Category I
Category II
Category III
Category IV
Category V

**Minutes:**

Details of this presentation can be found on the EURL homepage. It is important to remember that health categorisation is to be considered for specific diseases and for areas/zones and as such not at farm level, it makes no sense a downstream farm can be considered true negative for VHS if an upstream farm has an outbreak. Further in the light of the questionnaire where it is evident that in large areas most farms are placed as undetermined, does it then make sense to retain the way we think about listed diseases. From the questionnaire it can be seen, that in between similar production, the approach on how to categorise farms differs from country to country, though it can be seen that there is a major underreporting of farms, especially from central and parts of southern Europe there has been an increase in reporting and categorization, mainly due to a huge work performed by Germany and Poland where many small farms are present. The slow increase in categorization is a problem and in this context it has been a major constraint that there has not been any consequences of not getting this work done. Whether this will be better with the new Animal Health Legislation (AHL) is not clear, but there should optimally be an incitement to try and go for eradication and demonstrating freedom of disease.

**Questions:**

**Knut Roenningen**: With the new AHL there will be mandatory health visit on both registered and categorised farms.

# SESSION IV: Scientific research update

*Chairman: Dr. Richard Paley*

# MALDI-TOF MS: NEW DIAGNOSTIC TOOL FOR IDENTIFICATION OF FISH PATHOGENS

**Dalsgaard I. [1] and Nonnemann B.**

[1] *National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, 1870 Frederiksberg C*

*inda@vet.dtu.dk, benon@vet.dtu.dk*

**Abstract:**

Identification of fish pathogenic bacteria by traditional microbiology might be time-consuming due to different biochemical tests and appropriate conditions for bacterial growth. The aim of this study was to evaluate identification of different fish pathogens by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS). The focus has been on *Aeromonas salmonicida*, *Vibrio anguillarum*, *Yersinia ruckeri* and *Flavobacterium psychrophilum*, the main bacterial pathogens causing disease problems in Danish rainbow trout aquaculture. A rapid and correct diagnosis is necessary due to treatment with antibiotics. The reference spectra for some of the mentioned fish pathogens were already in the database by Bruker Daltonics: *A. salmonicida*, 3 strains and a subspecies *masoucida*; *V. anguillarum*, 5 strains; *Y. ruckeri*, 1 strain; *F. psychrophilum*, zero strains. So far we have established our own database with whole cell MALDI-TOF of well-characterized bacteria: *A. salmonicida*, 3 strains; *V. anguillarum*, 16 strains; *Y. ruckeri*, 3 strains; *F. psychrophilum*, 7 strains. The selected strains are from culture collections or clinical samples.

Routine bacterial diagnostics are done with commercial kits like API 20. This method takes time compared to MALDI-TOF technique, which can be made within minutes. The two identification methods have been compared using diagnostic samples. Both methods have some limitations mainly due to the available databases. An overview of the results found with these two methods will be presented.

Further we want to use this rapid method for subtyping some of the fish pathogens. The spectra representing the two biotypes of *Y. ruckeri* were created and compared but the observed peaks seem to be similar. Discrimination between *V. anguillarum* serotypes is of clinical relevance since environmental strains belong to other serotypes and are more resistant to antibiotics.

To conclude MALDI-TOF MS is a major advantage for identification of fish pathogens with its rapid and easy sample preparation. Future studies are needed for expansion of the database with more spectra of well characterized fish bacterial pathogens.

**Minutes:**
The Autoflex Speed MALDI-TOF system by Bruker Daltonics taken into use in September 2013 was presented. A short introduction was given explaining the use of pure single colonies on agar plates for diagnostics. Protein is extracted from the bacteria and a diagram of peptide sizes and intensity forms the basis for a score value in the Bruker database. Score values above 2 gives pure species identification, that is reliable according to the system. In relation to bacterial diagnostics of fish diseases there are several limitations as the Bruker database only include few bacterial fish pathogens (no *Flavobacterium psychrophilum*, 3 spectra for *Aeromonas salmonicida* subs. *salmonicida*, 1 spectrum for *Yersinia ruckeri* and 5 spectra for *Vibrio anguillarum*) This means it is necessary to add spectra from well characterized relevant bacteria in a local database to be able to use the system for bacterial diagnostics of fish diseases. This is an ongoing project at the department. Several examples of MALDI-TOF "mis-identification" from recent ring tests were presented. Discrepancies were found between supposed species, API-results and MALDI-TOF results in identification of some bacterial species.

**Questions:**

**Olga Haenen**: : I would like to inform that NL also works on MALDI-TOF in a Club5-project, together with VetDTU and SVA a.o., mainly to develop *Vibrio vulnificus* profiles for use in diagnosis, while the partner fish labs work on profiles of *Flavobacterium psychrophilum* and *F.columnare*, *Aeromonas salmonicida* subspp., and *Vibrio anguillarum*. Subtyping of bacterial species is also in view.
**Britt Hjeltnes**: As this is a commercial system will there be any feeding of local data into the Bruker database?
**Inger Dalsgaard**: So far the Bruker database and the local database are separate.

# VetMIC Aquatic - A BROTH MICRODILUTION PANEL FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING OF BACTERIA ISOLATED FROM AQUATIC ANIMALS

**Axén C.[1] and Pringle M[1]**

[1] National Veterinary Institute, SVA, SE-751 89 Uppsala, Sweden, charlotte.axen@sva.se, marit.pringle@sva.se

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**Abstract:**

Antimicrobial susceptibility testing of bacteria isolated from aquatic animals is important both for choice of treatment and for monitoring of resistance. Because of the variation in growth requirements among the relevant bacterial species standardization of methods takes time but is all the more important. Development of standards is ongoing by the CLSI (Clinical and Laboratory Standards Institute) Working Group on Aquaculture and one of the recommended methods is broth microdilution.

So far SVA has used test panels for terrestrial bacterial pathogens for testing of bacteria from aquatic animals. However the choice of antimicrobial substances and concentration ranges in these panels are not optimal. Hence a broth microdilution panel for this particular purpose was designed (Figure 1). The concentration ranges were chosen to, as far as possible, include MIC distributions for *Aeromonas* spp., *Flavobacterium* spp. and *Vibrio* spp. but may fit other bacterial genera as well.

The antimicrobials are dried in microtitre plates in serial twofold dilutions, packaged in foil pouches with a desiccant and stored in room temperature. The packages are hermetic and 60-66% of the air is evacuated with a vacuum pump. The shelf life of the panels is three years however oxolinic acid has only been tested after three months storage so far. Manufacturing procedure and quality control is performed according to CLSI standards. VetMIC Aquatic will be available for purchase during summer 2014.

**Figure 1.** Panel design VetMIC Aquatic, 100 µl/well gives the following concentrations (mg/L)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | **Otc** 4 | **T/S** 4/76 | **Oxo** 1 | **Am** 8 | **Ff** 8 | **Em** 2 | **Otc** 4 | **T/S** 4/76 | **Oxo** 1 | **Am** 8 | **Ff** 8 | **Em** 2 |
| **B** | 2 | 2/38 | 0.5 | 4 | 4 | 1 | 2 | 2/38 | 0.5 | 4 | 4 | 1 |
| **C** | 1 | 1/19 | 0.25 | 2 | 2 | 0.5 | 1 | 1/19 | 0.25 | 2 | 2 | 0.5 |
| **D** | 0.5 | 0.5/9.5 | 0.12 | 1 | 1 | 0.25 | 0.5 | 0.5/9.5 | 0.12 | 1 | 1 | 0.25 |
| **E** | 0.25 | 0.25/4.8 | 0.06 | 0.5 | 0.5 | 0.12 | 0.25 | 0.25/4.8 | 0.06 | 0.5 | 0.5 | 0.12 |
| **F** | 0.12 | 0.12/2.4 | 0.03 | 0.25 | 0.25 | 0.06 | 0.12 | 0.12/2.4 | 0.03 | 0.25 | 0.25 | 0.06 |
| **G** | 0.06 | 0.06/1.2 | 0.016 | 0.12 | 0.12 | 0.03 | 0.06 | 0.06/1.2 | 0.016 | 0.12 | 0.12 | 0.03 |
| **H** | 0.03 | 0.03/0.6 | 0.008 | 0.06 | bc | dc | 0.03 | 0.03/0.6 | 0.008 | 0.06 | bc | dc |

Otc, Oxytetracycline; T/S, Trimethoprim/Sulfamethoxazole; Oxo, Oxolinic acid; Am, Ampicillin; Ff, Florfenicol; Em, Erythromycin; bc, buffer control; dc, control well with distilled water.

**Minutes:**

As the "Vet MIC terrestrial"-panel is not optimal for testing bacteria isolated from aquatic animal diseases, a new panel was developed: "Vet MIC aquatic". This panel should support a correct treatment from the start ensuring that fish get well, and no unnecessary antibiotic is released in the environment. It is also important to have a relevant panel to monitor how antimicrobial resistance develops. CLSI has a working group on aquaculture and publishes manuals on resistance testing (disk diffusion, broth dilution (not fish bacteria yet))

Testing on oxolinic acid shelf life is ongoing, but otherwise a 3 year shelf life is applicated. Production starts in June, and ordering can be made to vetmic@sva.se, att. Märit Pringle.

More info on www.sva.se/en/Service-and-products/VetMIC/

**Questions:**

**Inger Dalsgaard**: Is part of the CLSI workgroup, and can inform that the report on broth microdilution in fish is very close to being published. But problems with testing *Flavobacterium psychrophilum* are foreseen.

# TARGETED DISEASE PROPHYLAXIS IN EUROPEAN FISH FARMING (TARGETFISH)

**Wiegertjes G.[1], Lorenzen N. [2*], Secombes C. [3], Collet B. [4], Fischer U. [5], Tafalla C. [6], Parra D. [7], Scapigliati G. [8], Boudinot P. [9], Evensen Ø. [10], Adams A. [11], Toffan A. [12], Buchmann K. [13], Vesely T. [14], David L. [15], Mulero V. [16], Smith P. [17], Aspehaug V. [18], Engell-Sørensen K. [19], Sober J. [20], Wallis T. [21], Rød T. [22], Flores M. [23], March J. [24], Stratmann A. [25], Christofilogiannis P.[26], Tobar J.[27], Henriksen N. [28], Sigholt T. [29], de las Heras A. [30]**

*[1]Wageningen University, The Netherlands, [2]Aarhus University, Denmark, [3]University of Aberdeen, United Kingdom [4]Marine Scotland, UK, [5]Friedrich Löffler Institut, Germany, [6]Instituto Nacional De Investigacion Y Tecnologia Agraria Y Alimentaria, Spain, [7]Universitat Autonoma de Barcelona, Spain, [8]Università degli Studi della Tuscia, Viterbo, Italy, [9]Institut National De La Recherche Agronomique, France, [10]Norwegian School of Veterinary Science, Norway, [11]The University Of Stirling, UK, [12]Istituto Zooprofilattico Sperimentale delle Venezie, Italy, [13]Københavns Universitet, Denmark, [14] Veterinary Research Institute, Czech Republic, [15]The Hebrew University of Jerusalem, Israel, [16]University of Murcia, Spain, [17]Tethys Aquaculture Limited, Greece, [18]PatoGen Analyses AS, Norway, [19]Fishlab, Denmark, [20]Naxo OÜ, Estonia, [21]Ridgeway Biologicals Limited, UK, [22]Rossi A/S, Denmark, [23]Ingeniatrics Tecnologias S.L., Spain, [24]BigDNA, UK, [25]W42 Industrial Biotechnology GmbH, Germany, [26]AQUARK, Greece, [27]CentroVet, Chile, [28]Danish Trout Association, Denmark, [29]BioMar A/S, Denmark, [30]Bionaturis, Spain*

*niels.lorenzen@agrsci.dk

**Abstract:**

TargetFish is a large collaborative project funded by the European Commission under the 7th Framework Programme for Research and Technological Development (FP7) of the European Union (Grant Agreement 311993 TARGETFISH). The project started November 2012 and will run for 5 years, bringing together leading European research groups that are experts on the fish immune system and enterprises from the Biotech and Veterinary sectors with a shared interest and experience with vaccination of fish. TargetFish will advance the development of existing (but insufficient) and new prototype vaccines against socio-economically important viral or bacterial pathogens of Atlantic salmon, rainbow trout, common carp, sea bass, seabream and turbot. TargetFish will also establish a generic knowledge- and technology-base for rational development of next generation fish vaccines. Improved vaccines will be brought closer to industrial application by addressing practical issues such as efficacy, safety and delivery route.

The main objectives of the project are to: 1) generate knowledge by studying antigens and adjuvants for different routes of administration while analyzing the underpinning protective immune mechanisms; 2) validate this knowledge with response assays for monitoring vaccine efficacy and safety, including issues associated with DNA vaccines; 3) approach implementation of prototype vaccines shortening the route to exploitation and 4) optimize vaccination strategies in order to obtain maximum protection in different sizes of fish. To achieve these challenging tasks, we brought together 30 partners from 10 EU member states, 2 associated countries and 1 International Cooperation Partner Country (Chile). In this large multidisciplinary consortium an approximate equal number of RTD and SME partners will cooperate closely while keeping an intensive communication with the large vaccine and nutrition industries via an Industry Forum.

**Minutes:**
The research group working on this project in Denmark is no longer part of the EURL, but is part of Aarhus University.
The 5 years Targetfish project was presented with focus on immunology and vaccines. The tasks include improving existing vaccines and development of new vaccine prototypes (next generation vaccines). The project has 30 partners from Europe and Chile, and industry is represented. The work address 6 major fish species farmed in Europe, with focus on salmon and rainbow trout. The work packages were presented, and some details in each WP were underlined. A major idea is shortening of the route from vaccine development to field use, and implementation of an industry forum is essential (Patrick Smith). An adjuvant producer (Seppic) was visited as part of the project.
More information on the homepage: www.targetfish.eu

**Questions:**
**Neil Ruane**: What is the strategy regarding vaccines covered by patents or with patent pending?
**Helle Franck Skall**: The idea is to develop new vaccines
**Uwe Fischer**: Patented vaccines are "included" as model of good working vaccines, and the project aims at looking into why some vaccines work and others do not – and then develop new vaccines on this basis.
**Angela Trent**: What is the time frame for WP5 (monitoring vaccine efficacy in vivo and in vitro)?
**Uwe Fischer**: Some WPs depend on others, but we try to start early with testing the prototype.
**Niels Jørgen Olesen**: In general there are some challenges in big projects (like H2020). In this case the steering group recommends early starting of field testing (industry close)
**Uwe Fischer**: The project should bring scientific ideas from researchers to industry.

# MOLECULAR TRACING OF AQUATIC VIRUSES - MOLTRAQ

**Mikkelsen S. S.[1], Schuetze H.[2], Korsholm H.[3], Bruun M. S.[1], Olesen N.J.[1]**

*susmi@dtu.vet.dk [1]DTU Vet National Veterinary Institute, Bülowsvej 27 1870 Frederiksberg C,*

*[2]Friedrich-Loeffler Institut, Insel Riems, Germany*

*Danish Veterinary and Food Administration, Tysklandsvej 7, 7100 Vejle [3]*

**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. Spatio-temporal and phylogenetic information will be used to create phylogeographic and scenario-simulation models to identify important factors for the spread of disease and 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 the OIE and EU reference laboratory for VHSV, the Danish national reference laboratory for fish diseases has a large collection of Danish VHSV isolates that span over more than 50 years. As part of MOLTRAQ more than 200 Danish isolates spanning from 1978-2003 has been sequenced and epidemiological information has been collected.

This information will form the basis for several different areas of research in the MOLTRAQ project. Data will be used to create a spatio-temporal computermodel for VHS in Denmark that will be able to provide information about control strategies in countries or areas with a similar spread of disease history. This work will be done in close collaboration with the National Veterinary institute in Norway and the Norwegian Computing Center.

Data will also be 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. Some of these data will be presented at the Annual Workshop.

Furthermore, data will be used to create phylogenetic and phylogeographic models at a wider European scale.

MOLTRAQ is funded under the EMIDA-ERA Net under the EU 7th Framework program (For more details about EMIDA: www.emida-era.net).

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).

**Minutes:**
An update on MOLTRAQ, a collaborative project with 6 partners, was presented.
The Danish contributions to the six work packages were mentioned, and selected details were presented.
More than 200 VHSV isolates from 1978 to 2003 have been sequenced (full length G-genes), and almost all sequences belong to genogroup 1a. Spatio-temporal models for VHS in Denmark have been developed and phylogenetic + phylogeographic models at a wider European level are made. A few cases from the Danish data material were presented with emphasis on genetic relatedness and epidemiological data.
Data will be uploaded to www.fishpathogens.eu.
More information can be found at www.moltraq.wordpress.com.

**Questions:**
**Richard Paley**: Fish pathogen looks like a useful tool for epidemiological studies. Can you comment on this?
**Susie Sommer Mikkelsen**: It is a powerful database, so even if we have no VHS in Denmark now, we will be able to make predictions in other countries. This modelling can be difficult due to differences in "structure" between countries, but the information can be used.
**Niels Jørgen Olesen**: It will also be possible to see "introductions" into DK from other countries – historically, but also as a tool in the future, as there is a risk of contracting VHS again. Also the 1b to 1a isolation is a rare occurrence, which is important for risk assessment.

# PKD INFECTIONS IN TROUT IN DENMARK WITH FOCUS ON INTERACTIONS BETWEEN WILD AND FARMED FISH

**Buchmann K.[1] & Skovgaard A.[1]**

University of Copenhagen, Department of Veterinary Disease Biology, Laboratory of Aquatic Pathobiology, Stigbøjlen 7, DK-1870 Frederiksberg C, Denmark kub@sund.ku.dk

**Abstract:**

Proliferative Kidney Disease PKD caused by *Tetracapsuloides bryosalmonae* is known to infect mainly salmonids. Rainbow trout *Oncorhynchus mykiss* is particularly susceptible and may exhibit severe pathological reactions in kidney tissue. This myxozoan has a two host life cycle including fish and bryozoans such as *Fredericella sultana*. PKD was previously occurring frequently in traditional Danish rainbow trout farms connected to natural water bodies but the infection status of wild brown trout *Salmo trutta* was largely unknown. Therefore we performed a field survey in 2008 and 2009 by electrofishing five streams in Denmark (located in Jutland, Funen and Zealand) and collecting juvenile *Salmo trutta* for examination. In addition, samples of rainbow trout from three fish farms in Jutland were included in the study. Infection was diagnosed by lectin histochemistry (by using biotinylated Griffonia simplicifolia lectin) and PCR. Two of the five investigated brown trout samples showed high prevalences (70 and 96 %, respectively) but the remaining three trout samples had merely less than 20 % prevalence. No external clinical signs were recorded but by autopsy the kidneys of more than 80 % of the fish in the highly infected samples had visually swollen kidneys. Infected rainbow trout displayed external clinical signs in addition to internal obvious swollen kidneys. Implementation of a new water recirculation strategy for freshwater trout farms in Denmark (model trout farms based on well or ground water supply and no connection to natural water bodies) is expected to lead to a decrease of infected farms due to elimination of bryozoans in the farms). The high prevalence of infections in wild brown trout may on the other hand sustain a continous infection source for bryozoans which again may liberate infectious stages targeting rainbow trout in traditional fish farms.

**Minutes:**
An investigation on Proliferative Kidney Disease caused by *Tetracapsuloides bryosalmonae* was presented.
Common occurrence in traditional rainbow trout farms connected to natural streams, and high mortalities associated with PKD have been observed in Swiss and Norwegian rivers. The life cycle has been shown to include freshwater bryozoans. Disease symptoms, lectin staining for visualization and susceptible species were presented. A survey was performed of the occurrence of *T. bryosalmonae* in Danish salmonids in natural streams. Only few salmon were infected, but a high percentage of other salmonids were found infected by PCR. Traditional earth ponds present a possibility for the life cycle to be complete whereas recirculation systems limit the life cycle due to lack of contact to bryozoans.

**Questions:**
**Brit Hjeltness**: In wild populations the temperature can have a huge impact on growth of salmon infected with PKD
**Kurt Buchmann**: We did not look for differences in symptoms associated to temperature. The temperatures at sampling were 8-10 °C in the streams.
**Thomas Wahli**: In Switzerland low mortality (<10%) is seen with river water temperatures <14°C, but up to 90% mortality if water temperature >15°C for more than 3 weeks. In the wintertime symptoms disappear.
**Niels Jørgen Olesen**: There is a strong seasonality in rainbow trout.
**Kurt Buchmann**: All samples in this survey were from autumn.

# SUSCEPTIBILITY TO VHS AND IHN OF REDFIN PERCH AND MARBLE TROUT

**Toffan A.[1] & Pascoli F.[1]**

*Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell´Università, 10 Legnaro Padova atoffan@izsvenezie.it*

**Abstract:**

The redfin perch, *Perca fluviatilis* (Linneus), is a freshwater species belonging to Perciformes family, commonly found in European and Italian rivers and lakes. The marble trout *S. marmoratus* (Cuvier) is an autochthonous species present in the Po basin, northern Italy, as well as in the Adriatic basin of Slovenia, Croatia, Bosnia-Herzegovina and Albania. Both species are of particular interest for the Italian aquaculture sector, the first for its high market value, the latter for resettlement in the wild, since it is an endangered species.

Viral haemorrhagic septicaemia virus (VHSV) and infectious haematopoietic necrosis virus (IHNV) are among the most important viral pathogens for freshwater aquaculture and both are endemic in Italy**.** The aim of the present study was to assess the ability of European strain of VHSV and IHNV to produce disease in redfin perch and marble trout experimentally infected, and to determine whether a carrier status can result in these species after bath infection. An already known susceptible species (*O. mykiss*) was used for comparison. To analyze the survival function from lifetime data, the Kaplan–Meier estimator was applied.

No mortality cases nor clinical signs were registered in the infected redfin perch, while rainbow trout showed high mortality rates with evident clinical signs, such as exophthalmos and severe haemorrhages. Virological and real time PCR performed on the surviving fish yielded always negative results, thus demonstrating the resistance of redfin perch to VHSV and IHNV under simulated natural conditions.

If confronted with the effects registered in the rainbow trout, the infection of marble trout showed low susceptibility to both VHSV and IHNV under simulated natural conditions, as supported by the results obtained with two independent laboratory test (virus isolation and rRT-PCR) and statistical analysis. Furthermore, our results highlighted the presence of a given number, although small, of chronically infected marble trout, confirming that this species should be definitely added to the list of susceptible species.

**Minutes:**
The aim of the present study was:

1. to assess the ability of VHSV and IHNV to produce disease and associated mortality in redfin perch and in marble trout
2. to determine whether a carrier status can result in these species after experimental infection

Perch were found to be resistant to Italian strains of VHSV and IHNV under simulated natural conditions. Moreover, this species does not seem to be a carrier of these diseases.
Marble trout showed low susceptibility to both VHSV and IHNV under simulated natural conditions, and a carrier status of this species for these diseases is possible.

**Questions:**
**Irene Ørpetveit**: Which virus strains were used?
**Anna Toffan**: The classical European strains – 1a for VHS.
**Richard Paley**: Perch has a low susceptibility to 4b

# FIRST CASE OF KOI SLEEPY DISEASE (KSD) BY CARP EDEMA VIRUS (CEV) IN THE NETHERLANDS

**Haenen O.[1], Way K.[2], Stone D.[2] and Engelsma M.[1]**

*NRL for Fish Diseases, CVI of Wageningen UR, P.O. Box 65, 8200 AB Lelystad, the Netherlands olga.haenen@wur.nl*
*NRL for Fish Diseases, Centre for Environment, Fisheries & Aquaculture Science (CEFAS), Weymouth, Engeland*

**Abstract:**

CEV (Carp Edema Virus) is a virus of carp and koi *Cyprinus carpio*. The virus was first detected and described in Japan in the 70's, where it caused a severe viral oedema of juvenile carp resulting in high mortalities (Murakami et al. 1976, Ono et al. 1986). CEV was shown to be a poxvirus by electron microscopy, and more recently has been shown to cause "Koi Sleepy Disease" (KSD) in older koi (Oyamatsu et al., 1997; Miyazaki et al., 2005). The lethargy manifests as sleepy behaviour, where the affected fish lie on the bottom of the pond and eventually die of anoxia (Miyazaki et al. 2005). Losses from KSD/CEV occur in spring and autumn in Japan, over a temperature range of 15 – 25°C, and mortalities may reach 80%.

In 2009 CEV was detected for the first time in Europe, in England in imported diseased koi, and again in 2011-2013, in diseased koi from hobbyist ponds. Low levels of CEV-like virus were also detected in healthy koi imports from Israel and Japan at ornamental fish wholesalers during 2013 in the UK. Furthermore, in 2012, a CEV-like virus was detected for the first time in common carp, displaying KSD-signs, obtained from a cluster of fishery sites in south- east England and a site in the English Midlands (Way & Stone, 2014).

In September 2013, the first Koi Sleepy Disease caused by CEV in the Netherlands was diagnosed by CVI in diseased koi kept at 20°C with "sleepy" behaviour and a high mortality rate (Haenen et al., 2014). Koi showed apathy, anoxia, anorexia, enophthalmus, gill necrosis with oedema, and many *Gyrodactylus* spp. in the gills. Internally, no abnormalities were seen. CEFAS confirmed our first detection of CEV in koi with their PCR. In this lecture, details on KSD and its diagnosis will be presented. The impact of CEV infections for Europe is still unclear.

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**Minutes:**

Characteristics and history of Carp Edema Virus were presented. Cases in Koi and Carp from NL were presented.

CEV has been present in Europe, at least since 2004: CEV in koi since 2008/2009 and CEV in carp since 2004. There are still many questions on CEV. The impact of CEV infections for carp populations in Europe is still unclear.

Therefore, surveillance and risk analysis on CEV in koi and carp, *Cyprinus carpio*, is important and should receive attention

**Questions:**

**Anna Toffan**: What are the best organs to sample?
**Olga Haenen:** Especially gills preserved in ethanol.
**Richard Paley**: What was the intensity of infection?
**Olga Haenen**: Ct-values were around 28.
**Uwe Fischer**: Are there genetic differences on isolates from carp (low temperature) and koi (high temperature)?
**Keith Way**: Different genetic profiles are found, and this could perhaps explain temperature differences?
**Torsten Boutrup**: Did you test the “original” koi’s for disease?
**Olga Haenen**: Not yet. They had no disease.

# VALIDATION OF A SERONEUTRALISATION TEST ALLOWING THE DETECTION OF ANTIBODIES SPECIFIC TO KOI HERPESVIRUS (KHV)

Cabon J. [1, 2], Castric J. [1, 2], Lamour F.[1, 2], Matras M. [3], and **Morin T.** [1, 2].

[1] *French Agency for Food, Environmental and Occupational Health & Safety, Ploufragan-Plouzané Laboratory, Viral Fish Pathology Unit, Plouzané, France, Thierry.MORIN@anses.fr*
[2] *Université Européenne de Bretagne, Rennes, France*
[3] *Department of Fish Diseases, National Veterinary Research Institute, 24 100 Pulawy, Poland*

**Abstract:**

Koï Herpesvirus (KHV) is the etiological agent of a contagious disease regulated in Europe (Directive 2006/88/EC) affecting common carp and varieties such as koï and ghost carp. This virus appeared almost simultaneously in Europe, the United States, Israel and Japan in the late 90s, with a geographical extension highly favored by the international trade. The most marked clinical signs are large skin ulcers, excess mucus production and hemorrhages in the fins. Symptomatic infections usually occur between 18 and 28°C, with mortality rates reaching 100%. Outside this temperature range, the virus can persist in a latent state in infected hosts which remain asymptomatic, contributing to the virus spreading. The KHV detection in these healthy carriers is difficult using the direct diagnostic methods recommended by the World Organization for Animal Health.

The objectives of this work were to develop and validate an indirect and non-lethal seroneutralisation (SN) test allowing the detection of KHV specific antibodies from sera of carps.

After phases of development, optimization and standardization, assessment of the analytical and diagnostic performance of this method was done using sera from healthy or experimentally infected carps. The KHV strain 07/108b used was efficiently neutralized by sera form carps infected with European, American and Taiwanese KHV isolates but no neutralization was observed using sera specific to other herpesviruses (Chanel Catfish, Herpesvirus Anguillae, Cyprinid Herpesvirus type 1). 100% of repeatability was obtained and diagnostic sensitivity and specificity calculated were respectively of 97.50% and 98.75%. Applied to KHV experimentally infected koï carps, we were able to detect 100, 95 and 65% of positive individuals respectively at 40 days, 3 months and 21 months post-contamination. Similar analysis were done on naturally infected carps, showing that the proportion of positive individuals increased with the time elapsed since the onset of the clinical signs. This SN test

could be used in a close future to improve the epidemiological surveillance and control of KHV disease in Europe.

**Minutes:**
A non-lethal test, allowing detection of specific antibodies against KHV was presented. The development was needed as, outside the optimal temperature range, there is persistence of the virus in a latent state in infected hosts which remain asymptomatic, contributing to the virus spreading. KHV detection in these healthy carriers is difficult using the direct diagnostic methods recommended by the OIE.
This test was used to screen more than 200 positive and negative sera which were obtained from farms with no history of KHV, by experimental infection of naïve fish, and finally sera obtained from fish infected with close related virus to test the specificity.
The parameters of diagnostic specificity, diagnostic sensitivity, repeatability and intra-laboratory reproducibility demonstrate the efficacy of this test.
After subsequent test of sera obtained from experimental condition, to monitor the single development of specific antibodies of infected fish, this technique demonstrated to be a reliable tool providing guarantees of reliability and robustness. At a farm scale has to be applied to a sufficient number of samples, it can be used as indirect diagnostic technique to determine the health status of fish for KHV, without sacrificing valuable animals for the farmer (parents).

**Questions:**
**Niccolo Vendramin**: What was the starting dilution of sera?
**Thierry Morin**: 1:40.
**Uwe Fischer**: How did you treat the sera to remove complement?
**Thierry Morin**: Treated 45°C for 20 min.
**Keith Way**: How did you determine if serum contained antibodies against CyHV-1?
**Thierry Morin**: In our test, we add a serum positive for the presence of anti-CyHV1 antibodies as control and each sample are generally tested in parallel for the presence of antibodies specific to CyHV1. No interference was observed.
**Niels Jørgen Olesen**: Did you test reproducibility (intra-lab)?
**Thierry Morin**: The test was used 9 times on the same panel of sera, changing different operators or time of performing the test.

# SUSCEPTIBILITY OF GOLDSINNY WRASSE (*CTENOLABRUS RUPESTRIS*) TO VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS (VHSV): EXPERIMENTAL CHALLENGES AND PATHOLOGY

**Iveta Matejusova, K. Urquhart, J. Simons, M. Ho, M. Hall, K. Lester, M. Fordyce, P. Noguera, D. Bruno**

*Marine Scotland Science, 375 Victoria Road, AB11 9DB Aberdeen, United Kingdom* Iveta.Matejusova@scotland.gsi.gov.uk

**Abstract:**

One of the main aspirations for continued development of sustainable Atlantic salmon (*Salmo salar* L.) aquaculture in Europe and worldwide is to significantly reduce problems related with the presence of salmon lice (*Lepeophtheirus salmonis*). As an alternative to chemical treatment, a biological approach to controlling sea lice was developed by industry, using cleaner fish such as wrasse (family Labridae). Following the isolation of viral heamorhagic septicaemia virus (VHSV) in close proximity to farmed Atlantic salmon in Shetland (Scotland) in 2012 (Munro et al., 2014, Journal of Fish Diseases) a potential of risk of disease transfer from cleaner fish was again emphasized.

The complexity of mixed infections observed in wrasse collected during the VHSV mortality event in 2012 in Shetland did not allow for clinical or histopathological changes to exclusively be attributed to VHSV. Thus fundamental experiments to investigate wrasse susceptibility to VHSV and describe histopathological changes related to presence and replication of this virus are needed. The present study describes experimental set up and results of two experimental challenges, where VHSV was administred to goldsinny wrasse (*Ctenolabrus rupestris*) by both intraperitoneal injection and immersion. Cumulative mortality, gross clinical signs, detection of VSHSV by real time PCR and histopathological changes associated with VSHV infection are described. The results obtained significantly contribute to understanding diseases in wrasse especially when the results suggest significantly different histopathology than observed for rainbow trout as a reference host. In addition, design and validation of wrasse endogenous control (ELF-1α) will be presented.

**Minutes:**
Wrasse used as cleanerfish for biological control of sea lice in salmon aquaculture.
Could Wrasse act as disease vector? Reports of diseases from the literature refer susceptibility to: *Aeromonas salmonicida*, *Vibrio anguillarum*, *Vibrio splendidus*, IPNV and Gill Amoebas. In December 2012 VHS mortality in wrasse (Shetland) occurred. Phylogeny showed type III, related to wild marine species.
Experimental challenges were performed to test Wrasse susceptibility to VHS. Challenge experiments confirmed Goldsinny wrasse susceptibility to VHSV genotype III. Goldsinny wrasse mortality associated with a presence of VHSV was confirmed in experiments. Clinical and histopathological changes were different to those described in rainbow trout.
Wrasse infected by intra peritoneal injection can survive and most likely recover from the infection. Wrasse infected with VHSV could be present on farms for long period of time without any problem/symptoms, thereby it can act as reservoir for other susceptible species.

**Questions:**
**Richard Paley**: Is the practice still going on?
**Iveta Matejusova**: Yes.
**Irene Ørpetveit**: Do you have comment on welfare using cleaner fish? Sometimes they can provoke skin lesion in farmed salmon.
**Iveta Matejusova**: This issue has not been raised, as far as I am aware.
**Niels Jørgen Olesen**: Have you considered including other VHS genotypes for risk assessment?
**Iveta Matejusova**: No, as funding only allows investigating what is relevant to industry.

# ISOLATION AND IDENTIFICATION OF VIRAL HEMORRHAGIC SEPTICEMIA VIRUS IN FARMED RAINBOW TROUT IN IRAN

**Ghasemi, M.[1], Zorriehzahra, J.[2], Mehrabi, M.R.[2], Haghighi Karsidani, S.[3]**

[1].*Iranian Fisheries Research Organization, (IFRO), Bandar anzali, Iran, Mohaddes mohades@yahoo.com*

[2].*Iranian Fisheries Research Organization, (IFRO), Tehran, Iran*

[3].*Islamic Azad University -Bandar Anzali Branch, Bandar anzali, Iran*

**Abstract:**

Viral hemorrhagic septicemia virus (VHSV), is an enveloped negative- strand RNA virus belonging to the genus novirhabdovirus, within in rabdoviridae family. That cause an acute, contagious disease with high mortality in an extensive host rang in both fresh water and marine environments.

In November 2012, one Rainbow trout farm situated in the south- west of Iran, reported unusually mortality in fingerlings and reared Rainbow trouts. Also same mortality was found in another Rainbow trout farm in the same province in December 2013.

Clinical signs included lethargy, dark coloration, ascites, pettechial hemorrhages in muscle and viscera, swollen spleen and kidney. Affected fish were delivered to virology laboratory of inland water aquaculture institute situated in Bandar Anzali for diagnostic investigation.

Presence of the virus was confirmed using cell culture, RT-PCR and IFAT. Specimens of kidney and spleen were homogenized, re-suspended in medium and clarified by centrifugation at 2000 rpm for 20 minutes in 4°C. Supernatants were inoculated on to monolayer of the BF-2 and EPC cell lines in 24 well multi dishes and identified by IFAT and RT-PCR.

CPE was observed 72 h post inoculation and VHSV was identified by IFAT and RT-PCR. Through on sequencing and phylogenetic analysis, isolates were more similar to 3 former submitted sequences in gene bank (AF143863, KF561228 and KC778774) and Genotype of all isolates in this study were identified Ia. This is the first isolation and identification of VHS from Rainbow trout in Iran.

**Minutes:**
More than 100000 tons of rainbow trout were produced in Iran in 2012 in fresh water. There have been two unconfirmed VHSV outbreaks in Iran (2008 and 2012)
First confirmed isolation of VHSV in Iran was presented. The virus was isolated on 2 rainbow trout farms in SW Iran. High mortality (<50%) observed on some farms. The isolates are under further characterization for genotyping and phylogenetic relationship to other VHSV isolates. The origin of the isolates is still unknown. Iran import significant number of eyed eggs from Europe (primarily Denmark and France) and import from Europe is suspected. Iran also import from Canada.

**Questions:**
**Helle Frank Skall**: Has there been earlier reports/suspicions from the same area?
**Mohades Ghasemi**: From neighbour province.
**Richard Paley**: Any contact between infected farms?
**Mohades Ghasemi**:Yes we are in contact with them monitoring the situation

# SESSION V: Update from the EURL

# EURL activities in 2013

**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 (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 (KHV) disease.

The 17th Annual Meeting of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark, 29-30 May 2013 at the premises of the Section for Fish Diseases at DTU Veterinary. A total of 52 participants from 28 countries attended over the two days period. There were five sessions with a total of 32 presentations, 7 of which were given by invited speakers, and two round table discussions. A report was submitted in August 2013.

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 fourth 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 (upon request from laboratories being accredited for these pathogens). PT2 was developed in order to test the proficiency of participating laboratories to identify ISA and KHV and in addition also spores of the oomycete *Aphanomyces invadans* causing EUS. The proficiency test is covering all 5 listed exotic and non-exotic diseases. 43 National Reference Laboratories (NRLs) participated in the proficiency test. A report was submitted primo March 2014. 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. Diagnostic manual for EUS was uploaded in 2012. But as the disease was delisted again in autumn 2012 only the most recent update of the OIE chapter on EUS is linked to the description of EUS on the webpage. Especially on KHV and ISA significant changes have been made. The manual on sampling and diagnostic procedures for the listed diseases are expected to be adapted by the Commission primo 2014 and will be 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 real time RT-PCR´s for detection of PMCV and PRV the causative agents og CMS and HSMI, respectively.

During 2013, resources were also 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 antisera; to update the EURL webpage (www.eurl-fish.eu); and finally to attend international meetings and conferences.

In 2013 the fish diseases activities of DTU Veterinary were moved from Arhus to Copenhagen, since DTU decided to close the department in Aarhus and move some of the activities to the headquarter in Copenhagen. Among the consequences were that almost all from the old permanent staff were substituted by new colleagues that had to be trained. New facilities were built for us (laboratories and aquaria) and had to be equipped and organised, unfortunately our research group conducted by professor Niels Lorenzen and his 6 co-workers was transferred from DTU to Aarhus University in order for them to be able to stay in town. The fish diseases group therefore now only consist of 5 academics and 4 technicians in permanent positions. The transfer, however, 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 other research facilities in the Copenhagen area.

**Minutes:**
2013 was a year of transition from Århus to Copenhagen of the EURL. It has been extremely hard but now EURL is settled in Copenhagen.
Our core activity is related to listed disease but we constantly keep awareness on emerging diseases – especially when culturing new fish species in intensive production systems. As guidelines for handling emerging diseases generally are not followed, and this is important to contain diseases, a working group is working on recommendations to the EU Commission.
No missions were done in 2012 and 2013, but this will be taken up again in autumn/next year.
We invite all member states to provide important topics to take up in this network – like borderlines between low-pathogenic and pathogenic virus.

# EURL WORKPLAN FOR 2014

**Niels Jørgen Olesen**, Anemone Olaja, Susie Sommer Mikkelsen, Torsten S. Boutrup and Niccolò Vendramin

**The work plan from this year is as follows:**

| | Description | Objectives |
|---|---|---|
| **1. Coordination and training** | | |
| **1-1** | Annual workshop | Organise and prepare for the 18th Annual Workshop for the National Reference Laboratories for Fish Diseases (NRLs) in 2014 |
| **1-2** | Annual workshop report | Produce a technical and financial report from the Annual Workshop 2014. |
| **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. |
| **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. |
| **1-5** | Scientific working group | Organise specific scientific meeting collating international experts to assess and provide recommendations on emerging diseases problems management and control. |
| **2. Proficiency test** | | |
| **2-1** | Proficiency test | Prepare the Annual Inter-laboratory Proficiency Tests year 2014 for the NRLs. The test will include VHSV, IHNV, EHNV, ISAV, and KHV and will also address other common viral pathogens i fish farming (IPNV, SVCV etc) |
| **2-2** | PT report | Collate and analyse information gained from the Inter-laboratory Proficiency Test |
| **3. Reagents and products** | | |
| **3-1** | Reagents | Supply reference reagents to the NRLs in Member States. |
| **3-2** | Antisera | Production of antisera against selected isolates when necessary |
| **3-3** | Pathogen library | Update and maintain a library of isolates of Infectious salmon anaemia virus (ISAV), Viral Haemorrhagic Septicaemia virus (VHSV) and Infectious Haematopoietic Necrosis virus (IHNV), Koi Herpes virus (KHV) and enzootic haematopoietic necrosis virus (EHNV) And other relevant putative emerging fish pathogens. |
| **3-4** | Tissue library | Maintain a library of tissue material from fish infected with listed pathogens |
| **4. Scientific advice and activities** | | |
| **4-1** | Webpage | Update the webpage for the EURL, www.eurl-fish.eu |

| | Description | Objectives |
|---|---|---|
| **4-2** | Diagnostic manuals | Update the diagnostic manuals for VHS, IHN, ISA, KHV disease, EHN and EUS on the EURL web page. |
| **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. |
| **4-4** | Pathogen characterization | Identify and characterise selected isolates of listed viruses (pathogenicity testing in vivo and in-vitro, serological and genetic characterisation). |
| **4-5** | www.fishpathogens.eu | Update and expand www.fishpathogens.eu with more pathogens. |
| **4-6** | Molecular epidemiology | Perform molecular epidemiology analysis to improve knowledge on diseases spreading mechanisms of viral pathogens. |
| **4-7** | Real-time PCR | Assessment and standardisation of real-time PCR tests for the diagnosis, identification and typing of the listed non-exotic fish diseases. |
| **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 |
| **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 |
| **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. |

**Questions**

**Anna Toffan:** It could be nice if EURL could recommend the precise and strict nomenclature of the virus, so this is harmonised between countries in the EU.

**Niels Jørgen Olesen:** This is a task for a working group (nomenclature group) – excellent idea!

# DRAFT EURL WORKPLAN FOR 2015

**Niels Jørgen Olesen**, Anemone Ojala, Susie Sommer Mikkelsen, Torsten S. Boutrup, Morten S. Bruun and Niccolò Vendramin

## EURL FOR FISH DISEASES, 2015

## OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2015

***1. Coordination and training***

1-1 Organise and prepare for the 19th Annual Workshop for the National Reference Laboratories for Fish Diseases (NRLs) in 2015.

1-2 Produce a report from the Annual Workshop 2015.

1-3 Collect and report data on the fish disease situation in EU, including all the listed non-exotic fish 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 2015

***2. Proficiency test***

2-1 Prepare the Annual Inter-laboratory Proficiency Test year 2015 for the NRLs. The test will include testing for VHSV, IHNV, EHNV, ISAV, KHV and in addition upon request SVC and IPN.

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

***3. Reagents and products***

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

3-2 Production of antisera against selected isolates when necessary

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 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 (serological and 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**

**Questions:**

**Knut Roenningen**:

The EURL-NRL network is extremely important tool for the EU Commission.

Some important perspectives from the Commission point of view:

1) The legal basis for movement of fish and aquatic animals should be harmonized/equalized across Europe.
2) Preparedness for non-listed diseases that can pop up should also be an important task for EURL.
3) Dissemination of information from EURL to member states

All in all the EURL should keep working on the same line as previous years.

# EURL TRAINING COURSE FOR 2014

**Niccoló Vendramin and Susie Sommer Mikkelsen**

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

**Abstract:**

Also for 2014 the EURL for fish diseases will organize training two training courses.

The courses available are:

- **Methods for implementation of surveillance procedures for listed fish diseases week 37 from Monday the 8th to Friday the 12th of September**

- **Real time PCR for diagnostics and surveillance of fish diseases in week 38 from Monday the 15th to Wednesday 17th of September**

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

More information are available on the EURL website at the following link

http://www.eurl-fish.eu/Activities/traning/Training_courses_2014

**Minutes:**
All expences wil be payed for 15 NRL participants. Further information are available at the EURL website.

**Questions:**
**Niels Jørgen Olesen:** If specific suggestions or needs arise for future training courses by EURL – please send e-mail to EURL.
Examples could be serology/antibody testing, sampling procedures etc.
For information a bioinformatics workshop on molecular tracing will be held the last week of January in Montpellier by MOLTRAQ.

# RESULTS OF THE PROFICIENCY TEST, PT1 AND PT2, 2012

**Niccoló Vendramin[1], Anemone Ojala, Troels Secher Rundqvist 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).
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.

**PT1** consisted of five coded ampoules (I-V). These ampoules contained IPNV, EHNV, SVCV, IHNV and VHSV, respectively. 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 2009. 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 2013 was conducted without major constraints. Despite the fact that the shipping company caused a delay in the delivery of the parcels 93% of parcels reached the respective laboratories of destination within 9 days after submission. Once again shipment to China demonstrated to be difficult and laborious taking about a month to reach the laboratories primarily due to border controls.
The overall performance of the participating laboratories was very high, and the fact that we this year included an ampoule with both IPNV and VHSV did not trouble most of the laboratories. It was, however, quite worrying that 6 of 43 laboratories detected virus in Ampoule V that only contain MEM without virus, and these laboratories should consider revising their procedures in order not to cross-contaminate their samples.

This year 40 participants were able to identify the EHNV isolate correctly using either sequencing or REA; however 2 laboratories sequenced the isolate but retrieved a sequence that was not correct. One laboratory did correctly isolate the virus but did not characterize it as they have indicated in their contingency plan that in case of EHNV suspicion they will forward the isolate to the EURL for Fish diseases.

**PT2** consisted of five coded ampoules (VI-X). The ampoules contained ISAV and KHV. Furthermore, one ampoule contained *Aphanomyces invadans* and one sterile pyrogen free water, see table 11. The test was designed to primarily assess the ability of participating laboratories to identify the notifiable fish pathogens ISAV, KHV and *A. invadans* (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. If present, only **inactivated** *A. invadans* was included in the ampoules.

**PT2 conclusion**

Considering that this was the fourth time that the EURL provided a proficiency test on ISAV and KHV identification, and the third time that the EURL provided a proficiency test on *A. invadans*, we consider that most participants obtained satisfying results.

Out of 36 laboratories testing for *A. invadans* 32 identified the pathogen in ampoule VI.

Out of 42 laboratories performing KHV identification, 41 laboratories identified KHV in ampoule VII and 42 correctly identified KHV in ampoule VIII.

Out of 43 laboratories 40 laboratories identified Not *A.invadans, KHV or* ISAV in ampoule IX.

Out of 42 laboratories performing ISAV identification 40 correctly identified ISAV in ampoule VI while 1 laboratory described coinfection ISAV and KHV scoring 1 point for this ampoule. Very significant improvement in the proficiency of identifying and typing these pathogens has been observed during these 4 years. In autumn 2012 the European Commission decided to de-list EUS and it is officially no more considered as an exotic disease in the Union. However we find that a certain level of preparedness for the introduction of this disease in European aquaculture should be maintained. As agreed at the Annual Workshop held in May 2013 this pathogen was included in 2013 but will not be included in 2014.

The results of the proficiency tests will be further discussed at this presentation.

**Minutes:**
EUS was included, but as it is not a listed disease it will not be included in 2014.
Some problems were seen with delivery, and perhaps the delivery company will be changed.
Some changes to the spreadsheet for the results are considered.
We recommend that laboratories never send out live material to non-accredited labs. Make sure that import permits are in place.
National labs can use the EURL material and make national/regional PT's for their own regional labs – but please inform EURL about this in advance by email.
About the idea to develop PT3, starting from the data collected during this workshop, a questionnaire about what people would like to be included, eg. SAV, but also other non-viral pathogens might be included.
A putative list could include SAV, AGD, Nodavirus.
Perhaps it should be designed as one package for Mediterranean countries, one for Salmon producers, etc.?

**Questions:**
**Irene Ørpetveit**: Why does the titration of virus have to be included? Sometimes it is almost possible to see that material is lost when opening the ampoules.

**Niels Jørgen Olesen:**: It is still a strong method, even if you lose 1/3 of the material it will not result in one-log drop. It will show the difference in cell sensitivity between labs.

**Anna Toffan**: Could it be possible to put more material in the ampoules, or perhaps provide 2 PT-tests (or ampoules) if requested?

**Niccoló Vendramin**: Could be possible.

# COMPARATIVE ANALYSIS OF SEQUENCES FROM PT 2013

**Mikkelsen S. S.**[1]

[1]*DTU Vet National Veterinary Institute, Bülowsvej 27 1870 Frederiksberg C, susmi@dtu.vet.dk*

**Abstract:**

Every year The European reference Laboratory offers two proficiency tests for all national Reference Laboratories in Europe as well as any other country that wants to participate. In 2013 43 laboratories participated in at least one of the two proficiency tests that cover all the listed fish diseases in Europe.

As part of the EURL proficiency test for fish diseases it is required to sequence any RANA virus isolates found in any of the samples. It is also highly recommended to sequence the ISA virus to determine whether it be HPRΔ or HPR0. Furthermore, it is recommended that any VHSV and IHNV isolates be genotyped.

As part of the evaluation of the proficiency results it was decided this year to look into the quality and similarity of the sequence results for selected viruses.

Ampoule III in the proficiency test 2013 contained an EHNV isolate. The EURL received 43 sequences from 41 laboratories. All but one sequence mapped to the MCP gene while the last sequence mapped to the Neurofilament gene. Approx. half of the sequences contained no errors while the rest differed with 88-99 percent similarity with most having 99% similarity. One sequence, when BLASTed, showed most similarity to European Sheatfish and not EHNV.

Generally, mistakes occurred at the ends of the sequences. This can be due to several factors. One is that the sequence has not been trimmed of the sequence primer sites. Another is the lack of quality control of the chromatogram. Finally, sequencing in just one direction can result in unclear determination of nucleotides at places with a bad quality score.

This talk will present some of the problems that can occur with sequencing as well as discuss potential pitfalls.

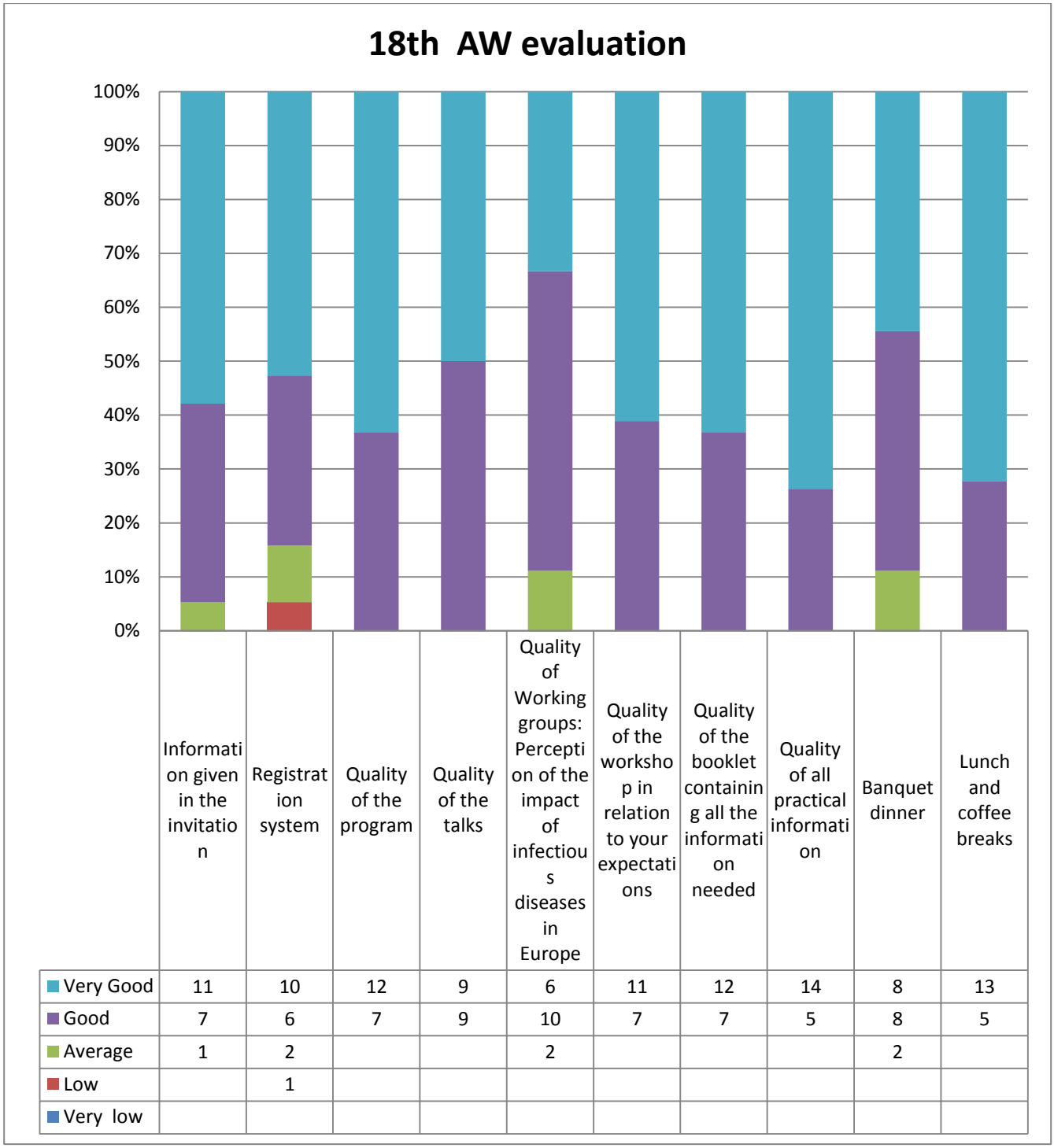
**Minutes:**
Important information to participating labs regarding sequencing of PT isolates

1) Trim the sequences – remove the primer sites
2) Send the assembled sequences to EURL (not the separate f- and r-sequences)
3) Next year EURL will ask for technique used and the chromatograms

**Questions:**
**Susie Sommer Mikkelsen**: Are member states interested in multiple alignments?
**Auditorium**: yes
**Niels Jørgen Olesen**: Alignment shows that small "mistakes" are often seen – this should be taken into account that similar mistakes may also be part of genebank sequences.

## WORKSHOP EVALUATION

A questionnaire was delivered to the participant asking to evaluate various aspect of the workshop. 19 questionnaires were anonymously retrieved, data compiled are shown hereunder. Specific comments will be considered for the next annual workshop organization.




| | Information given in the invitation | Registration system | Quality of the program | Quality of the talks | Quality of Working groups: Perception of the impact of infectious diseases in Europe | Quality of the workshop in relation to your expectations | Quality of the booklet containing all the information needed | Quality of all practical information | Banquet dinner | Lunch and coffee breaks |
|---|---|---|---|---|---|---|---|---|---|---|
| Very Good | 11 | 10 | 12 | 9 | 6 | 11 | 12 | 14 | 8 | 13 |
| Good | 7 | 6 | 7 | 9 | 10 | 7 | 7 | 5 | 8 | 5 |
| Average | 1 | 2 | | | 2 | | | | 2 | |
| Low | | 1 | | | | | | | | |
| Very low | | | | | | | | | | |

## GREETINGS AND CONCLUSION OF THE MEETING

The next meeting will be held at the end of May 2015. It will probably be organized in our new 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. Of course scientifically speaking it would be very interesting to visit other laboratories.

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